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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

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CONTENTS

ORIGINAL PAPER

JMS Journal of Medical Science

Morphology of rat brain neurons in subtotal ischaemia and introduction of L-NAME and omega-3 polyunsaturated fatty acids

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ABSTRACT

Introduction. Cerebral ischaemia leads to the development of numerous morphofunctional disorders of the cerebral cortex, which can be exacerbated by the introduction of a Nω-nitro-L-arginine methyl ester (L-NAME), which is a non-selective inhibitor of nitric oxide synthase (NOS).
Aim. To study the morphological features of rat brain neurons in subtotal ischaemia during the administration of L-NAME and naturally occurring omega-3 polyunsaturated docosahexaenoic acid (DHA).
Material and Methods. Subtotal cerebral ischaemia was modelled in rats by ligation of both common carotid arteries. L-NAME and DHA were given individually or in combination to separate groups of rats. L-NAME (5 mg/kg) was administered intramuscularly immediately before ligation and DHA (5 mg/kg) intragastrically during the week before ligation.
Results. The introduction of DHA alone had a corrective effect on the hippocampus under conditions of subtotal ischaemia, reducing the number of shadow cells and hyperchromic wrinkled neurons, without significantly affecting the size and shape of the neurons of the parietal cortex. However, the previous administration of DHA to rats with cerebral ischaemia receiving a NOS inhibitor did not abrogate the negative effects on the state of the neurons in the cerebral cortex.
Conclusion. The administration of DHA can modulate the morphological disorder of the hippocampus which occurs in subtotal cerebral ischaemia.

Introduction

Stroke is one of the most pressing problems in modern medicine [1,2]. Cerebrovascular diseases of ischaemic origin tend to grow, rejuvenate and are associated with a severe clinical course, high rates of disability, and mortality. The frequency of acute cerebrovascular accidents varies from 1 to

4 cases per 1,000 population per year. Cerebrovascular injuries are critical, requiring the concentrated efforts of different specialists, as well as the search for new approaches for the treatment of the disorders of ischaemic brain genesis [2]. One of the promising directions for solving this issue is to inhibit the formation of nitric oxide (NO). NO acts as a free radical therefore can exhibit both anti- and prooxidant properties, affecting vascular tone, haemostasis, and inflammation, showing dual effects. The non-selective inhibitor of NO synthase (NOS), Nω-nitro-L-arginine methyl ester (L-NAME) inhibits all isoforms of the enzyme, including endothelial, which leads to a decrease in antihypoxic resistance, an increase in platelet aggregation, and a decrease in cerebral blood flow [3,4].

Omega-3 polyunsaturated fatty acids (Omega-3) can have a corrective effect on the state of the endothelium, reducing the severity of oxidative stress by activating antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, and glutathione transferase), as well as acting as free radical scavengers [5,6]. The action of Omega-3 is associated with their effect on the function of cell membranes, transmembrane ion channels and the regulation of physiological processes. Furthermore, Omega-3 is involved in the implementation of basic neuronal functions, such as impulse transmission and receptor activation. Brain neurons, being electrically active cells rich in ion channels, are most sensitive to Omega-3 deficiency [7].

This study aimed to investigate the morphological characteristics of neurons of the parietal cortex and hippocampus of rats with subtotal cerebral ischaemia against the background of the administration of Omega-3 and L-NAME.

Material and Methods

Studies were performed on 30 male outbred white rats weighing 210±20 g according to the requirements of the Directive of the European Parliament and the Council No. 2010/63 / EU of 09.22.2010 on the protection of animals used for scientific purposes. Protocols were reviewed and approved by the Ethical Committee of the Grodno State Medical University (protocol No 1, 14.04.2013). The animals were kept in the vivarium in a ventilated room at 22°C, 50% humidity and illumination not more than 25 luxin, with no more than five rats per cage. The animals were fed cereals and legumes (oats, millet, barley, peas) and succulent feed (carrots, beets, cabbage) three times a day and had easy access to food and water.

The rats were divided into five groups, with six rats per group as described in **Table 1**. Subtotal cerebral ischaemia (SCI) was modelled by ligation of both common carotid arteries under conditions of intravenous thiopental anaesthesia (40-50 mg/kg). L-NAME was administered intramuscularly immediately before SCI at a dose of 5 mg/kg. Docosahexaenoic acid (DHA) in the form of a microalgae *Schizochytrium* sp. (Polski Lek S.A., Poland) preparation at a dose of 0,5g/kg was given intragastrically during the week before SCI. The control group consisted of false-operated rats treated with 0.5 ml isotonic NaCl solution.

The duration of the surgery to induct SCI was 60 minutes, after which the rats were decapitated. Samples of the anterior cortex of the cerebral hemispheres were quickly fixed in Carnoy's fluid to study the morphological changes in the neurons of the parietal cortex and CA1 field of the hippocampus in rats after SCI. Serial paraf-

Table 1. Experimental scheme

Animal group	Experimental scheme
Control	false-operated rats received 0.5 ml of isotonic NaCl solution
Group 1: SCI	ligation of both common carotid arteries under conditions of intravenous thiopental anaesthesia (40-50 mg/kg)
Group 2: SCI + L-NAME	L-NAME was administered intramuscularly at a dose of 5 mg/kg immediately before SCI
Group 3: SCI + L-NAME + DHA	L-NAME was administered intramuscularly at a dose of 5 mg/kg immediately before SCI and DHA (5 mg/kg) administered intragastrically during the week before surgery
Group 4: SCI + DHA	were additionally given intragastrically during the week before SCI with DHA at a dose of 5 mg/kg body weight

fin sections were stained with 0.1% toluidine blue according to the Nissl method.

The microphotography, morphometry, and densitometry of chromogen sediment in histological preparations were performed using an Axioscop 2 plus microscope (Zeiss, Germany), a digital video camera (LeicaDFC 320, Germany) and ImageWarp image analysis programme (Bitflow, USA). The localisation of the parietal cortex and the hippocampus cortex was determined using a stereotactic atlas [9]. At least 30 neurons of the fifth layer of the parietal cortex and the pyramidal layer of the CA1 field of the hippocampus were evaluated in each animal, which ensured a sufficient sample size for subsequent analysis. The number of large pyramidal neurons per unit area of paraffin sections of the cerebral cortex was determined. Cells were distinguished by the intensity of cytoplasm (chromatophilia) staining: normochromic – moderately stained, hyperchromic – dark, hyperchromic shrunken – very dark, with deformed perikaryons, hypochromic – lightly stained and shadow cells – almost transparent. The number of cells of each type per 1 mm^2 of brain tissue was counted. The size and shape of neuronal perikaryons were determined by area, form factor, and elongation factor.

Non-parametric statistical methods were used for data analysis (Statistica 10.0 software for Windows, StatSoft, Inc., USA) and the results are presented as Me (LQ; UQ), where Me is the median, LQ is the boundary of the lower quartile and UQ is the boundary of the upper quartile. The comparison between analysed groups was performed by Kruskal-Wallis test with post-hoc Dunn's tests. All tests were considered significant at $p<0.05$.

Results

The dimensions and shape of perikaryon neurons of the parietal cortex and hippocampus are shown in **Table 2**. The morphometry of the neurons in group 1 (SCI) revealed a significant decrease in the area of their perikaryons by 53% and 49%, with a 20% increase in elongation in each of the studied sections of the cerebral cortex, and an 11% and 22% decrease in roundness, respectively. It is assumed that these changes in the size and shape of neurons are due to water-electrolyte abnormalities, as well as the denaturation of the protein inside the cell, which forms the basis of the neurofibrils.

Table 2. Dimensions and shape of perikaryon neurons of the parietal cortex and hippocampus of rats

Animal group	Brain departments	
	parietal cortex	hippocampus
area, mkm^2		
Control	145(130; 154)[b]	109(100; 122)[b]
SCI	69(67; 74)[a]	56(55; 57)[a]
SCI + L-NAME	69 (59; 79)[a]	52(38; 58)[a]
SCI + DHA	68(50; 84)[a]	58(53; 84)[a]
SCI + L-NAME + DHA	68 (54; 80)[a]	57(40; 60)[a]
form factor, units		
Control	0,9(0,9; 0,9)[b]	0,9(0,9; 0,9)[b]
SCI	0,8(0,8; 0,8)[a]	0,7(0,7; 0,8)[a]
SCI + L-NAME	0,7(0,6; 0,7)[a]	0,8(0,8; 0,8)[a]
SCI + DHA	0,7(0,7; 0,8)[a]	0,8(0,6; 0,8)[a]
SCI + L-NAME + DHA	0,7(0,7; 0,8)[a]	0,8(0,7; 0,8)[a]
elongation factor, units		
Control	1,2(1,1; 1,3)[a]	1,2(1,1; 1,3)[a]
SCI	1,5(1,4; 1,5)[b]	1,5(1,4; 1,6)[b]
SCI + L-NAME	1,7(1,5; 1,8)[b]	1,7(1,6; 1,8)[b]
SCI + DHA	1,4(1,4; 1,5)[b]	1,4(1,4; 1,4)[b]
SCI + L-NAME + DHA	1,5(1,5; 1,5)[b]	1,5(1,4; 1,6)[b]

a, b – groups followed by the same letter do not differ statistically significantly

In group 2 (SCI + L-NAME), the form factor decreased by 22% in the neurons of the parietal cortex compared to group 1 (SCI). Compared with the control group, the area of neurons decreased by 52%, the form factor by 22%, and the elongation factor of neurons increased by 29% in the parietal cortex. No changes were detected in the hippocampus, and compared with the control group, there was a 52% decrease in perikaryon area and 11% decrease in the factor form, with a 29% increase in the elongation factor.

Compared to the control, in the parietal cortex of group 3, the area of neurons decreased by 53%, the form factor by 22%, and the elongation factor of neurons increased by 20%, whereas in the hippocampus, the area of neurons decreased by 48%, the form factor by 11%, and the elongation factor of neurons increased by 20%.

In the group 4 (SCI + L-NAME + DHA), there were no significant different area of neurons decreased by 53%, the form factor by 22%, and the elongation factor of neurons increased by 20% compared with those in the group 1 (SCI) and group 2 (SCI + L-NAME).

Regarding the chromatophilia of neurons in animals of the SCI group, there was a decrease in the number of normochromic neurons and an increase in the number of hyperchromic neurons, as well as degenerative forms, hyperchromic shrunken neurons and shadow cells in both the parietal cortex and the hippocampus (**Table 3**, **Figure 1**).

In the group 1 (SCI) in the parietal cortex, the number of hyperchromic neurons increased by 79%, hyperchromic shrunken cells by 80%, shadow cells by 67%. The hippocampus showed a 77% increase in the number of hyperchromic neurons, hyperchromic shrunken cells by 80%, and shadow cells by 67% compared to the control group.

In animals of group 2 (SCI + L-NAME), there was a 22% decrease in the number of hyperchromic neurons in the parietal cortex and a 17% increase in the number of hyperchromic shrunken neurons compared with the SCI group and in comparison to the control group, there was a 40% decrease in the number of normochromic neurons, an 73% increase in the number of hyperchromic neurons, hyperchromic shrunken neurons by 83% and shadow cells by 67%. There were no changes in the hippocampus compared with the SCI group but a 31% decrease in the number of normochromic neurons and a 79% increase in the number of hyperchromic neurons, hyperchromic shrunken by 82% and shadow cells by 67% compared with the control group.

Compared with the controls, the hippocampus of animals in group 3 (SCI + DHA) showed a 75% decrease in the number of hyperchromic shrunken neurons and an 84% increase in the number of hyperchromic neurons. Compared with the SCI group, there was a 20% decrease in the number of hyperchromic shrunken neurons, with a 31% increase in the number of hyperchromic neurons. This indicates the ability of DHA to correct the morphological changes induced in rats with SCI. No differences were observed between the parietal cortex of animals in group 4 (SCI + L-NAME + DHA) compared with the SCI and SCI + L-NAME groups, which indicates that DHA cannot correct the morphological changes in rats with SCI administered a NOS inhibitor.

Table 3. The number of different forms of neurons per 1 mm^2 by the degree of chromatophilia of the cytoplasm of the parietal cortex and hippocampus

Animal group	Brain departments	
	parietal cortex	hippocampus
normochromic neurons		
Control	3208(3178; 3245)[b]	3003(2989; 1945)[b]
SCI	1932(1920; 1945)[a]	2062(2009; 2298)[a]
SCI + L-NAME	1928(1910; 1960)[a]	2075(2004; 2345)[a]
SCI + DHA	2143(1942; 2143)[a]	2052(2001; 2167)[a]
SCI + L-NAME + DHA	1942(1932; 2143)[a]	2135(2001; 2269)[a]
hyperchromic neurons		
Control	201(201; 268)[a]	167(134; 201)[a]
SCI	938(804; 938)[c]	737(670; 938)[b]
SCI + L-NAME	737(670; 737)[b]	807(807; 874)[b]
SCI + DHA	1072(804; 1072)[c]	1072(1072; 1140)[c]
SCI + L-NAME + DHA	804(737; 1072)[c]	804(804; 938)[b]
hyperchromic shrunken neurons		
Control	134(67; 134)[a]	134(0; 134)[a]
SCI	670(670; 670)[b]	670(670; 670)[c]
SCI + L-NAME	806(806; 806)[c]	739(672; 807)[c]
SCI + DHA	603(536; 670)[b]	536(536; 536)[b]
SCI + L-NAME + DHA	670(536; 870)[b]	603(603; 672)[c]
shadow cells		
Control	134(0; 134)[a]	134(134; 134)[a]
SCI	404(269; 404)[b]	402(269; 402)[b]
SCI + L-NAME	404(269; 404)[b]	404(269; 404)[b]
SCI + DHA	269(269; 404)[b]	134(134; 269)[a]
SCI + L-NAME + DHA	404(404; 404)[b]	335(269; 404)[b]

a, b, c – groups followed by the same letter do not differ statistically significantly

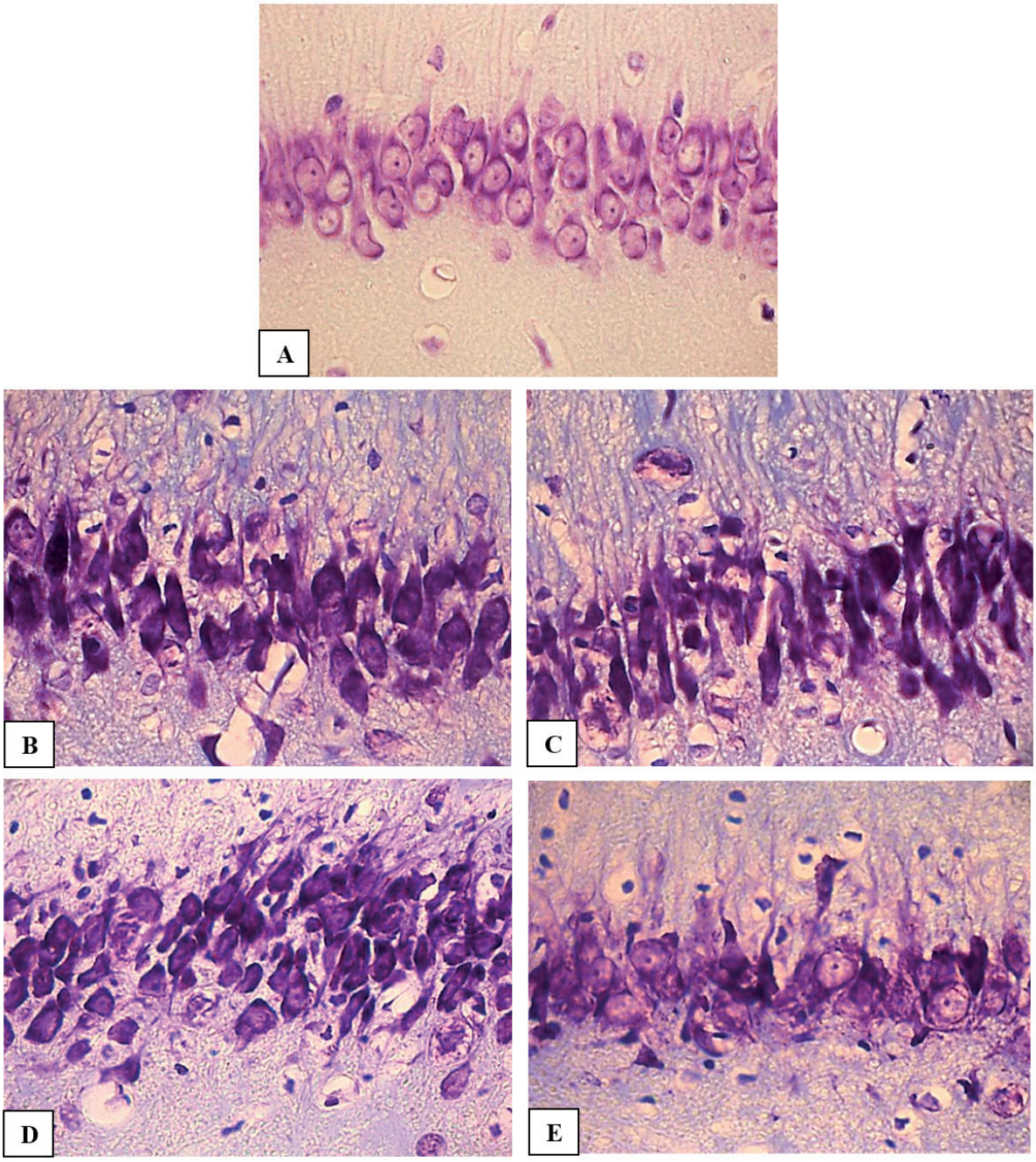

Figure 1. Neurons of the pyramidal layer CA1 of the rat hippocampus stained by Nissl. A – control, B – SCI, C – SCI + L-NAME, D – SCI + L-NAME + DHA, E – SCI + DHA. Scale bars and magnifications: A - 20 μm, X400

Discussion

Subtotal cerebral ischaemia induces significant morphological changes in the parietal cortex such as a decrease in the size and deformation of the perikaryons of neurons, the appearance of a large number of hyperchromic neurons, as well as the appearance of degenerative forms (hyperchromic shrunken neurons and shadow cells). Hyperchromic neurons are often regarded as markers of ischaemia [9, 10] and the intense staining of their cytoplasm is due to a significant predominance of the fraction of free ribosomes forming large clusters. The fixation of ribosomes to the membranes of the granular endoplasmic reticulum is an energy-dependent process provided by the ribophorin protein. Therefore, the degranulation of cisterns of the granular endo-

plasmic reticulum indicates increasing energy deficiency due to hypoxia. Degenerative changes in the granular endoplasmic reticulum lead to the accumulation of proteins in the cytoplasm, which denature under the influence of developing hypoxia and acidosis. Shrinkage of neurons in the cerebral cortex is the result of water loss due to energy and ionic disorders that decrease the size and increases the deformation of perikaryons. Hyperchromic shrunken neurons lose their functional activity and are subsequently phagocytosed by microglia [9]. The appearance of shadow cells is the next stage of hypochromia, the cause of which is oedema due to electrolyte changes caused by energy deficiency [11].

The introduction of a non-selective inhibitor of NO synthase L-NAME exacerbated the histological disorders of neurons that occur during SCI: an increase in the number of hyperchromic shrunken neurons, a decrease in size and deformation of their perikaryons. This effect may be due to an increasing degree of ischaemia due to decreased formation of NO, primarily in the endothelium and neurons, which impedes the development of vasodilator compensatory reactions [3,4]. This leads to the progression of disturbances in cellular metabolism, aggravation of water-electrolyte imbalance, manifested by deformation of neuron bodies, their wrinkling, and swelling [1,10].

Hippocampal neurons, as a phylogenetically more ancient part of the cerebral cortex, are less sensitive to hypoxia, therefore in this part of the brain, DHA had a corrective effect in rats with SCI, reducing the number of pathological forms of neurons (hyperchromic shrunken and shadow cells). A favourable effect on the state of hippocampal neurons in conditions of subtotal cerebral ischaemia may be due to a decrease in the production of thromboxane A_2 by platelets, an increase in the level of tissue plasminogen activator, and improved erythrocyte membrane fluidity, which leads to a decrease in viscosity and improved rheological properties of blood and cerebral circulation in general. DHA also exerts anti-inflammatory effects due to the incorporation of monocytes, leukocytes, endothelial cells into the phospholipid layer of the cell membranes, which is accompanied by a decrease in the production of inflammatory mediators and adhesion of leukocytes to the endothelial wall. In addition, Omega-3 affects the synthesis of prostaglandins that regulate vascular tone and inhibit vasoconstriction of blood vessels under the influence of catecholamines [5–7, 12–15].

Docosahexaenoic acid is involved in the biosynthesis of tissue hormones such as resolvin, which inhibits inflammation and neuroprotectin D1, an endogenous neuroprotector with antiapoptotic activity [7]. However, the administration of DHA to rats with SCI treated with a NOS inhibitor did not have a corrective effect on the neurons of the parietal cortex and hippocampus of rats.

The limitations of this study are due to the impossibility of following the dynamics of the adaptation of the brain during ischaemia and the metabolic changes of neurons [7].

Conclusion

Subtotal cerebral ischaemia leads to the development of morphofunctional disorders of the cerebral cortex, which can be modulated with the administration of DHA, reducing the number of shadow cells and hyperchromic wrinkled neurons, without significantly affecting the size and shape of the neurons of the parietal cortex.

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Conflict of interest statement
The authors declare no conflict of interest.

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ORIGINAL PAPER

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Depressive symptoms in acromegaly: factors that affect their incidence and severity?

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ABSTRACT

Aim. The study aimed to evaluate the influence of acromegaly on the prevalence and the severity of depressive symptoms in patients with active and inactive disease.

Material and Methods. The study group comprised 56 patients with acromegaly, which were divided into two groups based on growth hormone (GH) and insulin-like growth factor (IGF-1) levels, with controlled/cured and with uncontrolled acromegaly. The presence and severity of depressive symptoms were assessed using the Beck Depression Inventory-II (BDI-II).

Results. The mean score of BDI-II in the whole group of patients was 13.43±10.41, with no significant difference in the severity of depressive symptoms between patients with cured/controlled and uncontrolled acromegaly (p=0.620). Similarly, the lack of statistically significant differences was confirmed in patients with micro- and macroadenomas, as well as with and without hypopituitarism. There were no significant correlations between BDI-II scores and GH or IGF-1 levels, patient age or duration of the illness.

Conclusions. Depressive symptoms are common in acromegalic patients even if remission has been attained. They are most likely caused by psychological, non-organic causes. Patients diagnosed with acromegaly should undergo a screening BDI test as a part of comprehensive care, and in the event of elevated levels should be provided with a psychiatric consultation and psychological care.

Introduction

Acromegaly is a rare disease with a prevalence estimated at 4–13 cases per 100,000 population [1,2], mainly caused by a growth hormone (GH) producing pituitary adenoma. The consequence of the disease is excessive secretion of growth factors, mainly insulin-like growth factor-1 (IGF-1).

It is a chronic disease that progresses slowly, with symptoms developing gradually, and it usually takes about 5–10 years to determine a proper diagnosis. By this time, a spectrum of physical changes is already distinguishable and can visibly alter one's appearance. The main clinical features are enlarged hands and feet, thickened skin, facial changes including large ears, nose, tongue and lips, prognathism with wide spacing and premature tooth loss, excessive enlargement of superciliary arches and frontal tubers as well as other skeletal deformations (see **Figure 1**). These features are accompanied by metabolic disturbances, such as hypertension and disorders of carbohydrate metabolism, including diabetes, myocardial dysfunction, breathing disorders, visceromegaly and degenerative changes in the motor system. Acromegaly also significantly increases the risk of developing various tumours, both benign and malignant. Large pituitary adenomas can also cause visual impairment and other neurological disorders [3–5].

Metabolic disturbances, as well as changes in internal organs and appearance, maybe irreversible even after successful treatment. Consequently, patients with acromegaly often suffer from a decreased perception of general well-being, dissatisfaction with body image and tendency towards social isolation, sexual dysfunction and severe impairment of their quality of life [6–8]. Thus, acromegaly resulting in both physical and psychological limitations can increase the risk of depression and may have a negative impact on patients' quality of life even if they are in remission [6,9,10].

Additionally, it was confirmed that GH and IGF-I act on the brain through many mechanisms including several neurotransmitter systems, astrocyte intercellular communication, glucose energy metabolism, neuronal dendritic ultrastructure, and cerebral blood flow. IGF-I has a neuroprotective and regenerative role in the central nervous system, as it affects proliferation, cellular differentiation and various plasticity-related processes in several regions of the brain. Moreover, it enhances neurogenesis and increases oligodendrocyte recruitment of newborn cells in the hippocampus. These processes seem to be involved in various aspects of brain functions, such as ageing, undergoing stress, exercising

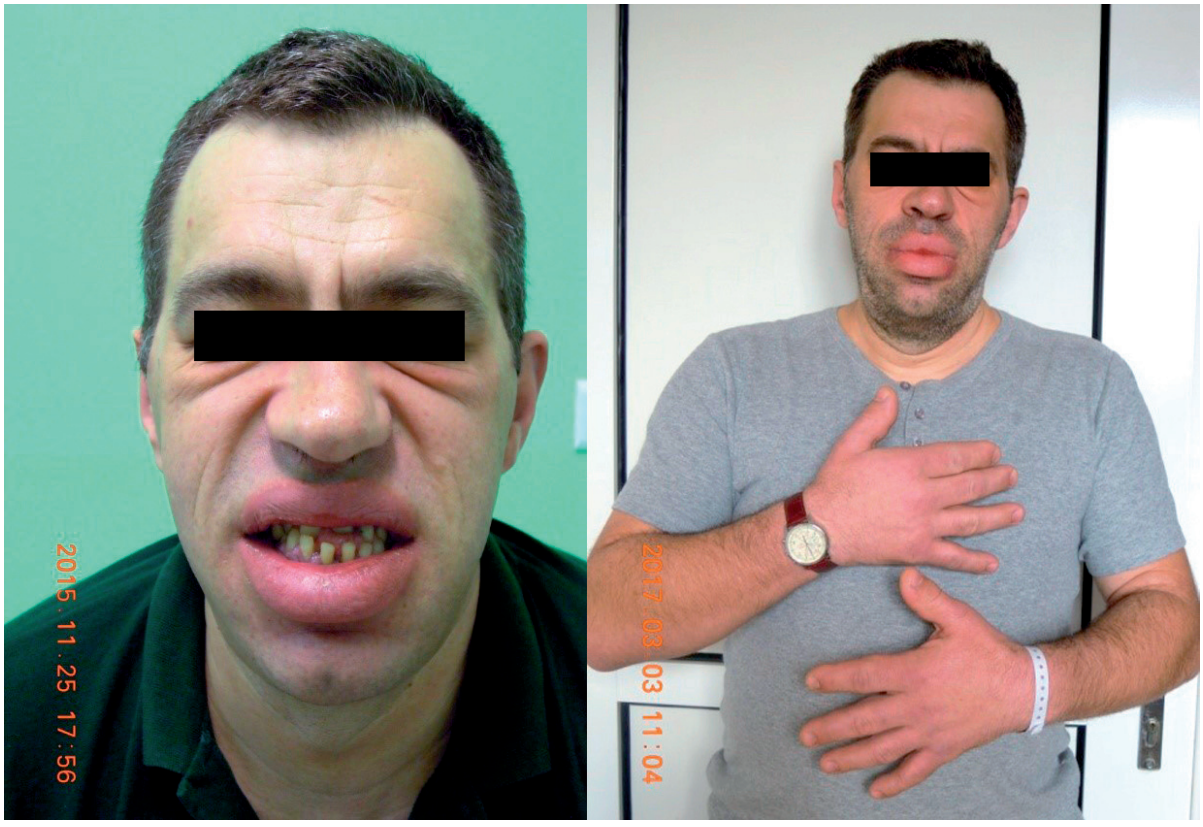

Figure 1. Typical symptoms of acromegaly in a 45-year-old patient

and learning [11]. Nonetheless, in a condition of chronic GH/IGF-1 excess, their effects could be paradoxically detrimental. Martin-Rodriquez et al. showed that acromegalic patients presented lower activity in the right dorsolateral prefrontal cortex and left parahippocampal cortex than healthy controls [12].

Aim

The aim of the study was to evaluate the influence of acromegaly on the prevalence and the severity of depressive symptoms in patients with cured or controlled and uncontrolled disease.

Material and Methods

The study group comprised 56 patients with acromegaly (37 women, i.e. 66.1% of the study group and 19 men, i.e. 33.9% of the study group), aged 25–82 years (mean 57.6±11.2) hospitalised in the Department of Endocrinology of Medical University in Lublin. Diagnosis of acromegaly was determined by the presence of typical clinical findings, failure to suppress the GH level to less than 1.0 ng/ml during an oral glucose tolerance test (OGTT), high levels of IGF-1 adjusted for age and gender, as well as the presence of adenoma in magnetic resonance imaging (MRI) pictures. In one case without visible pituitary adenoma, despite multiple imaging studies, the cause of over-production of GH was not determined. Disease duration ranged from newly diagnosed to 31 years (mean 8.8±8.4 years). The whole group was divided into two subgroups: the first with controlled or cured acromegaly (n=27) and the second with uncontrolled acromegaly (n=29). The characteristics of the study group are presented in **Table 1**.

According to Polish recommendations [5], acromegaly was considered cured as a result of surgery when both the circulating IGF-1 level was within the normal age and gender-adjudged range and GH was less than 1.0 ng/ml during OGTT. Simultaneously, it was considered controlled during pharmacological treatment when both the circulating IGF-1 level was within the normal age and gender-adjudged ranges and random GH was less than 1.0 ng/ml [5].

The presence and severity of depressive symptoms was assessed by the Beck Depression Inventory-II (BDI-II), a self-reported measure of the severity of depressive symptoms over the last two weeks. It has 21 items with a four-point scale ranging from 0 to 3 and the total score is the sum of each item-rating and can range from 0 to 63. Higher scores indicate greater symptom severity. The BDI-II can be used as a screening tool to detect depression in normal populations or as a tool to assess symptom severity in clinical populations. Depending on the population, there is a different interpretation of the BDI-II scores, for example, according to Smarr and Keefer [13], the following guidelines have been proposed: minimal range 0–13, mild depression 14–19, moderate

Table 1. Subject characteristics

Variables	The whole group n=56	Controlled / cured n=27	Uncontrolled n=29	p
Gender (male/female)	19/37	8/19	11/18	-
Age (years) mean ± SD	57.6 ± 11.2	60.7 ± 9.5	54.7 ± 12.1	p=0.044
Duration of acromegaly from diagnosis (years) mean ±SD	8.8 ± 8.4	9.7 ± 6.6	8.0 ± 9.7	p=0.442
GH (ng/ml) mean ± SD	7.14 ± 18.1	0.59 ± 0.58	13.01 ± 23.59	p=0.010
IGF-1 (ng/ml) mean ± SD	333.2 ± 278.2	151.3 ± 59.8	502.5 ± 295.1	p<0.001
Microadenoma	10	6	4	-
Macroadenoma	45	21	24	-
Tumour not visible	1	0	1	-
Treatment of acromegaly				
Previous surgery only	17	16	1	
Previous surgery + radiotherapy	2	1	1	
Previous surgery + medical treatment	18	7	11	
Medical treatment only	17	3	14	
None	2	0	2	
Hypopituitarism (appropriate hormone replacement therapy)	15	7	8	

depression 20–28 and severe depression 29–63. In a psychiatric population of the patients diagnosed with depression, a score of 0–13 is considered minimal depression [14,15] but as "no depressive symptoms" in the non-psychiatric population according to Oleszko et al. [16]. Our group of patients with acromegaly was classified as non-psychiatric.

The results were subjected to statistical analysis using STATISTICA 10.0PL. Two independent groups were compared using the Student's t-test. The $\chi 2$ test was used to compare two independent groups for sociodemographic and nominal variables. An analysis of ANOVA variance with multiple post hoc comparative Tukey's tests were used to compare three independent groups. The correlation between the severity of depressive symptoms and selected clinical variables was analysed using the Pearson correlation test. The significance level of $p<0.05$ indicating the presence of statistically significant differences or dependencies was accepted.

Results

BDI-II total score in the whole group of the patients ranged from 0 to 35, with a mean value of 13.43±10.41, which corresponds to the borderline value for diagnosing mild depression. Thirty-one patients (55.4%) scored 0–13 indicating no depression, while almost half the group (44.6%) presented scores suggesting potential depressive disorder: 8 (14.3%) mild depression (scores 14–18), 11 (19.6%) moderate depression (scores 20–26), and 6 (10.7%) severe depression (scores 29–35), as shown in figure 2. There was no statistically significant difference in the severity of depressive symptoms between patients with cured/controlled disease (mean score = 12.70±9.27) and uncontrolled acromegaly (mean score = 14.10±11.49; p=0.620). However, there was only one result corresponding to the diagnosis of severe depression in the subgroup with cured/controlled acromegaly and five cases in the subgroup with uncontrolled disease (**Figure 2**).

Similarly, the lack of statistically significant differences was confirmed in relation to the severity of depressive symptoms in patients with micro- and macroadenomas (p=0.559) as well as in patients with and without hypopituitarism (p=0.502).

There were no statistically significant correlations between the severity of depressive symp-

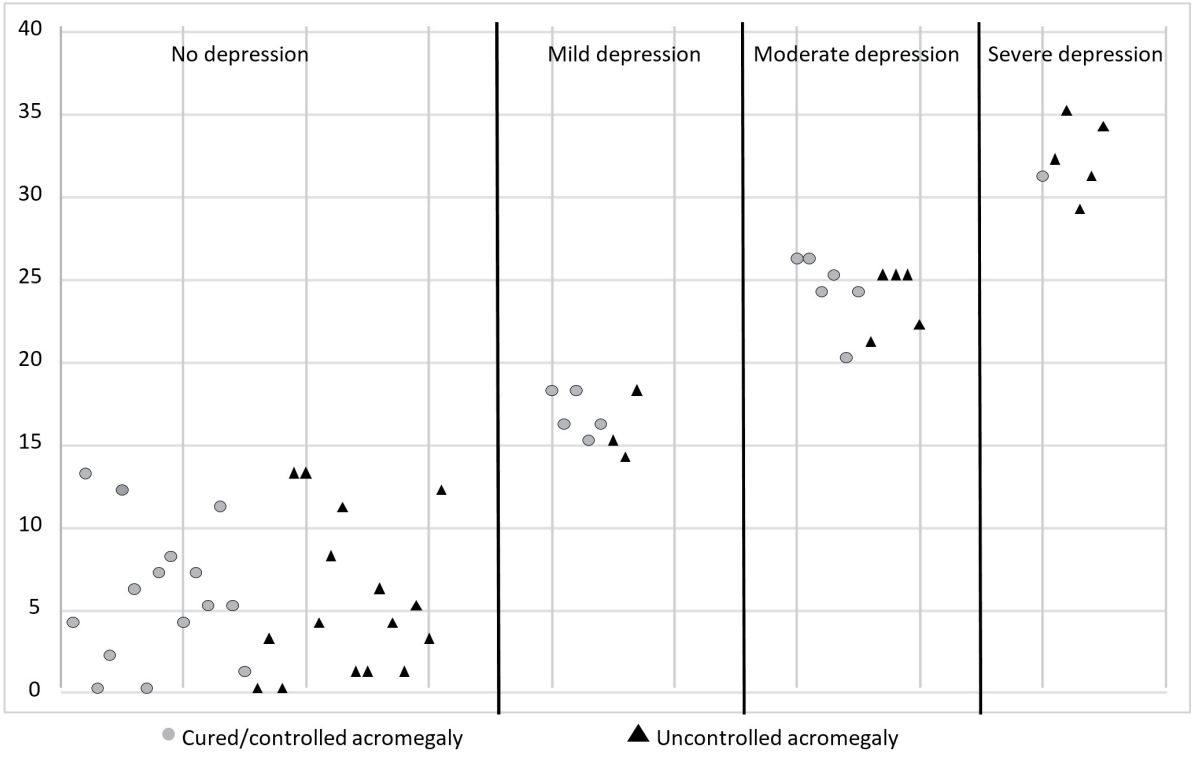

Figure 2. BDI-II scores in both groups of patients

toms and patient age, duration of illness or GH and IGF1 concentrations.

Discussion

There are very few reports concerning the psychiatric complications of acromegaly. Manfred Bleuler was probably the first to deal with this problem, describing personality changes characterised by lack of initiative and spontaneity with brief periods of impulsive behaviour and, at times, cheerfulness and self-satisfaction in 1951. He also observed brief mood swings with periods of anxiety along with bradyphrenia, egocentricity and lack of concern [17]. In the 1960s, 1970s and 1980s, there were also few reports concerning the problem of acromegaly and depressive symptoms [18–23]. Today, depression is a very widespread and valid problem as numerous studies have shown an increase in its incidence in the general population.

Depression commonly affects patients with various somatic illnesses, especially hypertension, ischaemic heart disease and diabetes [24] and can be associated with a reduced quality of life and a poorer course and prognosis of their illness [25–29]. Furthermore, a somatic disease can also be responsible for the occurrence of depression and co-occurrence of the mentioned disorders can significantly contribute to a delay in diagnosis, leading to worsening of the clinical course of both diseases [30].

BDI-II sores in the general population differ according to several reports but usually do not exceed several points. For example, in the study conducted on 576 students by Whisman [31], the mean BDI-II total score was 8.36±7.16 and a median BDI score of 9 was reported for a cohort of healthy female controls by Celik [7]. In a study of 341 healthy men in Poland by Łopuszańska [32], the mean score of depressive symptoms was 7.8±5.1 and positively correlated with age.

In our group of patients, the mean total score of BDI-II was 13.43±10.41, which was worse not only in comparison with the general population but also in comparison with other results in patients with acromegaly. For instance, with reference to the study conducted by Crespo [33], the total score of BDI-II in acromegalic patients was 9 (0–27), significantly higher when compared with controls [1 (0–17), p<0,001].

In our group, similar to other studies [6,7], the difference in BDI scores between controlled and uncontrolled patients was not significant. We also confirmed the lack of significant differences in relation to the severity of depressive symptoms in patients with micro- and macroadenomas, as well as in patients with and without hypopituitarism. There were no statistically significant correlations between the severity of depressive symptoms and patient age, duration of illness or GH and IGF1 concentrations.

To conclude, the occurrence and severity of depressive symptoms do not correlate with disease duration and activity, demographic data, biochemical parameters or the size of the pituitary adenoma, which, according to the authors, can be explained in the terms of psychological causes of depression.

There are few reports in the medical and psychological literature regarding mental and emotional disorders in patients with acromegaly despite their importance in this chronic and debilitating disease. In addition to the signs and symptoms of GH excess, patients display a loss of initiative and spontaneity, mood swings, self-esteem disturbances, distorted body image, interpersonal relations disorders, and fear of social withdrawal [34,35]. In a study of 118 patients with acromegaly, Biermasz et al. proved that joint complaints had contributed to a reduced perception of life quality for these patients. Moreover, patients with a history of myocardial infarction had reduced scores for general health, depression and fatigue, while diabetes mellitus was associated with reduced scores for anxiety and sleep [36]. T`Sjoen et al. confirmed the marked impairment of the patients' quality of life, especially in relation to appearance with a simultaneous lack of correlation regarding biochemical markers of disease activity [8]. Moreover, patients report a chronic impairment of well-being as a consequence of lasting cosmetic and orthopaedic deformities despite normalisation of GH/IGF-I secretion [34,36]. Our results also suggest that depressive symptoms are probably associated with the changes that arise in the organism and self-perception of the patient, or with the fear of having a severe chronic disease. It is unlikely that they are related to disease activity or GH and IGF-1 direct effects on the brain.

Currently, an improvement in surgical techniques and pharmacological treatment has made

it easier to gain control of acromegaly. However, the assessment of the treatment's efficiency is still based more on hormonal tests and imaging techniques estimating tumour shrinkage and less on evaluating the mental health and well-being of the patient, which we suggest is equally important. A similar opinion is shared by other authors who have proved that depression and anxiety seem to have a significant impact on the quality of life in acromegaly, greater than biochemical parameters [37]. Therefore, it is advisable to consider adequate treatment, which, as De Sousa proved with escitalopram and cognitive therapy [38], can bring very beneficial results.

Our study has some limitations. Acromegaly is a relatively rare disease so the study group is small and restricted to data from only one centre. The heterogeneity of the study population and the possible impact of treatment (surgery, radiotherapy, pharmacotherapy, hypopituitarism) should also be considered. Also, the study did not include a control group of healthy people. Nevertheless, the problem seems to be very important and requires further analysis.

Conclusions

This study shows that regardless of the disease activity, depressive symptoms are common in acromegalic patients. Also, as the occurrence and severity of depressive symptoms do not correlate with the demographic data, the biochemical parameters or the size of the pituitary adenoma, the authors believe that they may have potential psychological, non-organic causes. They could be associated primarily with the adverse changes that acromegaly causes in the appearance of patients and with the complications of the disease, which emphasises the need for early diagnosis of acromegaly. Furthermore, to optimise the management, dimensions that reflect mental state and quality of life should be evaluated in addition to biochemical and radiological parameters in acromegaly. Patients diagnosed with acromegaly should undergo a screening BDI test as a part of comprehensive care, and in the case of obtaining elevated results, they should be provided with a psychiatric consultation and psychological care.

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Conflict of interest statement
The authors declare no conflict of interest.
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ORIGINAL PAPER

JMS Journal of Medical Science

Identification of factors associated with hand hygiene adherence as a support for creating curriculum for nurses training – a multivariate analysis

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ABSTRACT

Aim. Handwashing is the easiest way to prevent infection but is often neglected. The purpose of the study was to identify the barriers limiting the respect for hygiene procedures by nurses.

Material and Methods. The study involved direct quasi-participant observation and a questionnaire of 11 nurses in six wards of three hospitals in Poland.

Results. In total, 1,195 observations were conducted in which 3,355 activities requiring hygiene procedures were observed over 8 months. The nurses' knowledge of proper hand hygiene and infection prevention principles were unsatisfactory, with an average value of correct answers in the knowledge test of 8.7 (Max=15). The univariate analysis indicated the following barriers in hand hygiene: emergencies, allergies, or too few dispensers. In multivariate analysis, the application of hygiene procedures depended on the level of education (higher education – worse compliance with the rules) and subjective conviction that handwashing/glove use was important.

Conclusion. Educational programmes on hand hygiene should focus on the World Health Organisation indications that glove use is not a substitute for handwashing.

Introduction

Hand hygiene is a general concept that includes either handwashing with soap and water, antiseptic handwash, antiseptic hand rub, or surgical hand antisepsis. Hand hygiene conserves our health, preventing the spread of respiratory and gastrointestinal infections. Recommendations for proper hand hygiene have been developed by major global institutions dealing with infection prevention and control, e.g., *Healthcare Infection Control Practices Advisory Committee* (HICPAC) and *World Health Organization* (WHO) [1,2].

It is widely recognised that hand hygiene is the cheapest way to prevent the spread of infections, including nosocomial infections. Even during the COVID-19 pandemic, the *Centres for Diseases Control and Prevention* (CDC) also issued recommendations on handwashing:

› After being in a public place and touching an item or surface that may be frequently touched

by other people, such as door handles, tables, gas pumps, shopping trolleys, or electronic cashier registers/screens, etc.

› Before touching eyes, a nose, or a mouth to prevent germs from entering our bodies [3].

Therefore, strict compliance with this procedure is extremely important. Unfortunately, it is sometimes forgotten that viruses and bacteria exist and pose a serious threat in the present world. Many authors have reported negligence in the field of hand hygiene in healthcare institutions. The non-compliance relates to the fact that hand hygiene is rare, procedures are performed improperly or the handwashing time is too short [4-6]. The reasons for this have also been described [2,7-8]. The current study is distinguished by the following features:

› It is a combination of a survey and observational study while minimising the Hawthorne effect,

› There is a lack of research in Polish literature to help identify thematic areas for the development of interdisciplinary educational programmes in the field of hand hygiene. Such a study may also help to concede educational priorities in other countries.

This study aimed to identify the barriers limiting the compliance of hygiene procedures by nurses.

Materials and Methods

The study involved the direct observation of nurses in hospital wards over eight months. After the observation, the nurses received questionnaires that assessed their knowledge and attitudes towards hand hygiene and asked them to state the barriers they perceived prevented full compliance of hand hygiene with current recommendations.

Participants

All nurses from six wards in three hospitals in central Poland.

Measures

2.2.1. Direct quasi-participant observation of each nurse and registration of all activities was performed in the observation unit (1 hour). If at the 60th minute of the observation unit, the nurse was performing any activity that required hand hygiene, the observation continued until the completion of that activity. Three random observation periods were adopted for each employee three times: in the morning, afternoon, and evening. In this case, random selection consisted of observing the first nurse encountered in the ward in a given observation unit. The observation in each ward was preceded by a weekly adaptation of staff to the observer without recording behaviour. The nurses did not know the real purpose of the study to minimise the Hawthorne effect. Elements of hygienic behaviour under observation have been described previously [9-11] and were according to current recommendations [1-2]. The workload was calculated and defined as:

Activity indicator: number of circumstances requiring hand hygiene per unit of time (an hour) [12].

The indicator of effective workload taking into account the time of performing individual medical activities as well as cleaning and administrative work: as a general percentage of time devoted to work per unit of time (an hour) (author's definition).

Observations were recorded on coded sheets.

Questionnaire

The questionnaire consisted of 15 items regarding hand hygiene issues, with 'true', 'false' or 'don't know' responses. The questions concerned the role of hands in the transmission of infections, transient and constant bacterial microflora, situations in which hand hygiene is required, and the use of protective gloves, the effectiveness of soap and disinfectants, the possibility of not washing hands when protective gloves are used. In addition, two questions were asked: how important it is for the respondent to wash their hands and how important it is for the respondent to wear protective gloves in specific situations. Identification of factors influencing non-compliance with hygiene procedures was made based on the questions regarding barriers limiting the adherence to handwashing and disinfection procedures, and the use of protective gloves.

Data analysis

The results obtained were subjected to statistical analysis using the statistical package R [13]. The following parameters were calculated for the

knowledge test: mean value (Xm), standard error of the mean value (sd), minimum value (Min), and maximum value (Max). The logistic regression model was used in the analysis of the co-contribution of many factors determining hygiene behaviour. The multivariate model included the following variables: level of knowledge (test), personal beliefs about the essence of hand hygiene, and the use of protective gloves, education, work experience, and the indicator of effective workload. The level of significance was $p \leq 0.05$.

The coding of observation sheets and surveys allowed the attribution of specific survey responses to the level of compliance with hygiene procedures by a particular nurse. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Medical University of Łódź (Resolution No. RNN/113/06/KE).

Results

Characteristics of the study group

The observed group consisted of 125 nurses working in six wards of the selected hospitals, with 55 nurses in surgical departments, and 70 nurses in non-surgical departments. The questionnaire was completed by 111 nurses (6 with higher education, 35 with post-secondary education, 70 with secondary education). Five nurses had worked in the profession for less than 5 years, 33 nurses had worked for 6-15 years, 44 nurses had worked for 16-25 years, and 29 nurses had worked for more than 25 years. In total, 111 nurses who were observed and agreed to complete the questionnaire qualified for further multifactorial analysis.

Preliminary analysis

The average value of correct answers in the knowledge test provided by nurses was 8.7 ± 2.3 (Min = 0; Max = 14). The level of knowledge of nurses was not affected by the nature of the ward they worked in (p = 0.51) or their level of education (p = 0.64). Nurses with 16-25 years of experience were most knowledgeable (**Table 1**).

Table 1. The level of the nurses' knowledge depending on the variables studied (max = 15)

	Average	± sd	Min	Max
Type of ward	(p=0.51)			
surgical	8.7	2.7	0	14
non-surgical	8.7	1.9	4	12
Education	(p=0.64)			
Higher	8.5	1.6	6	10
Post-secondary	9.0	1.6	5	13
Secondary	8.6	2.7	0	14
Seniority (years)	(p=0.043)			
≤5	7.6	1.8	6	10
6-15	8.7	2.2	3	14
16-25	9.3	2.4	2	13
>25	8.1	2.4	0	12

Note: ± sd – standard deviation; Min – minimum; Max – maximum; p – level of significance

Table 2. Multivariate model results for hand hygiene and the use of protective gloves

Variable	Hand hygiene before patient contact			Hand hygiene after patient contact			Use of protective gloves		
	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
Knowledge	1.02	0.85-1.22	0.81	0.95	0.85-1.05	0.32	1.07	0.92-1.23	0.37
Q1	1.12	0.91-1.38	0.28	1.09	0.97-1.22	0.14	1.17	1.01-1.36	0.036
Q2	1.46	1.11-1.93	0.004	1.20	1.03-1.39	0.019	1.08	0.89-1.31	0.44
Education									
Higher	1.00	(ref.)	0.34	1.00	(ref.)	0.023	1.00	(ref.)	0.017
Post-secondary	0.40	0.08-1.92		0.52	0.21-1.30		0.27	0.08-0.92	
Secondary	0.33	0.08-1.37		0.36	0.16-0.80		0.21	0.07-0.63	
Seniority									
1-5	1.00	(ref.)	0.24	1.00	(ref.)	0.15	1.00	(ref.)	0.22
6-15	4.25	0.59-30.47		2.16	0.98-5.35		2.98	1.02-8.75	
16-25	5.42	0.75-38.96		2.31	0.99-5.39		2.40	0.82-7.01	
>25	4.16	0.53-32.35		1.65	0.66-4.12		2.74	0.86-8.74	
Workload indicator	1.04	0.99-1.10	0.12	1.00	0.96-1.03	0.78	1.04	1.00-1.08	0.061

Note: OR – Odds Ratio; 95% CI – 95% Confidence Interval; p-level of significance; Q1 – question "how important is washing your hands for you?"; Q2 – question "how important it is for you to wear protective gloves?"

According to the nurses, the most common factors that may affect non-compliance with hand hygiene were: emergencies (75.68%), skin irritations, allergies (49.55%), and lack of washbasins and dispensers (29.73%). Similarly, the reasons for not wearing protective gloves were: skin irritations and latex-allergies (64.86%), emergencies (57.66%) as well as the deterioration of manual ability (53.15%).

Multivariate analysis

During the observational study, 1,195 observations were performed, recording a total of 3,355 activities requiring hygiene procedures. The nurses applied proper hand hygiene in only 15% of cases, and protective gloves in 48.3% of the circumstances requiring it. The multivariate model indicated that education significantly influenced the handwashing and not wearing protective gloves, with more highly educated nurses practising hygiene procedures less frequently. In addition, the nurses for whom the use of protective gloves was very important at work washed their hands less often both before and after contact with a patient. Also, the conviction of the importance of handwashing significantly affected the use of protective gloves (**Table 2**).

Discussion

The nurses' knowledge regarding the principles of proper hand hygiene and the importance of hand hygiene in the prevention of infections was unsatisfactory, as was the case in other Polish studies [14-16]. The importance of educational programmes in achieving effective hand hygiene has been the subject of many studies around the world.

Pittet et al. conducted a comprehensive three-year educational programme aimed at increasing effective hand hygiene, thus indicating the reduction of hospital infections and hospital transmission of bacterial microflora, including methicillin-resistant *Staphylococcus aureus (MRSA)*, achieved a significant increase in compliance with adopted procedures from 47.6% in 1994 [12] to 66.2% in 1997 [17]. During the same period, the overall hospital infection rate decreased from 16.9% to 9.9%, and the number of reported MRSA strains dropped from 2.16 to 0.93/10,000 person-days [17].

A steady increase in hand hygiene was observed in a hospital in Buenos Aires from 23.1% to 73.8% after the implementation of an educational programme in intensive care wards over a two-year period. At the same time, the number of nosocomial infections decreased from 47.55 to 27.93/1,000 person-days [18]. Grayson et al. also reported a decrease in the frequency of MRSA infections with an increase in the frequency of compliance with hand hygiene procedures from 21% to 47% over two years in six Australian hospitals [19].

Gordin et al. observed a decrease in nosocomial MRSA and VRE *(vancomycin-resistant Enterococcus)* infections over a 6-year period, after widespread access to alcohol disinfection of hands [20]. Similarly, Johnson et al. reported an increase in the level of compliance with hygiene procedures (from 21% to 42%) along with a decrease in hospital MRSA infections as the result of an educational programme combined with the improvement of the availability of hand disinfectants, indicating the importance of hand disinfection in preventing cross transmission of microorganisms [21].

Cross transmission is favoured by the survival of microflora on hospital surfaces. Most Gram-positive bacteria, such as *Enterococcus spp.* (including VRE), *S. aureus* (including MRSA), or *Streptococcus pyogenes,* and Gram-negative bacteria such as *Acinetobacter spp.* or *Klebsiella spp.* can survive on surfaces for many months. Spore-producing bacteria such as *Clostridioides difficile* survive the longest on surfaces [22]. Currently, the biggest challenge in fighting infections is the development of resistance by microbes, and recently, the most serious threat has been the spread of *Carbapenemase-producing Enterobacterales* (CPE). In Poland, the isolated number of strains producing carbapenemase increases every year, including NDM (New Delhi metallo-β-lactamase), VIM (Verona integron-encoded metallo-β-lactamase), KPC (*Klebsiella pneumoniae* carbapenemase), and OXA-48 (OXA-48-like carbapenemases) [23]. Infections caused by multidrug-resistant (MDR) organisms are associated with increased patient mortality [24].

In health promotion, it is essential to diagnose the problem, then implement appropriate prevention programmes. Hand hygiene is a similar matter. First, the barriers to appropriate hand hygiene

must be identified to allow the design of targeted educational programmes. To the author's knowledge, such research that would combine multifactor analysis using tools in the form of participant observation and a questionnaire has not been published in Poland.

In this study, the univariate analysis indicated the following barriers in hand hygiene: emergencies, allergies, or too few dispensers, which is in line with other studies [25]. To reduce these barriers, there would be a need to focus on the distribution of individual hygiene dispensers, which every nurse could carry and use when necessary. However, such actions do not always bring the expected results [26]. Perhaps less allergenic agents or better-quality gloves should be purchased, which would be more acceptable to users. Finally, hospital wards should be equipped with more dispensers. Surprisingly, the multivariate analysis revealed that nurses who thought it important to wash their hands wore protective gloves significantly less often. A similar dependence was observed in two subsequent models: the nurses, for whom it was important to use protective gloves, significantly washed/disinfected their hands less often both before and after contact with patients. This finding indicates that the belief that the use of protective gloves can replace hand washing and disinfection. Fuller et al. also observed that the rate of compliance with hand hygiene was significantly lower when gloves were worn [27].

The recommendations indicate that putting on gloves does not negate hand hygiene procedures [1], therefore, the identification of barriers limiting the respect of hygiene procedures may be more effective in multivariate models that take into account the strength of simultaneous interaction of several factors. Hand hygiene training programmes should therefore consider the factors that nurses perceive as barriers (e.g. poor quality disinfectants, poorly placed dispensers) as well as those that result from in-depth analysis.

Conclusions and Perspectives

Educational programmes on hand hygiene should focus on the indications of the World Health Organization that glove use is not a substitute for handwashing [1].

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Limitations of the Study

The study was designed to avoid the Hawthorne effect, however, it cannot be completely excluded. Also, it should be remembered that the data obtained from surveys are always subjective.

Author Contributions

Anna Garus-Pakowska conceptualised and designed the study, analysed and interpreted the data, drafted the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

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REVIEW PAPER

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The role of STAT3 in the colorectal cancer therapy

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ABSTRACT

Colorectal cancer is a type of a malignant tumor in the digestive system and its incidence rate in the United States and the European Union increases by an average of 4.2% to 4.6% annually. Colorectal cancer is a common tumor affecting rather elderly than younger individuals. An increasing number of studies prove that deregulation of the signaling pathway and abnormal expression and activation of genes can be the main reason for the development of colorectal cancer. Signal transducer and activator of transcription (STAT3) is a transcription factor of signal transduction and transcriptional activation of target genes and plays important roles in proliferation, differentiation apoptosis and other physiological processes. Several data confirm that abnormal activation of STAT3 is involved in the development of tumors. Identifying compounds that inhibit STAT3 is a promising strategy for cancer chemoprevention and treatment of colorectal cancer. In this review, the roles of STAT3 in pathogenesis and treatment of colorectal cancer are discussed.

Introduction

Colorectal cancer (CRC) is the second most common cancer diagnosed in women and the third most common in men. The prevalence of CRC increases at an average rate of 2.5% annually. Moreover, the incidence of CRC worldwide is predicted to increase to 2.5 million new cases in 2035 [1]. Epidemiological studies have shown the strong dependence of the disease incidence on gender, males, and increasing age. Additionally, diet, lifestyle, medications, smoking, obesity and a sedentary lifestyle were associated with an increased risk of CRC. Moreover, genetic changes may play a crucial role in CRC pathogenesis [2].

CRC develops through a multistage process characterised by the accumulation of aberrant protein expression, which results in the formation of tumour cells [3]. Recently, increasing attention has been focused on transcription factors contributing to oncogenic signalling pathways such as signal transducer and activator of transcription (STAT3) [4, 5]. Persistent STAT3 activation is described in several neoplasias, including CRC. Blocking STAT3 in cultured CRC cells inhibits cell proliferation and induces apoptosis [6]. Although STAT3 is required for the survival of normal intestinal epithelial cells, long-term interference with STAT3 activation could promote gastrointestinal damage. Hence, STAT3 is a potential therapeutic target for CRC [7, 8].

Structure of STAT3

STAT3 belongs to the STAT family proteins and contains six domains: N-terminal domain (ND), coiled-coil domain (CCD), DNA binding domain (DBD), the linker region, Src homology domain (SH2), and a C-terminal transcriptional activation domain (TAD) (**Figure 1**). The ND domain stabilises the dimerised STAT3, promoting the formation of tetramers of two STAT3 dimers for more stable binding with DNA. The CCD domain mediates STAT3 direct binding to the receptor and promotes STAT3 phosphorylation on the 705-tyrosine site (Y705). The DBD domain initiates transcriptional activation of the target genes, while the SH2 domain plays a critical role in signal transduction. The TAD domain possesses conserved phosphorylation sites at Tyr705 and Ser727, and SH2 can recognise phosphotyrosine residues, thus are closely related to STAT3 activation [6, 9].

STAT3 possess four isoforms: STAT3α, STAT3β, STAT3γ, and STAT3δ, with STAT3α being the most common and consists of ND, CCD, DBD, Linker, SH2 and TAD domains. STAT3α is associated with the proliferation and transformation of cells [9].

Activation of STAT3

The classical STAT3 signalling pathway is activated through the binding of interleukins, cytokines or growth factors to their corresponding cell surface receptors (**Table 1**).

In normal conditions, STAT3 is situated in the cytosol, dimerising and translocating to the nucleus after being activated via phosphorylation of the tyrosine705 residue. In the nucleus, it controls the transcription of several apoptotic and cell cycle regulatory proteins [10]. STAT3 can be activated through Janus kinase (JAK), Ras/mitogen-activated protein kinase (MAPK) and non-receptor tyrosine kinase signalling pathways [11]. The JAK phosphorylates tyrosine residues on STAT3, especially at the Y705 site, leading to activation and dimerisation of STAT3, subsequent transport to the nucleus and binding to the GAS sequence for the initiation of the transcription of target genes [12]. Ras-MAPK phosphorylates the serine residue in STAT3 on S727, which leads to STAT3 dimerisation and its translocation to the nucleus, it also binds to DNA sequences in the promoters of genes [13]. The non-receptor tyrosine kinases such as activated Src kinase and MAPK family members (p36, ERK, JNK), PKCδ,

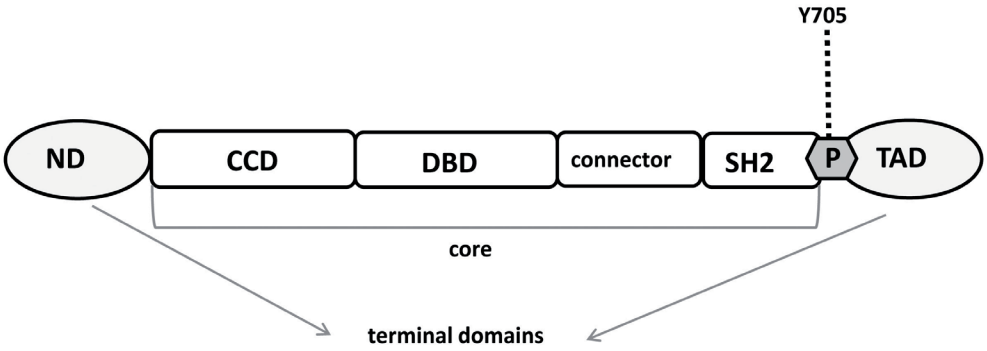


Figure 1. Structure of STAT3 protein. Functional domain: N-terminal domain **(ND)**; coiled-coil domain **(CCD)**; DNA binding domain **(DBD)**; Src homology domain **(SH2)**; C-terminal transcriptional activation domain **(TAD)**. Post-translational modification occurs at the 705-tyrosine site **(Y705)**

Table 1. The inducers of STAT3

Interleukins	Cytokines	Growth factors
IL-6, IL-7, IL-10, IL-20	– leukaemia inhibitory factor (LIF), – ciliary neurotrophic factor (CNTF), – interferon γ (IFN- γ), – tumour necrosis factor (TNF-α), – monocyte-1 chemotactic protein (MCP-1), – macrophage inflammatory protein-1α (MIP-1α), – stem cell factor (SCF), – oncostatin M (OSM)	– epidermal growth factor receptor (EGFR), – hepatocyte growth factor receptor (HGFR), – fibroblast growth factor receptor (FGFR), – platelet-derived growth factor receptor (PDGFR), – insulin-like growth factor receptor (IGFR), – vascular endothelial growth factor receptor (VEGFR)

mTOR phosphorylate STAT3 on S727 in the C-terminal domain [14].

Additionally, STAT3 is also acetylated on a single lysine residue located at position 685 by histone acetyltransferase p300. This acetylation regulates both transcriptional activity and homodimer stability. Other factors, such as UV radiation or sunlight, carcinogen, stress, smoke and infection are also known to play a significant role in STAT3 activation. STAT3 is negatively regulated by specific factors, including the suppressor of cytokine signalling (SOCS) and the protein inhibitor of activated STAT (PIAS) [15, 16].

Role of STAT3 in the pathogenesis of colorectal cancer

CRC cells and normal colon cells differ in their hallmarks. In normal cells, the activation of STAT3 is rapid and transient, whereas, in CRC cells, abnormal activation of STAT3 accelerates CRC cell proliferation, blocks their differentiation and inhibits apoptosis which leads to the occurrence and development of CRC. Several studies showed that STAT3 activation contributes to cellular proliferation and survival in the case of CRC. Persistent activation of STAT3 induces upregulated expression of CyclinD1, c-Myc and survivin and accelerates cell cycle progression in colon cancers [17–19]. The STAT3 signalling pathway suppresses apoptosis in CRC through upregulation of the expression of anti-apoptotic proteins such as Bcl-2 (B-cell lymphoma-2), Bcl-xl (B-cell lymphoma-2-like 1), and Mcl1 (myeloid cell leukaemia sequence 1) to prevent apoptosis of CRC cells [20, 21]. Inversely, inhibition of STAT3 decreases cell proliferation and promotes apoptosis in CRC [22]. Additionally, recent studies have demonstrated that increased phosphorylated STAT3 (phospho-STAT3) expression was detected in patients with colorectal carcinoma. However, the prognostic value and clinicopathological parameters of phoshoSTAT3 expression in CRC remain undefined [2].

Tang et al. [23] reported that the positive expression of JAK1 and STAT3 proteins in patients with colon cancer was not associated with sex, age, tumour differentiation degree and neurovascular invasion, but was dependent on the clinical stage of cancer, tumour infiltration depth and lymph node metastasis. The survival time of CRC patients with positively-expressed JAK1 and STAT3 proteins was significantly shorter compared to patients with negatively-expressed JAK1 and STAT3. Thus, the JAK/STAT signal may be used as a novel tumour marker and prognostic factor for the diagnosis, assessment and prognosis of colon cancer [23].

STAT3 promotes cell invasion by activating the transcription of matrix metalloproteinases, mainly MMP-2 and MMP-9. In the case of CRC, a correlation between increased MMP-2 and MMP-9 expression and a poor outcome has been proven [24]. Several studies refer to the utility of serum MMPs as markers for CRC invasion. Dragutinović et al. [25] confirmed the higher levels of MMP-2 and MMP-9 proteins in the sera of patients with CRC compared to controls with no CRC. Additionally, Kryczka et al. [26] described the upregulation of MMP-2 expression in invasive CRC. The opposite effect was observed in the case of MMP-12, which is also called metalloelastase, which does not belong to any of the MMP subfamilies. According to the studies in animal and human models, increased MMP-12 expression is associated with both reduced tumour growth and increased overall survival [27, 28].

STAT3 activation can also contribute to angiogenesis through its effects on vascular endothelial growth factor (VEGF) [29]. However, there is still controversy in terms of the relationship between serum VEGF and VEGF receptor (VEGF-R) tumour expression in CRC [30]. Evidence from preclinical and clinical studies indicates that VEGF is the predominant angiogenic factor in human CRC and is associated with the formation of metastases and poor prognosis [31].

The JAK/STAT/SOCS-signalling pathway plays a critical role in immune response and regulation of inflammation. Additionally, components of the pathway, such as STAT3, have been shown to promote cell growth and survival through impairment of the expression of genes involved in apoptosis, cell cycle regulation and angiogenesis [32]. SOCS3 is an important signal inhibition factor in the JAK2/STAT3 pathway. The reduction or deletion of SOCS3 expression causes sustained activation of STAT3 in many malignant tumours [33]. Other studies indicate that posphoSTAT3 in CRC is higher than in surrounding tissues, whereas the expression of SOCS3 is lower or absent in CRC tissues. The activation of this signalling

pathway promotes the transformation of colitis to CRC. SOCS3 protein inhibited the activation of the JAK/STAT3 pathway by negative feedback regulation of tyrosine phosphorylation of STAT3, which inhibits the growth of tumour cells. STAT3 activation also promoted hypermethylation of SOCS3 gene promoters in DLD1, HT-29 and SW480 cancer cells [34].

STAT3 as a target in CRC therapy

As STAT3 plays an important role in the development of CRC, it could be used as an essential target in the diagnosis and treatment of CRC. These inhibitors are not implemented in clinical practice but are suggested to be useful. However, clinical studies are required to assess their usefulness, efficiency and potential anticancer activities.

Table 2 presents the chemical structure of potential inhibitors of STAT3. In this review, we focus on the representatives of different groups of compounds possessing the possible implications of targeting STAT3 in colon cancer. Moreover, the mechanism of STAT3activation is multifaced, thus **Figure 2** presents the suggested therapeutic intervention strategy in the STAT3 pathway during CRC therapy.

Nowadays, STAT3 inhibitors can be classified as indirect and direct (Table 2). The indirect strategy can block molecules and induce the STAT3 pathway, indirectly inhibiting the signal transduction functions of STAT3, mostly through inhibiting the function of JAKs, in turn, there are sev-

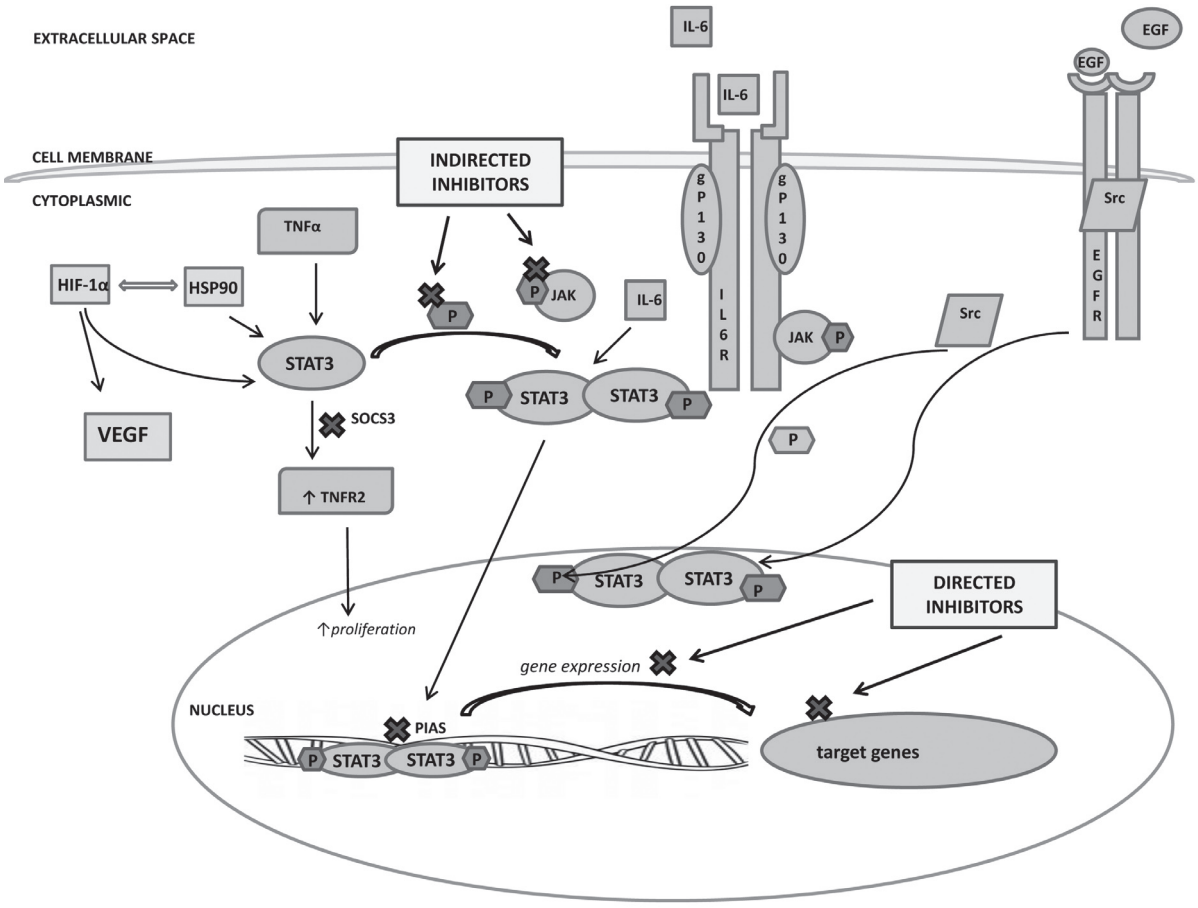


Figure 2. Therapeutic intervention strategy in the STAT3 signalling pathway for CRC therapy. Under physiological conditions, STAT3 is localised in the cytoplasm and nucleus and can be activated by **IL-6** and other cytokines, including **TNF-α**. In response to stimulation of STAT3 by cytokines, JAK1 is usually involved in the dimerisation of STAT3, as well as nuclear transport and binding to DNA. Growth factors, like **EGFR, VEGFR**, and non-receptor tyrosine kinases **(Src)** can also stimulate STAT3. Activation of STAT3 is controlled by inhibitors of cytokine signalling **(SOCS)** and protein inhibitors of active STAT, protein inhibitors of activated STAT **(PIAS).** Signal transduction is initiated by dimerisation of glycoprotein 130 **(gp130)** due to the effect of growth factor or interleukin [36]. The activation of STAT3 can also be dependent on the functionality of the hypoxia-inducible factor 1-α **(HIF-1α)**. Both STAT3 and HIF-1α are heat shock protein 90 **(HSP90)** client proteins, which transcribe **VEGF** [37], **EGF, IL-6R and TNF2**

Table 2. Inhibitors of STAT3

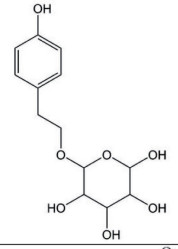
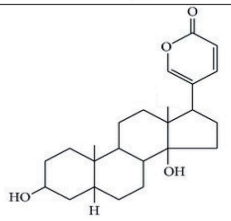
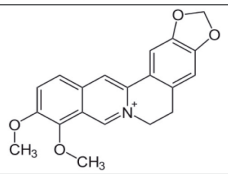
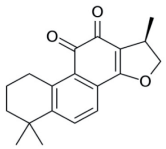
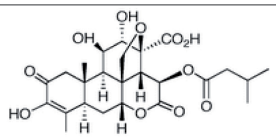
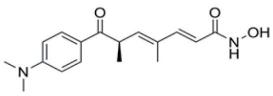
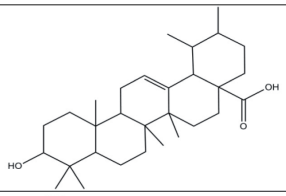
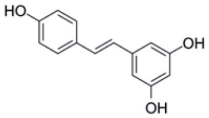
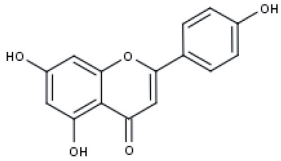
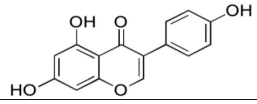
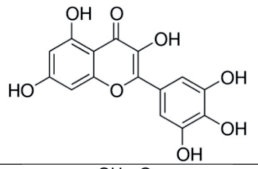
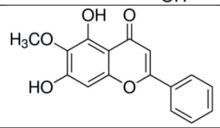
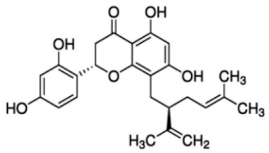
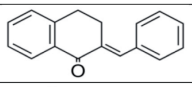
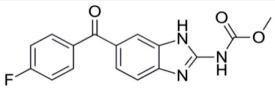
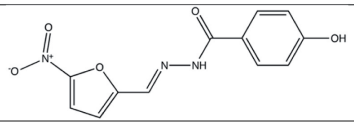
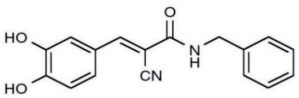
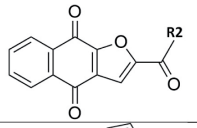
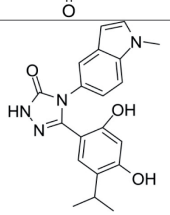
	INDIRECT INHIBITORS OF STAT3		
	Inhibitors	**Synonyms**	**Chemical structure**
Natural inhibitors	Salidroside	(2R,3S,4S,5R,6R)-2-(hydroxymethyl)-6-[2-(4-hydroxyphenyl)ethoxy]oxane-3,4,5-triol	
	Bufalin	(3β,3β)-3,14-dihydroxybufa-20,22-dienolide	
	Berberin	5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium chloride	
	Cryptotanshinone	(1R)-1,6,6-trimethyl-2,7,8,9-tetrahydro-1H-naphtho[1,2-g][1]benzofuran-10,11-dione	
	Bruceantinol	(1R,2S,3R,6R,8R,13S,14R,15R,16S,17S)-3-[(E)-4-acetyloxy-3,4-dimethylpent-2-enoyl]oxy-10,15,16-trihydroxy-9,13-dimethyl-4,11-dioxo-5,18-dioxapentacyclo[12.5.0.01,6.02,17.08,13]nonadec-9-ene-17-carboxylic acid	
	Trichostatin A	2E,4E,6R)-7-(4-dimethylaminophenyl)-N-hydroxy-4,6-dimethyl-7-oxohepta-2,4-dienamide	
	Ursolic acid	3-beta-3-hydroxy-urs-12-ene-28-oic-acid	
	Resveratrol	3, 5, 4′ -trihydroxy-trans-stilbene	
	Apigenin	4′ ,5,7-trihydroxyflavone	
	Genistein	5,7-dihyroxy-3-(-4-hydroxyphenyl)-4H-1-benzopyran-4-one	

Table 2. Continued

	Myricetin	3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4-chromenone	
	Oroxylin A	5,7-dihydroxy-6-methoxy-2-phenyl-4H-1-benzopyran-4-one	
	Sophoraflavanone G	(2S)-2-(2,4-dihydroxyphenyl)-5,7-dihydroxy-8-[(2R)-5-methyl-2-(prop-1-en-2-yl)hex-4-en-1-yl]-2,3-dihydro-4H-chromen-4-one	
Synthetic inhibitors	Benzylidenetetralones	(2E)-2-(phenylmethylidene)-1,2,3,4-tetrahydronaphthalen-1-one	
	Flubendazole	methyl(5-(4-fluorobenzoyl)-1H-benzimidazol-2-yl)carbamate	
	Nifuroxazide	4-hydroxy-N-[(E)-(5-nitro-2-furyl)methyleneamino]benzamide	
Small-molecule inhibitor	AG-490	2-cyano-3-(3,4-dihydroxyphenyl)-N-(phenylmethyl)-(2E)-2-propenamide	

DIRECT INHIBITORS

	Inhibitors	Synonyms	Chemical structure
Synthetic inhibitors	Napabucasin	2-Acetylnaphtho(2,3-b)furan-4,9-dione	
Small-molecule inhibitors	Ganetespib	3-(2,4-Dihydroxy-5-isopropylphenyl)-4-(1-methylindol-5-yl)-5-hydroxy-4H-1,2,4-triazole	

eral direct strategies according to different target domains, including the SH2 domain [35–37].

Indirect inhibitors

Natural inhibitors

Salidroside is a glucoside extracted from *Rhodiola rosea* [38], which inhibits the proliferation and cell cycle and reduces migration and invasion of colon cancer SW1116 cells through blocking the phosphorylation of JAK/STAT3 [39]. Moreover, Li and Chen [2017] suggest that salidroside downregulates phosphoSTAT3 in HCT116 cells, which is correlated with the induction of autophagy.

Bufalin is a steroid isolated from Chinese toad venom, which inhibits JAK/STAT3 signalling through decreasing the level of phosphoSTAT3 and downregulates the Bcl-2 protein. Bufalin blocks the proliferation of colon adenocarcinoma SW620 cells and induces G2/M cell cycle arrest of these cells [40, 41]. Similar results were reported by Qiu et al. [42], that bufalin reduces the viability of HCT116 cells in a dose- and time-dependent manner.

Berberin is an alkaloid isolated from *Hydrastis canadensis* and can decrease phosphorylation of JAK and STAT3 proteins in CRC cells [43]. Other studies demonstrated that berberin affects the expression of *MMP-2* and *MMP-9*, but the mechanism has not been elucidated [44, 45]. Liu et al. [43] argue that berberin reduces COX-2/PGE2 levels, consequently decreasing JAK2/STAT3 activation, leading to dampened expression of downstream target genes MMP-2 and MMP-9, reducing invasiveness and metastasis in CRC. A similar effect was presented by Hu et al. [46] and Hallajzadeh et al. [47], where the reduction in the JAK2/STAT3 signalling as a consequence of attenuating the COX-2/PGE2 expression by berberin was observed.

Cryptotanshinone is a quinoid diterpene isolated from *Salvia miltiorrhiza* Bunge. It inhibits the activation of STAT3 pathways through inactivating phosphorylation of STAT3 in SW480, HCT116 and LOVO CRC cell lines. Moreover, cryptotanshinone attenuates the expression of *Bcl-2, CyclinD1* and *survivin* in HCT116 and SW480 cells. The mechanism of action of cryptotanshinone by direct interaction with STAT3 can also rely on the inhibition of EGFR phosphorylation at higher doses of cryptotanshinone [48].

Bruceantinol is a triterpenoid isolated from *Brucinea javanica*, which reduces the level of phosphorylated STAT3 and downstream target expression of *Mcl-1, c-Myc,* and *survivin in vitro*. A reduction of phosphoSTAT3 was observed in mice with CRC xenografts treated with bruceantinol [49].

Trichostatin A is a hydroxamic acid produced by *Streptomyces hygroscopicus* and an inhibitor of class I and II histone deacetylases**.** The hyperacetylation of histones is associated with SOCS1 and SOCS3 promoters in CRC cells [50]. According to Xiong et al. [50], trichostatin A can increase the level of SOCS1 and SOCS3 expression in SW1116 and HT-29 colon cancer cell lines. Consequently, it negatively modulates the JAK2/STAT3 pathway, subsequently downregulating Bcl-2 and survivin and decreases growth and apoptosis of CRC cells.

Ursolic acid is a pentacyclic triterpenoid, abundant in apples, pears and prunes [51]. Studies conducted by Wang et al. [52] confirmed that ursolic acid selectively inhibits STAT3 phosphorylation at Y705 in CRC cell lines, HT-29, HCT116 and SW480. Moreover, some studies confirmed the antiapoptotic properties of ursolic acid in HT-29 cells via inhibition of Bcl-xl, Bcl-2 and Cyclin D1 expression [53, 54].

Resveratrol is a plant polyphenol naturally occurring in grapes, red wine, and peanuts [55]. It can inhibit cell proliferation and promote cell apoptosis via the STAT3 signalling pathway in DLD1 and HCT15 colon cancer cells. Li et al. [56] demonstrated that resveratrol inhibits cell growth in CRC by inhibiting the serine/threonine-protein kinase AKT and its downstream signalling targets. AKT serves as an upstream regulator of STAT3. Additionally, the expression of phosphorylation of STAT3 at the Tyr705 site was suppressed by treatment with resveratrol in a dose-dependent manner [56].

Apigenin is a type of flavone identified in several types of berries and vegetables, which inhibits the nuclear localisation of STAT3 through the reduction of the expression of phosphoSTAT3 (Tyr705) in HCT116 colon cancer cells [57].

Genistein is a major isoflavone in soy and soy-based food products that are regularly consumed in Asian countries [58]. Genistein promotes apoptosis in HT-29 colon cancer cells by modulating caspase-3 and p38 MAPK signalling pathway [59]. Genistein also abolished the activation of STAT3, preventing translocation into the nucleus by downregulating the activity of JNK [60].

Myricetin is a common dietary flavonoid abundantly found in plants. It deregulates the JAK1/STAT3 signalling pathway that controls many processes such as cell growth, differentiation, senescence and apoptosis [61]. Myricetin directly binds with the catalytic domain of the JAK1 protein and inhibits the phosphorylation of STAT3 and JAK1. Moreover, myricetin has been found to increase EGF-induced autophosphorylation of EGFR at Tyr845, Tyr992, Tyr1045, Tyr1068, and Tyr1173, as well as inhibit the autophosphorylation of endogenous EGFR sites. The results indicated that myricetin exerts its chemopreventive effect by directly interacting with JAK1 and STAT3 proteins [62].

Oroxylin A is an O-methylated flavone found in the roots of *Scutellaria baicalensis*. It inhibits colitis-associated carcinogenesis through modulating the IL-6/STAT3 pathway in AOM/DSS mouse model and HCT116 cells. This study confirmed that oroxylin A induces Bax and Bcl-2 binding in colon cancer Caco-2 cells [57].

Sophoraflavanone G is a plant material isolated from *Sophora leachiana*, *S. exigua* or *S. moorcroftiana. S. pachycarpa or S. flavescens.* Treatment of HCT116 cells with this small-molecule significantly inhibited tyrosine phosphorylation of STAT3, as confirmed by western blot analysis, where the level of phosphoSTAT3 protein was decreased in comparison to the control [63].

The ethanol extract of *Prunella vulgaris* L., termed **Spica Prunellae,** is a well-known traditional Chinese formulation, which can inhibit the STAT3 phosphorylation of Y705, increase the Bax/Bcl-2 ratio, reduce Cyclin D1 and subsequently inhibit CRC cell proliferation and promote apoptosis [64–66].

Synthetic inhibitors

Benzylidenetetralones are synthetic, cyclic chalcone analogues [67]. Natural and synthetic chalcones have also shown anticancer activity caused by their inhibitory potential against targets such as the JAK/STAT signalling pathway [68]. Benzylidenetetralones decrease the expression of Bcl-xl, consequently inducing cell cycle arrest and apoptosis in the HCT116 CRC cell line [67].

Flubendazole is a well-known anthelmintic drug, which blocks IL6-induced nuclear translocation of STAT3, leading to the inhibition of the transcription of STAT3 target genes, such as *MCL1* and *VEGF*. Flubendazole inhibition of STAT3 phosphorylation is partly dependent on the upstream kinases JAK2 and JAK3. Also, flubendazole reduces the expression of *P-mTOR, P62, Bcl-2*, and upregulates *Beclin1* and *LC3-I/II*, major autophagy-related genes, thereby inducing potent cell apoptosis in CRC cells. Furthermore, flubendazole displays a synergistic effect with the chemotherapeutic agent 5-fluorouracil in the treatment of CRC [69].

Nifuroxazide is a nifuran antibiotic, which downregulates the phosphorylation of tyrosine residues (Y705) on STAT3 as well as impairing the expression of MMP-2 and MMP-9 in HCT116 and HT-29 human CRC cell lines [70].

Small-molecule inhibitors

AG-490 is a pharmacological inhibitor of kinase JAK2, which decreases VEGF secretion in SW1116 and HT-29 cells, functioning likes the JAK/STAT3 pathway in angiogenesis [71]. Additionally, the downregulation of phosphoJAK1, phosphoJAK2 and phosphoSTAT3 was observed after treatment with AG490. This leads to a decline in Bcl-2 and survivin expression [71,72]. Furthermore, STAT3 inhibition by AG-490 treatment has been found to increase cell sensitivity to chemotherapeutic agents [73].

Direct inhibitors

Natural inhibitors

Curcumin is a natural polyphenol, the yellow pigment in *Curcuma longa* L. It reduces binding of STAT3 to DNA in CRC cells [74], thereby abrogating its phosphorylation and nuclear translocation, as well as the subsequent expression of target genes. This approach has mainly focused on targeting the SH2 domain, an important domain by which STAT3 maintains its biological functions [75].

Synthetic inhibitors

Napabucasin was the first compound to undergo a series of clinical trials (NCT03522649, NCT02753127, NCT01776307, NCT02851004, NCT01830621, NCT02641873) to determine its efficacy and safety in patients with metastatic CRC [76]. A napabucasin derivative with a 2-(piperidin-1-yl)ethylamino-group substituted at the R2 position significantly inhibited tumour growth in a mouse model. Molecular docking suggested that this compound bound to the SH2 domain of STAT3 in CT26 colon carcinoma mouse cell line [77].

Small-molecule inhibitors

Ganetespib is a small-molecule inhibitor of heat shock protein 90 (HSP90) activity. Ganji et al. [78] demonstrated that ganetespib inhibits STAT3 and disrupts angiogenesis of CRC cell lines HCT116 and HT-29, also downregulating the expression of VEGF transcription factors, hypoxia-inducible factor 1-α (HIF-1α) and STAT3. Both STAT3 and HIF-1α are dependent on HSP90 and transcribe VEGF, therefore, HSP90 inhibition by ganetespib also affects the expression of HIF1α and STAT3, leading to decreased transcription of pro-angiogenic cytokines such as VEGF in CRC [79].

Conclusions

STAT3 is an important signal transducer and activator of transcription which is widely involved

in numerous cellular physiological processes, such as proliferation, differentiation and apoptosis. Available data indicate that STAT3 is involved in the pathogenesis of colorectal cancer, hence, it may be useful in colorectal cancer diagnosis, treatment and prognosis. However, further studies are needed to determine if STAT3 is of use in cancer diagnosis and prognosis of disease development, as well as the possible beneficial effects of STAT3 targeted therapy. There is an increasing evidence STAT3 inhibitors, such as phytochemicals or synthetic compounds, may be potential therapeutics for colorectal cancer, so further research regarding the inhibitory properties of natural or synthetic compounds is justified.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

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The role of bisphenol A and its analogues as endocrine disruptors influencing the thyroid gland: a short review

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ABSTRACT

Bisphenols (BPs) are common plastic additives widely used in industry, hence, human exposure to BPs is inevitable. The best known BP is bisphenol A (BPA), the production of which and its analogues has been increasing worldwide. This chemical is classified as an endocrine-disrupting chemical, inferring with hormonal homeostasis. Indeed, BPA is associated with the development of oestrogen-dependent neoplasms, infertility, metabolic disorders and neurobehavioral disturbances. However, there is a lack of evidence regarding the impact of BPA and its analogues on the thyroid, with most studies mainly performed on animals or in vitro. This review aims to summarise the knowledge regarding the relationship between BPA and its analogues on the thyroid gland.

Introduction

Bisphenols (BPs) are common plastic additives widely used in industry. They are products in the manufacture of polycarbonate plastics, such as water bottles, toys, food boxes, teethers, baby pacifiers, thermal paper, inner linings of beverage and food containers, dental sealants, epoxy resins [1-6]. People are exposed to BPs mainly due to contaminated food, especially in high temperatures or acidic conditions [6, 7]. Nonetheless, the other possibilities of exposure to BPs include transdermal or inhalation routes [8].

The most common BP is bisphenol A (BPA; 4,4'-isopropylidenediphenol), which is composed of two benzene rings and two 4,4'-OH substituents (**Figure 1**). Since its industrial application, many studies have revealed the harmful effects of this chemical on human health, especially on hormonal homeostasis. Subsequently, BPA has been classified as an endocrine-disrupting chemical (EDC) [5, 9-11]. BPs act as xenoestrogens, impacting the development of oestrogen-dependent neoplasms (e.g., breast or endometrial cancers) [12-14], as well as being associated with infertility [15] and polycystic ovary syndrome [16]. More-

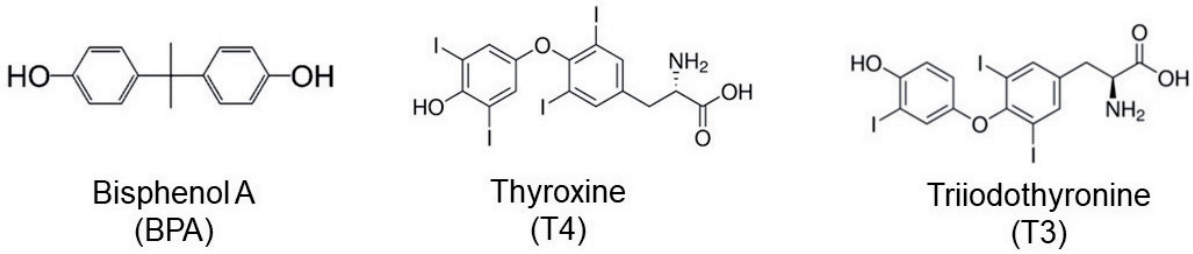

Figure 1. Chemical structures of bisphenol A, thyroxine and triiodothyronine

over, BPA may contribute to the development of metabolic disorders (e.g., insulin resistance, diabetes, obesity) [17] and neurobehavioral disturbances [18]. Furthermore, the specific molecular structure that mimics thyroid hormones allows BPA to influence thyroid hormone homeostasis [19-21] but most of these studies were conducted in vitro [19, 22, 23] or on animals [24, 25].

Types of BPA analogues

Since the use of BPA in products for children has been prohibited, the large-scale production of BPA analogues has escalated. Ideally, substitutes intended to replace a specific chemical should be less toxic than the original substance. Unfortunately, many chemical replacements introduced into the industry have never been studied and are often more harmful than the original chemical.

BPA analogues are compounds with a chemical structure similar to BPA, which means they include at least two phenyl rings, but their substituents differ depending on the type of the analogue (i.e., methyl, bromine or chlorine substituents in 3,3′ or 3,5-positions of the phenyl rings). BPA analogues include bisphenol F (BPF), bisphenol B (BPB), bisphenol Z (BPZ), bisphenol C (BPC), bisphenol P (BPP), bisphenol M (BPM), bisphenol AP (BPAP), bisphenol AF (BPAF), bisphenol AD (BPAD), tetrabromobisphenol A (TBBPA), tetrachlorobisphenol A (TCBPA), tetramethylbisphenol A (TMBPA), and dimethylbisphenol A (DMBPA) [19, 22, 23, 26].

Metabolism of BPA

After oral consumption, BPA undergoes first-pass metabolism in the intestine and liver, then it is metabolised by UDP-glucuronosyltransferase in the liver. After glucuronidation, BPA is eliminated via renal clearance within 24 hours [27]. Nonetheless, there is a concern about the other **routes** of human exposure to BPA, mainly via inhalation or transdermally, which bypass the first pass in the gastrointestinal tract, hence there is a longer exposure to unconjugated BPA. It is well documented in the literature that BPA is ubiquitous and has been measured in a variety of human body fluids [28] including placenta, maternal milk and amniotic fluid [29, 30]. The detection rates of BPs differ according to the detection method used (LC-MS, GC-MS, HPLC, HPLS-MS/MS) and the form of BPs (conjugated, unconjugated or total). Importantly, there is a lack of information regarding the metabolism of BPA analogues in humans.

Mechanisms of action of BPs on the thyroid

BPs can interact with the thyroid gland via a variety of routes, therefore, the potential crosstalk needs to be considered at multiple levels. BPA is the first environmental chemical known to bind to the thyroid receptor (TR) and affect thyroid hormone homeostasis in vitro [25]. Lee et al. [22], as well as Moriyama et al. [21], suggested that BPA can influence thyroid hormones at the transcriptional level. Moreover, Schmutzler et al. [31] reported that BPA interferes with thyroid function by inhibiting recombinant thyroid peroxidase (TPO) activity. Furthermore, an in vitro study of Kudo et al. [32] found the antagonistic ability of BPA derivatives to triiodothyronine (T3) in binding to transthyretin (TTR), which is the transport protein for thyroid hormones.

Influence of BPs on thyroid function

The results regarding the influence of BPs on thyroid function are conflicting and depend on the study design (in vivo or in vitro) and the examined group (human, animals, cell lines). According to in vitro studies of rat pituitary (GH3) cells, BPs (BPA, BPAF, BPAP, BPB, BPC, BPF, BPM, BPP, BPZ) have an agonistic effect on thyroid hormones, which is dose- and time-dependent [23]. Co-exposure of GH3 cells to 17β-oestradiol enhanced this effect. Similarly, in another study [22] on rat pituitary (GH3) and thyroid follicular (FRTL-5) cells, the authors suggested that the results were different according to cell type, with BPA and its analogues (BPAF, BPAP, BPB, BPF, BPM, BPP, BPS, BPZ, BPC) significantly downregulating *tshβ, tra, trβ, dio1* or *dio2* genes in GH3 cells, whereas in FRTL-5 cells, the genes responsible for hormone synthesis were upregulated. Furthermore, in the first in vitro study on the influence of BPA on thyroid [21], BPA antagonised T3 action at the transcriptional level in a dose-dependent manner. Kitamura et al. [19] reported that in the rat pituitary cell line GH3, selected BPs exhibited thyroid hormonal activity (TBBPA, TCBPA, TMBPA), while others (BPA, BPF, BPS, BPAF, BPAD, BPB, DMBPA) did not show such effect. The authors suggested that the chemical structure (the type of substituents of the phenyl rings of BPs) is crucial for the thyroid hormonal activity, particularly, hydroxyl groups in 4,4'-positions and methyl, bromine or chlorine in 3, 3', 5 and 5'-positions of the phenyl rings.

The results of the animal studies are inconsistent. Perinatal exposure to BPA in pups [33] or adult polecats [34] did not show any statistically significant influence on thyroid hormones, whereas there was a positive relationship between concentrations of BPA and thyroxine (T4) levels in rats [25]. Also, Lee et al. [26] demonstrated that selected BPs disrupted thyroid hormone levels by increasing T3 and T4 in embryo-larval zebrafish, suggesting that the potency of BPA analogues could be even stronger than that of BPA. It is of note that BPA derivatives could be more harmful than BPA as they act in much lower concentrations than BPA itself.

Regarding human studies, the data considering the relationship between BPs and thyrotropin (TSH) and T4 are conflicting. According to correlations between TSH and BPs in humans, there is an inverse relationship in both sexes [35] and only in women [36], suggesting that exposure to BPs may lead to the development of hyperthyroidism. Also, Meeker et al. [37] measured BPA concentrations in the urine of 1346 adults and 329 adolescents (aged 1–19 years) from the National Health and Nutrition Examination Survey (NHANES) in the period 2007–2008, observing a suggestive inverse association (but without statistical significance) between urinary BPA and TSH. In contrast, Andrianou et al. [28] suggested a positive correlation between BPA with its derivatives and TSH, which could lead to the development of hypothyroidism. Furthermore, there was a positive association between urinary BPA and serum TSH in lean individuals [38]. Taking into the consideration that BPs influence thyroid hormones in humans, the authors of several studies in pregnant women reported that BPA levels were positively [35] or inversely [20] correlated with maternal T4 levels, with two other studies found no association [36, 39].

BPs influence on the formation of thyroid nodules

Nodular goitre and thyroid cancer are related to endogenous oestrogen activity [40-42], hence, as a xenoestrogen, BPA could impact the formation of thyroid nodules. Zhou et al. [43] showed that higher BPA concentrations in urine are potentially linked to the genesis of nodular goitre and papillary thyroid carcinoma (PTC), with women with nodular goitre and PTC having higher concentrations of BPA than men. Moreover, females from the PTC group presented lower urinary BPA levels than those of the nodular goitre group. Furthermore, Marotta et al. [44] described a dose-independent correlation between BPAF and the risk of development of differentiated thyroid cancer in subjects with thyroid nodules. Li et al. [45] also showed a significant association between BPA and a higher risk of thyroid nodules in Chinese women, but only in subjects with positive TgAb and TPOAb, whereas Wang et al. [46] observed a negative correlation between urinary BPA and the risk of forming multinodular goitre, but not of solitary thyroid nodules in schoolchildren. In another study, Andrianou et al. [28] reported no association between BPs and higher risk of thyroid nodules in adult females.

BPs influence on autoimmune thyroid disease

Several studies have assessed the relationship between BPA and the development of autoimmune diseases, including autoimmune thyroid disease [47-49]. BPA can affect the immune system directly and indirectly [50]. Özaydın et al. [48] proved the influence of BPA on the alteration of immune parameters, such as cytokine profile and the distribution of $CD8^+$ and $CD4^+$ T lymphocytes in rats which can result in the development of immunodeficiencies and autoimmune diseases. Also, two case reports described the possible relationships between BPA exposure and immune system-related diseases [51, 52]. Chailurkit et al. [47] also documented the independent, statistically significant association between BPA and thyroid peroxidase antibodies (TPOAb).

Conclusions

In conclusion, the results of the studies concerning the impact of BPs on the thyroid are conflicting and are dependent on the study design and the detection methods used. It seems that BPA derivatives could be even more harmful to humans than BPA as they could act in much lower concentrations than BPA itself. As the exposure to these endocrine disruptors is inevitable, there is a strong need for large randomised human trials to establish the potentially detrimental effects of BPA and its analogues before their industrial application.

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Conflict of interest statement
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Oliceridine — a unique drug among opioid analgesics

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ABSTRACT

Oliceridine is an opioid with a different mechanism of action compared to classical opioid agonists, e.g. morphine, as it does not act through active metabolites and activates the β-arrestin pathway to a small extent, thereby reducing dangerous side effects and broadening the therapeutic window. This is particularly important as, despite the wide availability of drugs from various therapeutic groups, the effectiveness of analgesics in many patients remains low. Oliceridine is a novel and effective analgesic providing rapid analgesia, with a favourable safety and tolerability profile concerning respiratory and gastrointestinal adverse effects compared to morphine, and may provide a new treatment option for patients with moderate to severe postoperative pain where an intravenous opioid is required.

Introduction

The treatment of pain is a significant challenge in modern medicine, particularly as sustained pain diminishes patients' quality of life. In addition, the lack of effective pain treatment may cause accompanying symptoms, such as anxiety, depression, reduced physical/mental performance, and limited social relationships. Therefore, there is a need to identify new analgesic substances with fewer side effects [1].

It is well known that conventional opioids have a narrow therapeutic window, with similar doses exerting the desired therapeutic effects as well as causing adverse effects [2], which is mainly related to their mechanism of action. At the cellular level, they bind to μ-opioid receptors (MOR) belonging to G-protein coupled receptors (GPCRs) and non-selectively activate two crucial intracellular signalling pathways: the G-protein pathway, associated with analgesia, and the β-arrestin pathway, associated with opioid-related adverse effects and feedback inhibition of G-protein-mediated analgesia observed as receptor desensitisation. Therefore, the analgesic effect is proportionally combined with adverse effects [3], hence, there is a significant unmet need for a predictable and powerful analgesic with improved safety and tolerability compared with currently available therapies.

Aim

This manuscript provides an overview of the information regarding oliceridine, a new selective opioid drug. The work was based on a review of international literature contained in PubMed, Google Scholar, Scopus and Web of Science literature databases and limited to the English or Polish language.

Mechanism of action of oliceridine versus opioids

When classical opioids bind to MOR, the conformation changes detach the Gα and Gβ subunits of the heterodimeric G-protein. The Gα subunit inhibits the activity of adenylate cyclase, reduces the production of cyclic AMP (cAMP) and activates downstream signalling pathways, while the Gβ subunit inhibits voltage-gated calcium channels. Modulation of pre- and postsynaptic calcium channels causes a reduced influx of Ca^{2+} ions into the cell while reducing neuronal excitability, which is exacerbated by the blockage of sodium channels and TRPV1 (transient receptor potential vanilloid 1) channels [1,4]. When stimulated by an agonist, GPCR can activate many G-protein molecules, and if unchecked, leads to the uncontrolled stimulation of cell signalling pathways. The processes that follow activation of the PCRR leading to the inhibition of its catalytic activity (called desensitisation) are regulated by the so-called β-arrestins. They belong to the group of cytosolic proteins that "arrest" the residues involved in G-protein binding, prevent the attachment of subsequent G-protein subunits to the receptor and their activation [5]. In this way, they prevent excessive activation of the receptor, leading to the suppression of the transmitted signal, but are also responsible for the occurrence of side effects, as evidenced in β-arrestin 2 knockout mice which exhibited enhanced analgesia while significantly reducing levels of respiratory depression and constipation following morphine administration [3-7]. Therefore, it is now believed that MOR ligands, which primarily activate the G-protein pathway, by showing limited β-arrestin2 recruitment may be more effective therapeutic agents with preserved analgesia and reduced side effects [8,9]. It should be also emphasised that the weaker receptor desensitisation due to its decreased level of β-arrestin2-mediated internalisation reduces the adverse effects of GPCR agonists, slows down the development of tolerance to analgesic effects and improves the patient's quality of life [8]. The ability of these ligands to activate one selected signalling pathway has been defined as "functional selectivity", "collateral efficacy", or "biased agonism" [10-14]. A new drug belonging to this group with an atypical chemical scaffold is TRV-130 (also known as oliceridine) that acts as a full agonist for G-protein activation but exhibits markedly reduced β-arrestin recruitment than conventional opioids [15-18].

Unique pharmacological effects

Oliceridine is characterised by rapid brain penetration which is consistent with the lipophilicity of the drug, achieving the peak analgesic effect at 5 minutes after administration compared with 30 minutes for morphine, but the duration of action for both drugs is similar, that is, approximately 90 minutes. Behavioural experiments in rodents also revealed that oliceridine has 3-10 times greater analgesic activity [15]. In a tail-flick assay, it was a 4-fold more potent analgesic than morphine, with less tolerance and opioid-induced hyperalgesia after 4 days of administration of increasing doses [19] or 3-day repeated administration, indicating a lack of tolerance development to the analgesic effect [20], whereas in the hot plate test, oliceridine was 10-fold more potent than morphine [21]. Moreover, experiments in mice models indicated that oliceridine did not worsen allodynia or gait disturbances after trial fractures in contrast to morphine [19,22].

Initially, the analgesic properties of oliceridine in rodents were considered similar to those of morphine. However, this compound induced fewer side effects, especially on the respiratory and gastrointestinal tract without the development of tolerance to its analgesic effect [23,24]. The lack of tolerance for analgesic effects of oliceridine is of therapeutic importance because even long-term use does not require an increasing dose to achieve the desired therapeutic effect, which also significantly reduces the potential side effects [19].

Therapeutic application

The promising results from behavioural studies led to controlled open-label trials, which demonstrated the efficacy and safety of oliceridine for the management of moderate to severe pain following bunionectomy (APOLLO-1) [23] or abdominoplasty (APOLLO-2) [25].

In the APOLLO-1 study, effective analgesia was observed for oliceridine at a dose of 0.1, 0.35 and 0.5 mg, however, the analgesic effect comparable to morphine was observed after treatment by two higher doses. In this trial, the respiratory safety burden showed a dose-dependent increase across oliceridine regimens (mean hours: 0.1 mg: 0.04; 0.35 mg: 0.28; 0.5 mg: 0.8) but none were statistically different from morphine (1.1). Likewise, adverse effects observed as gastrointestinal adverse reactions were also induced by oliceridine in a dose-dependent manner [23].

In the APOLLO-2 study, oliceridine at the dose of 0.35 and 0.5 mg showed a favourable safety and tolerability profile regarding respiratory and gastrointestinal adverse effects in comparison to morphine. Likewise, the respiratory safety burden showed a dose-dependent increase after oliceridine treatment (mean hours: 0.1 mg: 0.43; 0.35 mg: 1.48; 0.5 mg: 1.59) in comparison to 1.72 for morphine. Gastrointestinal adverse events induced by oliceridine also increased in a dose-dependent manner but the effects were lower than for morphine [25].

Moreover, in these two pivotal efficacy studies in hard- and soft-tissue surgical models, oliceridine demonstrated a rapid (2–5 minute onset of action after intravenous administration) analgesic efficacy statistically significant vs. placebo [23,24]. Thus, oliceridine may provide an important new treatment option for the management of moderate to severe postoperative pain. Additionally, high skin-permeation of oliceridine suggests the possibility of research in terms of its use via a transdermal patch [15].

Metabolism

Additionally, unlike classical opioids, oliceridine undergoes hepatic metabolism to the inactive metabolites TRV0109662 and M22 [26,27] via the action of cytochrome P450 isoenzymes (CYP3A4 and CYP2D) during oxidation and finally, glucuronidation. Most metabolites are excreted in the urine, some with faeces. Meanwhile, it is well known that classical opioids act through active metabolites excreted by the kidneys, which may lead to their accumulation and damage to nephron structures. Due to the different metabolism of oliceridine, it can be safely used in patients with renal failure without dosage adjustments. However, considering the hepatic metabolism of this compound, it is necessary to reduce its dose in severe liver failure [17,27].

Side effects and precautions

The first clinical trials in healthy volunteers showed less pronounced side effects compared to classical opioids, demonstrating that oliceridine was safe and well-tolerated in a medically heterogeneous patient population, including the elderly, obese, and patients with comorbid conditions such as diabetes and sleep apnoea. The most common adverse reactions were nausea, vomiting, dizziness, headache, constipation, pruritus, and hypoxia [27]. Cardiovascular side effects such as lowered blood pressure and reduced heart rate were dose-dependent [18]. It should be emphasised that the prolongation of the QT interval in the ECG recording and respiratory depression were risk factors against the approval of oliceridine [28]. Unfortunately, although oliceridine may be safer than conventional opioids, prevailing evidence suggests that it will retain opioid-like abuse-related effects particularly during repeated treatment [250,21,29].

Additionally, special attention should be paid in the case of prolonged use of oliceridine during pregnancy, since it can result in neonatal opioid withdrawal syndrome [30]. Furthermore, concomitant use with benzodiazepines or other CNS depressants, including alcohol, may result in life-threatening complications such as profound sedation, respiratory depression, coma, and even death [31]. Moreover, oliceridine is contraindicated in patients with significant respiratory depression, acute or severe bronchial asthma in an unmonitored setting or the absence of resuscitative equipment, known or suspected gastrointestinal obstruction and hypersensitivity to oliceridine.

Finally, oliceridine was approved by the U.S. Food and Drug Administration (FDA) in adults for the management of acute pain severe enough to require an intravenous opioid analgesic and for whom alternative treatments are inadequate.

Summary

Currently, opioid analgesics are of great importance in medicine and their associated side effects do not exclude them from basic pharmacotherapy of high-intensity pain. However, oliceridine is a promising new analgesic drug with unique functional selectivity ("biased" agonism) which allows the therapeutic effect to be separated from the side effects previously considered inextricably linked to the mechanism of opioid action. Therefore, it may offer new therapeutic possibilities as a more effective and safer compound, especially for patients with moderate to severe postoperative pain requiring an intravenous opioid.

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Conflict of interest statement
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COVID-19: prevention and future initiative within nursing homes

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ABSTRACT

The high COVID-19 mortality rate in nursing homes in the United States and internationally prompted a comprehensive mini literature review concerning the prevalence, preventative protocol, and proactive initiatives against the highly infectious COVID-19. PubMed articles published between January and June 2020 and data sourced from government ministries of health concerning COVID-19 in nursing homes were used for this review. The prevalence and mortality rate in seven countries were compared. The underlying theme of the articles reviewed addressed four focus areas for the prevention of infectious disease spread: diagnostics, protection of residents in nursing facilities, administration and staff protection, and legislative advocacy. Adaptations and solutions may reduce the current transmission of COVID-19 within nursing homes, as well as in the future.

Introduction

An unexpected demographic group that has been significantly impacted by the coronavirus disease 2019 (COVID-19) pandemic are the elderly in nursing homes and long-term facilities. The rapid spread within a long-term skilled facility was highlighted by the first report published on 28th February 2020 in King County, Washington [1]. For the most part, COVID-19 cases in nursing homes are related to the facility location and size, not quality metrics [2]. The illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affects the elderly far more severely due to the multiple comorbidities, cognitive and behavioural issues, and living situations [3]. The focus of this literature review is to highlight the prevalence and mortality rate of COVID-19 within nursing facilities, and in turn, review which preventative measures could lower the transmission of an infectious outbreak in the future.

Material and Methods

Prevalence and mortality rates within nursing homes were collected from countries' government health ministry COVID-19 data pages. The selected countries had data on COVID-19 cases within nursing facilities.

A literature review was performed using the PubMed database, focusing on preventative measures against COVID-19 within nursing homes. The search criteria were limited to articles and data published between January 2020 to June 2020 using the following keyword(s): nursing home(s), long-term living facility, elderly, older individual(s), COVID-19, SARS-CoV-2, prevalence, mortality rate. An article was excluded if it had no substantial protocol for infection prevention or failed to address the nursing facility population. Articles were selected for review if they identified an issue regarding infection spread or proposed a protocol to preserve the health of residents and the workforce.

Results

Table 1 summarises data collected from seven countries: Belgium, Spain, Germany, Singapore, Australia, Canada, and the United States. The data comprised of the number of cases and deaths in affected long-term care facilities (LTCF) and showed that the population within care facilities in these countries had been significantly affected by COVID-19, with deaths among residents

Table 1. COVID-19 cases and deaths in affected facilities that include residential care centres, long-term care facilities, and nursing homes around the world

Country	Report date	Affected facilities	Total number of COVID-19 cases	Confirmed COVID-19 cases in LTCF	COVID-19 related deaths in LTCF	Total number of COVID-19 deaths	% of LTCF deaths
Belgium [4]	15th June 2020	Residential care centres	60,100	N/A	4,472	9,661	46%
Spain [5]	15th June 2020	Residential care centres	246,272	N/A	19,549	28,323	69%
Germany [6]	15th June 2020	Facilities for the care of older, dibbled, or other persons in need of care, homeless shelters, community facilities for asylum	186,461	17,300	3,439	8,791	39%
Singapore [7]	3rd May 2020	LTCF run by governments, non-profit organisations, and the private sector	18,205	N/A	2	18	11%
Australia [8]	21st June 2020	Australian government subsidised residential aged care facilities	7,461	71	29	102	28%
Canada [9]	22nd April 2020	Long-term care or other residential care settings (including retirement homes and assisted living facilities)	40,179	6,519	1,240	1,974	62%
United States [10]	14th May 2020	Medicare skilled nursing facility/Medicaid nursing facility	1,480,873	107,389	29,497	89,219	33%

* official data on COVID-19 related deaths among home care residents is not available for all countries due to discrepancies among testing availability and polices in documenting deaths

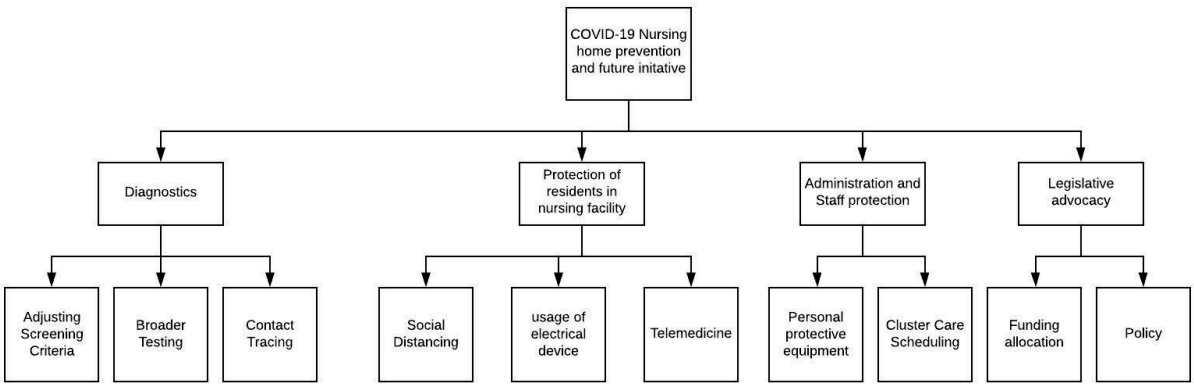

Figure 1. Summary of the nursing home preventative actions against COVID-19 based on the available studies

Table 2. Studies associated with COVID-19 in nursing homes found through PubMed

Study	Objective	Method	Preventative Action	Results	Summary
McMichael et al [1]	Investigation of confirmed cases of COVID-19 in a skilled nursing facility in King County, Washington on 28th February 2020	Public Health-Seattle and King County, aided by the CDC launched an investigation; contact tracing initiated where COVID-19 positive individuals were interviewed to collect information regarding symptoms, severity, other chronic illnesses, travel history, and close contacts; diagnostic testing; survey to assess clusters of influenza-like illness among residents, staff, transfers, and other facilities in the area; survey assessed issues that may have contributed to infection spread	Diagnostic broad testing and adjusting screening criteria; administration and staffing cluster scheduling, personal protective equipment	4 cases confirmed at Facility A within King County with 45 residents and staff displaying symptoms on 28th February 2020 As of 18th March, 167 persons positive for COVID-19 linked to Facility A; most individuals had a range of symptoms besides 7 cases; the mortality rate was 33.7%; most individuals had underlying chronic illnesses; 3 other facilities were epidemiologically linked to Facility A Survey identified that vulnerability of facilities, staff who worked while symptomatic, staff working in multiple facilities, issues with PPE, and delayed recognition of cases	COVID-19 rapidly spreads once introduced into a skilled nursing facility, which has many negative consequences Steps against infection spread need to be implemented quickly Broad testing, staff management, PPE supply should be practised
Kimball et al [11]	Investigation of asymptomatic, presymptomatic SARS-CoV-2 cases in long-term care skilled nursing facility	1st case with a long-term care skilled nursing facility occurred on 28th February 2020 On 13th March, CDC performed system assessments and SARCoV-2 testing to assess utilisation of symptoms screen as a clinical assessment of COVID-19; residents categorised as asymptomatic or symptomatic at the time of testing and preceding 14 days	Diagnostic broad testing and adjusting screening criteria	76 of 82 residents tested positive; among 23 (30%) residents with positive results, 10 (43%) had symptoms and 13 (57%) were asymptomatic	Symptom-based screening misses the identification of all COVID-19 cases, even a person who is asymptomatic or presymptomatic can have a high quantity of viral RNA
Dora et al [12]	Investigation of confirmed cases of COVID-19 On 28th March 2020, two residents in a long-term care skilled nursing facility at Veterans Affairs Greater Lost Angeles Healthcare System had positive test results for COVID-19	During 29th March 29–23rd April, all staff and residents were tested every week by reverse transcription-polymerase chain reaction (RT-PCR) testing of nasopharyngeal specimens	Diagnostics broad testing and contact tracing	99 residents (19%) and eight of 136 (6%) staff members tested positive; isolation protocol implemented; additional testing on 13th, 22nd & 23rd April showed no new positive cases	Broad testing of residents and staff members of a long-term care skilled nursing facility aided in the rapid identification of hotspots Isolation and grouping of these residents lead to a reduction in transmission within the facility Serial testing of residents performed until all were negative

Table 2. Continued

Study	Objective	Method	Preventative Action	Results	Summary
Banskota et al [13]	Show benefits of using mobile technology such as application to better quality of life during isolation of older adults	Apps review categorised as: social networking, medical, health and fitness, food and drinks, and visual and hearing Apps needed a rating of 4.5 or higher and at least 3000 reviews in the Apple Store Further screened based on function, cost, and ranking based on reviews	Protection of residents within the nursing facility through the use of electrical devices	Top apps social networking: Facetime and Skype medical: telemedicine (Teladoc, K Health Primary Care, Doctor on Demand), prescription management (GoodRx, Medisafe medication management) health and fitness: calm, headspace, MyFitnessPal, and Yoga: down dog food and drinks: Doordash and Instacart visual and hearing: Be my eyes- helping blind and Glide-live video messenger	Many apps available on many mobile device platforms can help older adults handle isolation by staying connected with others and maintain autonomy
Tan et al [14]	In Singapore, deaths of LTCF residents due to COVID-19 accounted for 14% of total fatalities as of 14th April 2020	Following preventative strategies implemented rapidly across all LTCF: early management of LTCF residents with respiratory symptoms, transfer protocols between hospital and LTCF, increased temperature screening, restriction of visitors, social distancing, and segregation of staff and residents	Administration and staffing cluster scheduling, personal protective equipment	After a month, COVID-19 found in 6 nursing homes in Singapore; an increase in LTCF correlated with exponential community transmission meaning staff were at risk of catching the virus and spreading it to the residents	Staff management is very important in preventing the spread of infection
Quigley et al [15]	Preparedness of Nursing Homes across the nation	Emailed a 30-item survey to NH drawn from national surveys conducted in 2013 and 2015 (N=942); first email sent on 30th March 2020 and reminder on 5th April 2020	Administration and staff personal protective equipment and legislative allocation of funds	Fifty-six NH responded nationwide, representing 29 states within the United States Guidance and Preparedness: NH used 2–5 guidance documents by CDC, WHO, local government Greatest COVID-19 preparedness concern: lack of supplies (43%), especially PPE, staff shortages (34%), and resident health and safety (14%) Financial effects: most indicated increased costs for supplies (58%) and employee hours (38%), or fewer admissions (27%)	Results indicate further need for NHS to continue preparedness, with a particular focus on the lack of supplies, especially PPE

accounting for 11–69% of all COVID-19 related deaths (**Table 1**). Of the seven countries, Spain had the highest percentage of LTCF deaths, with the percentage of deaths in LTCF in Belgium, Germany, Australia, and the United States between 28–45%, highlighting the vulnerability of LTCF communities. Examples of effective preventative measures can be drawn from Singapore, where the percentage of deaths in LTCF was 11%.

Sixty-two articles matched keywords, but only six articles are summarised in **Table 2**, and **Figure 1** quantitively addresses COVID-19 issues within nursing homes and provides preventative guidelines.

Discussion

The purpose of this literature review was to comprehensively address the high number of cases and deaths within nursing homes, the issue of infection prevention, and future mitigation of infectious spread. The underlying theme of the articles addressed four areas: diagnostics, protection of residents in nursing facilities, administration and staff protection, and legislative advocacy.

Diagnostics

The initial screening for COVID-19 is usually based on clinical presentation, with the most common symptoms being fever, cough, and shortness of breath [11]. Many elderly individuals present with atypical symptoms of satiety or altered mental status that can be confused as a symptom of age or a chronic illness [16]. Adjusting the screening criteria for COVID-19 to include these atypical symptoms needs to be implemented for the timely identification of cases. Furthermore, asymptomatic individuals can also have a high viral load [11]. Broad facility testing and serial testing of residents and staff should be implemented to identify clusters of infection [12], as the early identification of hotspots and initiation of infection protocols can significantly reduce the transmission within facilities [12]. Furthermore, an investigation of positive individuals can help identify weaknesses in the prevention protocol.

Protection of residents in nursing facilities

COVID-19 can rapidly spread among residents and staff once introduced into a skilled nursing facility, which may have many negative consequences. Hence, many facilities implemented social distancing to slow down the communicable spread of COVID-19, with residents isolated from each other, restricted family visitation, and limited interaction with staff [12]. Though isolation reduces the spread of infection, it has some negative ramifications. The loss of communication with others, especially loved ones, lack of information, and loss of autonomy can psychologically impact a resident in a nursing home leading to depression [13]. However, the use of devices can help address this issue, as various apps that focus on social networking, telemedicine, prescription management, health and fitness, and food and drink can be utilised to improve the residents' quality of life [13]. Many residents of nursing homes are considered vulnerable COVID-19 due to their underlying health conditions, so telemedicine can be used to provide continuity of care by limiting the cross interaction with health care providers that may have been exposed to COVID-19 [16].

Administration and Staff Protection

Staff management is essential in preventing the spread of infection, as there is the potential for staff to acquire the virus through community transmission, then spread it to the facility residents [14]. To minimise cross interaction between residents and their multidisciplinary care team, cluster care scheduling of staff should be implemented whereby the staff are assigned to specific patients for the duration of their shift [17]. To protect the staff members who are putting themselves at risk, an adequate supply of personal protective equipment and additional resources need to be accessible. In addition, if the supply chain is limited, staff need to be educated on how to reuse resources throughout their shift in a safe manner [15].

Legislative advocacy

Unfortunately, often the elderly demographic is forgotten during a health crisis, therefore, emphasis needs to be placed on public health planning by collaborating with geriatric health experts, nursing home leadership, and government [18]. Reform and policies focusing on funding allocation need to be made so that resources, like personal protective gear, testing supplies, wages for increased staff, and smart device purchases, can be used within a nursing home to minimise the negative outcomes of an infectious outbreak.

If implemented, the above solutions can reduce the transmission of COVID-19 and help prepare facilities for future outbreaks.

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Zoonoses and their traces in ancient genomes – a possible indicator for ancient life-style changes?

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ABSTRACT

Humans are constantly exposed to health risks inherent to the environment in which they live, thereby including non-human fauna. Zoonoses are infectious diseases caused by agents such as bacteria, parasites, or viruses being transmitted to humans from wild animals and livestock. The close proximity of animals and humans facilitate the spread of zoonoses, so it is intriguing to hypothesize that populations accustomed to different lifestyles will also vary in the prevalence of zoonotic agents. The Neolithic era in human history is characterised by a dramatic transition in lifestyle, from hunting and gathering to farming. Thus, with the changes in the reservoir of animal species humans were exposed to zoonotic agents potentially penetrating human populations. Due to the rapid development of sequencing technologies and methodology in ancient DNA research, it is now possible to generate complete genomes of ancient specimens and pinpoint those genomic regions or epigenetic signatures that might be influenced by past zoonotic transmissions. Unravelling such traces, particularly on a population-scale, will help to overcome the lack of generalisation that hampered previous research focusing exclusively on the model fossils in human evolution, and facilitate a better understanding of the aetiology of diseases, including those caused by zoonotic agents.

Humans are constantly exposed to health risks inherent to their environment or facilitated by direct interactions with members of our species or other organisms. Zoonoses are infectious diseases that pose a severe risk to health and while being manifested in human populations, they have their origin in non-human fauna [1]. Bacteria, parasites, viruses or other agents are transmitted to humans from wild animals and livestock, thereby causing serious illnesses like Ebola and SARS [2, 3]. Zoonoses are tightly linked to human-animal interactions and contemporary exposure can directly be assessed via stool or blood screens, and whenever a genetic marker

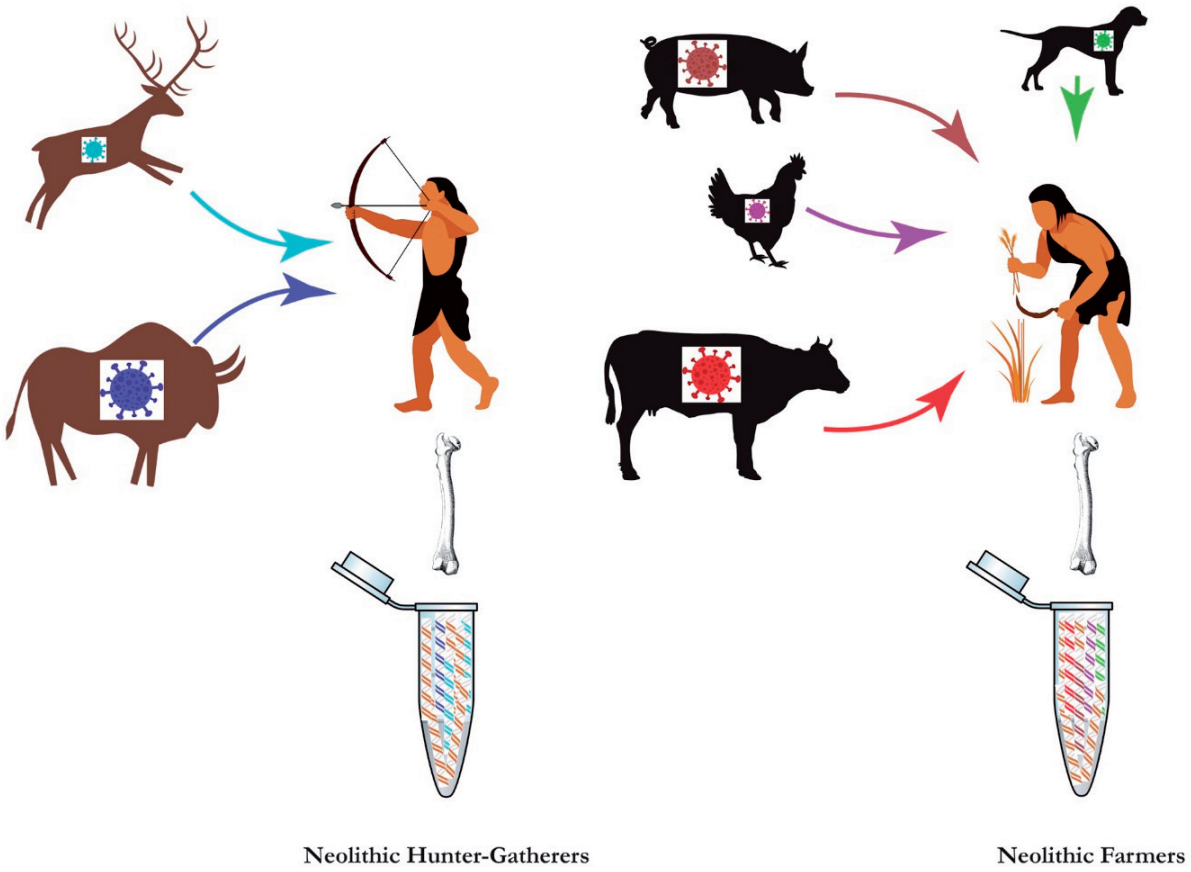

Figure 1. Graphical depiction of the hypothesis of population differences in zoonoses exposure and detection in Neolithic humans

of the agent becomes available, it can be traced through space and time. A classical example is the emergence and trajectory of various Ebola outbreaks [4], however, while Ebola is most likely associated with recent exposure to the virus, other zoonotic agents entered human populations a long time ago.

It is now intriguing to speculate that various human lifestyles or the transition between them might have facilitated the emergence of zoonoses. One such crucial periods in human history was the Neolithic, in which the shift from hunting-gathering to farming occurred. During this era, the agricultural revolution sustainably altered our diet and how communities were organised, thereby allowing cultures to thrive. Most importantly, humans started to interact with animals that ultimately became our domesticates (5). Archaeological evidence and simulation data support the idea of increased zoonotic prevalence due to lifestyle transitions [6, 7], however, to explicitly test this hypothesis and evaluate the zoonotic burden of our Neolithic ancestors, we must exploit their respective ancient genomes. First attempts to detect disease agents in ancient genomes have provided encouraging results, suggesting an onset of tuberculosis and plaque as early as 5000 years ago or identifying the trajectory of HBV transmission and its origin 100,000 years ago [8, 9]. We believe that incorporating population-wide genomic data generated from actual Neolithic human samples would greatly expand current knowledge with respect to zoonoses, possibly providing new evidence regarding the health implications of the Neolithic Revolution. Herein, we discuss the prospects of novel Paleogenomic and Paleoepigenomic approaches in light of the evolution of zoonoses [10].

One major hurdle that needs to be addressed relates to the characteristics of ancient DNA, namely its fragmentation, various damage patterns and low endogenous DNA content [11]. Recent developments in sequencing technologies [12] have enabled us to not only account for these patterns but also to make use of them. It has long been accepted that ancient DNA extracts are

a mixture of endogenous DNA, environmental, microbiological and modern contaminants [13]. Generating billions of DNA snippets facilitates the investigation of the origin of literally every single DNA fragment obtained from an ancient sample. The prospects are unlimited, and the recent drop in per-base sequencing costs render the 1,000 $ genome rather science than fiction. The length of the molecules sequenced from ancient materials can be indicative of the age of the specimen with shorter fragments representing older material [14], which further helps to differentiate endogenous from contaminant DNA. A comparison of these fragments with genetic databases allows a taxonomic classification of those not mapping to the respective reference genome (i.e. the human genome). Intriguingly, this approach might readily uncover disease agents the specimen was exposed to in the past [15, 16]. However, the quality of the genome is further determined by its coverage, a measure of how often a single nucleotide has been sequenced from a sample. This is crucial, as the higher the coverage in a particular genomic region, the better the chances of identifying rare variants, including those with health relevance (e.g. encoding viral/pathogen interacting proteins [17]). Despite a qualitative difference in the genes affected by such variances, we would further predict a difference in frequencies observed in populations differentially exposed to zoonotic agents. Alternatively, by screening the endogenous genome, we could detect genetic material potentially incorporated from actual zoonotic agents. Parasites infecting livestock and wild animals can act as media species for horizontal gene transfer [18-20], an often underappreciated but widespread phenomenon [20]. If so, we can hypothesise that two populations accustomed to different diets and ways in which they interact with animals will differ in terms of parasitic infections and horizontally transferred DNA fragments. The methodical approach has been established on modern genomes [18] and its application for ancient DNA should pose no major obstacles. Despite the merits of investigating endogenous DNA, assessing the metagenomic composition of, for instance, dental plaque will further help to not only highlight dietary components but also to evaluate the oral fauna, thereby assess host-pathogen interactions and provide a direct health indicator from the past [21].

A highly covered, high-quality ancient genome is the basis of Paleoepigenomic analyses [12, 22]. It has been suggested that populations with low genetic variation might exhibit higher epigenetic variation leading to the hypothesis that epigenetic mechanisms might act as a fast compensatory mechanism for the adaptation to novel environments [23, 24]. If these patterns are now translated into the Neolithic, it is tempting to speculate that changes in the methylation landscape, the only means to detect epigenetic changes in the past [12], and in particular, those affecting immunological responses might have been triggered by a decreased vicinity and a prolonged exposure to livestock in novel farming communities. Prior to the emergence of next-generation sequencing technologies [25], we simply lacked the means to address Paleoepigenomics questions, but high-quality ancient genomes have now produced intriguing results alluding to epigenetic changes in our archaic human ancestors. Gokhman and colleagues provided a first ancient methylation map [22] and were able to identify thousands of differentially methylated sites in the genomes of Denisovan, Neanderthals and modern humans, and by using these maps they proposed a detailed morphological profile of Denisovans [26].

As these studies solely focused on the charismatic models in human evolution, they can merely present an individual assessment, thereby lack generalisation. Consequently, the investigation of population-wide patterns of genomic variation prevalent in the past will help to circumvent such singularity and address patterns of broader relevance for the emergence and prevalence of zoonotic agents during the lifestyle transition in the Neolithic. Such elaborate investigations will not only elevate ancient DNA research to a next level but also allow the evaluation of the effects of lifestyle changes in the past on the aetiology of modern diseases, and our knowledge of the variety and severity of zoonotic agents.

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Conflict of interest statement
The authors declare no conflict of interest.

Author contribution
All authors have discussed the ideas and contributed to the manuscript.

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