PEDIATRIA POLSKA 88 (2013) 230–235

Available online at www.sciencedirect.com

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Original research article/Artykuł oryginalny

The content of serotonin cells in duodenal biopsies of autistic patients

Zawartość serotoniny w błonie śluzowej dwunastnicy pacjentów autystycznych

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ARTICLE INFO

Article history:
Received: 04.02.2013
Accepted: 13.03.2013
Available online: 21.03.2013

Keywords:
- Autism
- Serotonin
- Gastrointestinal disorders

Słowa kluczowe:
- autyzm
- serotonina
- zaburzenia żołądkowo-jelitowe

Abbreviations:
- ECH cells – enterochromaffin cells
- ECH 5-HT cells – enterochromaffin serotonin cells
- SERT – serotonin transporter
- 5HT – serotonin
- GI – gastrointestinal

ABSTRACT

Introduction: Autistic spectrum disorders (ASD) don't have the same etiology. Platelet hyperserotonemia remain the most common neurochemical abnormality in these patients. The main producer and storage of peripheral serotonin are enteric enterochromaffin cells – serotonin cells. Platelet hyperserotonemia may result from disorders in the synthesis and/or release of enteric serotonin. An increased number of people with ASD have gastrointestinal disorders. Some of them have a serotonergic background. **Aim:** The aim was to assess the serotonin cells in the duodenal mucosa of patients with ASD. **Material and methods:** Study group: 30 children with ASD, including 73% with *duodenitis chronica*. Control group (patients without ASD): 45 patients, 56% with *duodenitis chronica*. Immunohistochemical assessment of the number of serotonin cells was performed.' **Results:** Children with ASD and *duodenitis* have fewer serotonin cells than autistic children with a normal picture of the duodenum. Children with ASD and *chronic duodenitis* have fewer serotonin cells than patients from the control group. Patients from the control group, suffering from *chronic duodenitis* have an increased number of serotonin cells in relation to children without inflammatory lesions in the duodenum. **Conclusions:** The serotonergic profiles of the GI tract of autistic patients and their peers without autistic symptoms are different. In the course of *chronic duodenitis* in patients with ASD the number of serotonin cells falls while in persons without autistic features it increases significantly. *Chronic duodenitis* contributes to an increase in the number of serotonin cells in persons without autistic features while decreasing it in patients with ASD.

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http://dx.doi.org/10.1016/j.pepo.2013.03.005

Introduction

Serotonergic disorders in ASD were pronounced after the conduction of the following examinations: biochemical, pharmacological, behavioural analyses, molecular biology concerning serotonin receptor and transporter, serological and neuroimaging diagnosis (positron emission tomography, PET; functional MRI, fMRI) [1–6]. Estimations of the level of 5HT in peripheral blood in autistic patients in the developmental age indicate prepubescent platelet hyperserotoninemia [7, 8]. In adult patients with ASD, lower than the control values with a decrease in platelet serotonin reuptake have been observed [9]. Simultaneously conducted neuroimaging, pharmacological (SSRI) and behavioural profile examinations suggest central hyposerotoninemia [10]. So far the significance of platelet (PLT) hyperserotoninemia has not been established. The serotonergic profile changes as it grows (function of receptor/neurotransmitter systems, types of 5HT receptors, their activity, number, location, serotonin level). In autistic persons this process is probably disturbed from the neurogenesis [8, 10].

In postnatal life, due to the blood–brain barrier, peripheral and central 5HT are two different deposits. The main producer and a storeroom for the peripheral 5HT are the intestinal enterochromaffin cells (ECH), and specifically their subgroup referred to as serotonin cells (ECH 5HT). 2% of 5HT in our bodies is stored in the CNS, 95% in the intestines (90% in ECH and 10% in the enteric nervous system – ENS), the remaining part is in blood PLT [11]. 5HT is mainly secreted paracrinely from ECH 5HT onto the gastrointestinal (GI) mucosa. It penetrates into the intestinal lamina propria (it impinges on the peripheral nerves' endings and affects the enteric immune system) and diffuses into the peripheral blood. Small amounts can be found in intestinal lumen (trace amount detected in faeces) [12]. 5HT secreted from ECH is subject to active SETR-mediated reuptake. Molecularly identical SERT is present on blood PLT, cells of the mucosa of the intestines and lungs, and in the central, peripheral and enteric nervous system. It has been suspected that it is SERT that is responsible for serotonergic disorders in autistic persons. Conducted molecular analyses do not confirm the above theory [13]. Free 5HT in peripheral blood is subject to first pass metabolism in the liver and to a lower degree in the lungs. It is only the 5HT, hidden in blood PLT that avoids the metabolism [12]. Due to the few-day half-life ($T_{1/2}$) of 5HT and the short time of life of PLT, the PLT level of 5HT reflects the current availability of 5HT for PLT. It should be accepted that PLT 5HT is a reflection of the intestinal production [11]. 5HT is broken down in the body by MAO – A into 5-hydroxyindoleacetic acid (5HIAA), which is subsequently extracted from urine. An indirect proof of an increased serotonin turnover is increased extraction of 5-HIAA [14].

Recently an increased number in ASD patients suffering from problems relating to the GI tract in comparison to the population of persons without the autistic features has been observed. The most common disorders include abdominal pains, disorders in gastrointestinal motor activity and nutritional problems. Both endoscopic and histopathological examinations have confirmed on several occasions an increased number of patients with autistic disorders, suffering from chronic inflammation of the abdomen, the duodenum and the colon [15–18]. Moreover, autistic patients present the signs of microbiological gut dysbiosis [19, 20].

Serotonin is one of the GI transmitters (signaling molecule), which plays a vital role in the perception, motor activity and secretion of the GI tract. The role of 5HT in the ailments (dyspepsia), motor activity disorders (vomiting, diarrhoea, constipation) and gastrointestinal disorders (mainly functional GI disorders, irritable bowel syndrome = IBS) has been proven [21, 22]. The clinical picture of serotonergic disorders corresponds with GI problems of the patients with ASD.

Janusonis conducted a theoretical analysis of biological parameters related to the serotonin system. Using a mathematical model he proved that the content of 5HT in blood platelets depends on the PLT reuptake of serotonin, the amount of free plasma serotonin subject to the first pass metabolism in the liver and lungs, intestinal production of serotonin and the volume of the enteric wall [7]. Because, theoretically, the cause of platelet hyperserotoninemia may be a disorder of the synthesis of serotonin and/or of the release of the enteric serotonin, we made an attempt to assess the proportion of the ECH 5HT cells in the duodenal mucosa.

Material and methods

Characteristics of the study and control group: The total of 75 patients were included in the retrospective analysis: 30 children with autistic spectrum disorders (ASD) and 45 of their peers without the symptoms of ASD (not – ASD). The study was retrospective. The study followed the permission of the Bioethic Committee of the SMU in Katowice (number of the consent L.dz.NN-013-42/03).

The children were patients of the Department of Gastroenterology of the Clinic of Paediatrics of the SMU in Katowice between 2004 and 2006. During clinically indicated hospitalisation, the upper GI endoscopy and the collection of specimens of the mucosa in the descending part of the duodenum were performed. The study group (ASD) and the control group (non-ASD) are homogenous in terms of sex and age.

Study group: a total number of 30 persons (16 AD/14 AA); males $n = 19$, females $n = 11$; age between 3 and 13 years old; average age of 8 years; in 8/30 persons a normal picture of the mucosa was reported (ASD-SN) and in 22/30 of persons were presented with symptoms indicating an inflammation (chronic duodenal inflammation in 9 patients, chronic duodenal inflammation with infiltration of eosinophiles in 13 persons; ASD-Dch).

Control group: a total number of 45 persons; males $n = 28$, females $n = 17$; age between 3 and 13 years; average age of 8 years; the patients from the control group were selected retrospectively based on the relevant medical documentation; they were patients without ASD, where a histopathological examination revealed a normal picture of the duodenal mucosa, corresponding with the picture obtained from

the study group that is: a normal picture of the duodenum in 20 patients (non-ASD – SN), chronic inflammation of the duodenum in 25 patients – including 13 with infiltration of eosinophiles (non – ASD – Dch).

The excluding criteria for both groups included: celiac disease (CD), intake of drugs affecting the level of 5HT (within 30 days preceding the examination), active epilepsy, the presence of an organic lesion in the area of the abdomen and/or the pelvis, confirmed by an imaging examination, and having been on a gluten-free diet within the 6 months preceding the examination.

Method

Both histopathological and immunohistochemical analyses were performed of the specimens of the descending part of the duodenum collected from the patients. The histopathological analysis of the specimens of the duodenal mucosa and the assessment of the content of serotonin in the mucosa were performed at the Department and Institute of Pathological Anatomy of the SMU. Immunohistochemical staining was performed in accordance with the following scheme: parts of tissue of the size of 4 μm cut on silanised slides were heated up in a laboratory heater at 60 °C for one hour and next deparaffinized in Xylene. At the next stage they were placed in a number of alcohols of decreasing concentration, after which the specimens were hydrated and the immunohistochemical analysis commenced. Endogenous peroxydase was inhibited for five minutes with 3% hydrogen peroxide. After rinsing the sections in TBS solution (DAKO, cat. no S 3001) they were incubated with the first antibody (Serotonin, DAKO cat. no 1530) at room temperature in a ready dilution. The following stages of the immunohistochemical reaction were performed using the LSAB 2 developing kit (DAKO cat. no K 0675). DAB chromogen (DAKO cat. no K 3468) was used for the colour developing reaction. After rinsing in distilled water the sections were dyed with Meyer hematoxylin for one minute and rinsed in running water for 15 min. The preparations were then dehydrated in a number of alcohols of increasing concentrations, overexposed in Xylene and closed in DPX. Dyed serotonin cells were counted in 5 fields of vision when enlarged 200 times and numbered in relation to the number of tubules in the same fields of vision. The obtained results were compared to those obtained from the control group – homogenous in terms of age and sex with the study group, without developmental disorders, and for which the performed endoscopy showed a normal picture of the GI mucous membrane.

Results

Both histopathological and immunohistochemical analyses were performed on the same section and by the same group of pathomorphologists. The specialists had not been informed about the patients' pervasive developmental disorders when analysing the sections (Figs. 1 and 2).

Children with ASD and the inflammation of the duodenum have significantly fewer serotonin cells compared to autistic children with a normal picture of the duodenum ($p = 0.0436$). In the control group patients with duodenitis chronic have an increased percentage of serotonin cells compared to children without the inflammation of the

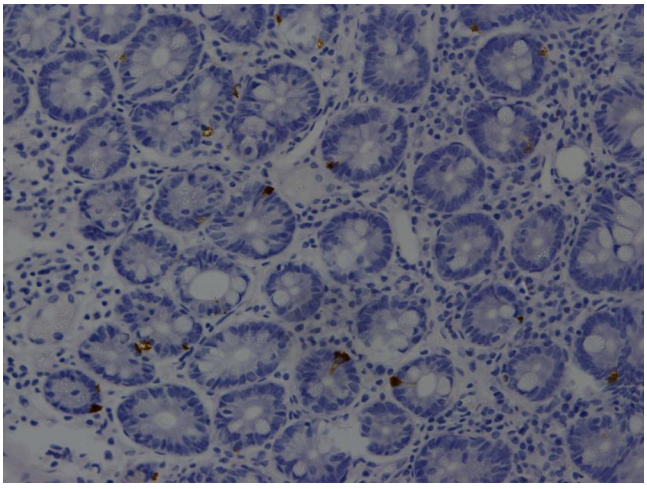

Fig. 1 – Serotonin in the duodenal mucosa. Enlarged 80 times

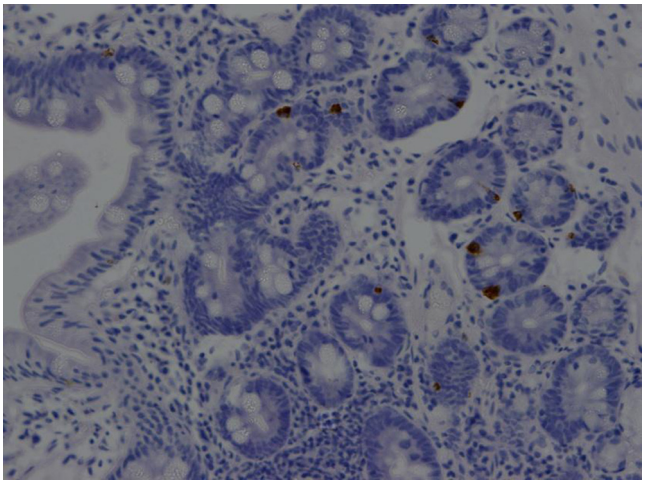

Fig. 2 – Serotonin in the duodenal mucosa. Enlarged 200 times

Table I – Indication of the cell/tubule in the examined groups

	Autistic children		Control groups	
	A–SN	A–Eo + Dch	SN	Eo + Dch
N	8	22	20	25
Average	0.511	0.268	0.299	0.588
SD	0.327	0.192	0.160	0.314
Median	0.456	0.266	0.241	0.519
Comparison NS – $p > 0.05$	$p = 0.0436$		$p < 0.001$	
	NS		$p = 0.0041$	

duodenum ($p < 0.001$). At the same time, children without the autistic features, with pronounced duodenitis chronica have considerably more serotonin cells that autistic children with the same pathology ($p = 0.0041$) (Table I).

Discussion

Serotonin is a signalling molecule of both central and peripheral character. Specialists that are mainly concerned with it are neurologists, psychiatrists and gastrologists. Dysfunctions of serotonin transporters/receptors and an abnormal level of the enteric serotonin may be the cause of nausea, abdominal pains and malfunctions of the motor activity of the upper and lower GI tract [11, 23]. As has been determined, the level of serotonin, the number of the ECH cells, TpH – 1 and SERT change depending on the level of the GI tract [24, 25]. A number of scientists have analysed the percentage of ECH cells in patients with various GI disorders. The related research concerns mainly the assessment of the colonic mucosa and was conducted in the population of adults, therefore it is difficult to compare them to the results obtained during our research. Patients examined by us, without autistic symptoms and with histopathologically confirmed chronic duodenitis show a statistically considerable increase in the number of ECH cells, which partially confirms the so far conducted observations. An inflammation within the GI tract leads to an increase in the 5HT levels, in the number of ECH cells and an increased secretion of 5HT from them [26, 27]. However, according to some scientists, chronic and severe inflammation may cause a decrease in 5HT levels in the colonic mucosa, with reduction of the number of ECH cells [23, 28]. Our patients – autistic with chronic inflammation of the duodenum – showed a statistically significant decrease in the number of ECH cells. However, in this group it is difficult to establish the duration of symptoms.

The authors found two examples of research where the number of ECH cells in biopsies of the upper GI tract were analysed. However the patients presented in the research were diagnosed with different primary disorders. Coleman et al. [21] analysed the 5HT metabolism in the duodenal mucosa of patients with untreated caeliac disease, concluding a significant increase in the number of ECH 5HT cells and the presence of other factors, manifesting the enteric overproduction of serotonin. Faure et al. [23] analysed 5HT transmission in patients at the developmental age with functional dyspepsia, examining the number of ECH 5HT cells in the mucosa of the corpus ventriculi and did not report a difference in relation to the control group.

The obtained result, confirming a significant decrease in the number of 5HT cells in the mucosa of autistic patients with duodenitis chronica, is considered surprising for scientists, as in patients with ASD a significant increase in the number of serotonin cells that could explain platelet hyperserotonemia, should be expected. Lesions within the area of the colonic mucosa in the form of an increased number of ECH cells and of T lymphocytes are characteristic for IBS. Based on the experiments conducted on animals, it was established that the inflammation of the intestines with a persisting high number of ECH 5HT cells depends on the role of T lymphocytes in the inflammatory process [21, 26, 27]. In the immunohistochemical examinations of the biopsies of the duodenal mucosa of autistic children, lymphocytic infiltrations with an increased number of T leukocytes and deposits of G-immunoglobulin both within the area of the epithelium and the membrane of the small intestine were reported [17, 18]. So, it could have been assumed that T-cell induced inflammation would result in an increase in the number of ECH-5HT cells.

The reduction of the number of ECH 5HT cells may have been induced by the intensity of the inflammatory process. However the abnormalities observed in autistic patients in endoscopic and histopathological examinations were not significantly intensified and remained disproportionate between the disorders presented by the patients. At the same time we know that serotonin is referred to as a molecule of visceral oversensitivity [22]. In patients with gastrointestinal disorders within the area of the duodenal wall, hyperserotonemia (constant? temporary?) may be expected.

In 13 out of 22 autistic patients with histopathologically pronounced duodenitis chronica, eosinophilic infiltrations were observed in the duodenal mucosa. Both 5HT and eotaxin are chemotactic factors of eosinophil granulocytes [29]. However Trajkovski et al. observed considerably higher levels of total Ig and specific antibodies against nutrients in the classes of IgE, IgG and IgM compared to the healthy population [30]. An increased number of autistic patients with gastrointestinal disorders, coexisting with allergy and food intolerance was also reported in our previous research [5]. So at the moment the analysis of the described phenomena remains difficult and requires further research.

The results presented by us are considered preliminary research. We are aware of the existing limitations. As we have presented, only the number of ECH 5HT cells was analysed, without the measurement of other indicators. It is also difficult to compare our results to other scientific research. The research that was available to us, refers mainly to the examinations of the colon of adults, which considerably hinders the comparison (the duodenum, children). In order to answer our questions, it seems crucial to repeat the analysis of ECH 5HT cells (together with the assessment of the total number of ECH cells), to determine the remaining 5HT parameters (including SERT of the gastrointestinal mucosa and of platelets, the content of 5HT in platelets of peripheral blood and enteric mucosa), to conduct a more thorough immunohistochemical diagnosis of the specimen and a complex microbiological diagnosis.

Conclusions

The serotonergic profiles of the GI tract of autistic patients and their peers without autistic symptoms are different. In the course of chronic duodenitis in patients with ASD the number of serotonin cells falls while in persons without autistic features it increases significantly. Chronic duodenitis contributes to an increase in the number of serotonin

cells in persons without autistic features while decreasing it in patients with ASD.

Authors' contributions/Wkład autorów

BK – study design, data collection, analysis and interpretation, write an article. EJ – study design, acceptance of final manuscript version. MK, MC-K – data collection, analysis and interpretation. UG-C – endoscopic examination with biopsy for histopathological examination. HW – acceptance of final manuscript version.

Financial support/Finansowanie

None declared.

Conflict of interest/Konflikt interesu

None declared.

Ethics/Etyka

The work described in this article have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

The own research were conducted according to the Good Clinical Practice guidelines and accepted by local Bioethics Committee.

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