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ANALYSIS OF A PROCESS OF PEROXIDATION, CASPASE-3 AND CASPASE-8 IN PATIENTS WITH AUTOIMMUNE THYROIDITIS

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Abstract

The article presents the results of a comparative analysis of peroxidation process activity of caspase-3 and caspase-8 in patients with thyroid adenoma (TA) and autoimmune thyroiditis (AIT). While studying the processes of peroxidation in the tissue of the thyroid gland, we revealed that the abnormal tissue is characterized by a significant growth of parameters of protein oxidative modification (POM) at the same time, the activity of antioxidant enzymes (AOE) is significantly reduced, and more likely in patients with AIT. It is shown that in patients with AIT there is a significant increase of activity of caspase 3 and 8 both compared to macroscopically unchanged tissue and to thyroid adenoma. Possible mechanisms of the detected disturbances have been discussed.

Key words: autoimmune thyroiditis, thyroid adenoma, apoptosis, peroxidation, caspases 3, 8.

Introduction

In the process of their vital activity the cells are exposed to many damaging factors of endogenous and exogenous nature. It is undoubtedly that various toxic influences or metabolic disorders lead to the development of oxidative stress, and in this case the future of the cell is determined by a balance of various adaptive metabolic processes induced by a pathological factor as well as by genetic and constitutional features of its biochemical systems [1, 6, 7, 10]. One of the responses of the cell in case of not only the action of toxic effects, but also a imbalance in necessary growth factors, hormones, cytokines, when DNA is damaged, other structural elements of the cell or when the cell cycle course is interrupted, is activation of the genetic program of the cell death, that is apoptosis [2 - 4].

According to the literature, the earliest stage of apoptosis is oxidation of proteins and cell membranes, which is influenced by an excessive production of reactive oxygen species (ROS) [1, 6, 7, 18, 20]. The processes that allow the cell to adapt to the negative impacts and determine the possibility of its further existence or death, are believed to depend on the characteristics of the mechanisms of induction and regulation of apoptosis and its implementation [8, 9, 11].

Mechanisms of apoptotic death in autoimmune thyroid diseases have been a subject of an active study for the last decades [6, 7, 12-15, 17].

Intensity of studies on apoptosis in recent years has been linked to a number of circumstances. First of all, there were methodological possibility of registering various manifestations of apoptosis and analysis of its molecular mechanisms are closely related to mechanisms other current events (for example, activation of peroxidation) [13, 14, 20]. In addition, the study of apoptosis has been very productive for understanding a number of important processes, including immune homeostasis and oncogenesis [6, 7, 12, 16, 24]. Finally, due to apoptosis, it became necessary to reconsider a number of conceptual bases of pathophysiology [12, 13].

To date, a number of mechanisms of thyrocytes apoptosis induction have been found. Normally, apoptosis occurs in 2 major ways: internal way, mediated by mitochondria, resulting from activation of caspase 9, while the outer way is mediated by activation of the Fas-receptors (CD95) and includes the activation of caspase 8 [6, 7, 21, 22]. Both ways converge to the activation of effectors' caspases, executors of apoptosis (caspase 3, 6, 7), which leads to DNA degradation and cell death [6, 7, 9, 11].

Proteins whose degradation causes irreversible changes in the cell are targeted by

effectors' caspases. [6, 7]. The action of caspases is specific: under their influence only certain proteins degrade to fragments of a certain length [9, 20, 21]. That is why caspases make up a central component of apoptosis program, their activation leads to the final stage of the cell death, namely, to DNA fragmentation and degradation of structural proteins of the cytoskeleton and cell membranes as well as to inactivation of other proteins that ensure normal functioning of the cells [6, 23, 25]. The appearance of such protein fragments serves as a biochemical marker of apoptosis [6, 13, 14]. However, the literature does not give any serious data on the relationship of lipid peroxidation processes and activation of caspase cascade in patients with AIT.

The purpose of the study

To study pro- and anti-oxidative activity, caspase-dependent mechanisms of apoptosis' induction in the thyroid tissue of patients suffering from autoimmune thyroiditis.

Materials and methods

During 2013-2015 75 women complaining of discomfort in the neck have been examined. We evaluated the hormonal status (with thyroid stimulating hormone (TSH), free T4 thyroxin, free triiodothyronine T3) levels of antibodies to thyroglobulin (AB-TG) and to thyroid peroxidase (AB-TPD), the volume and structure of the thyroid gland (TG) according to ultrasound examination.

We isolated a group of 25 women (1^{st} group), in which, according to the ultrasound, fine-needle aspiration biopsy (FNAB) and histological findings after the surgery thyroid adenoma was diagnosed. We identified this group due to the fact that this pathology is one of the most common forms of nodular goiter.

50 women were diagnosed with AIT (2^{nd} group). The indications for the surgery in this group were: enlargement of the thyroid gland with symptoms of compression and narrowing of the trachea and esophagus; some nodes compressed organs of the neck; progressive growth of goiter, despite ongoing conservative therapy for 1.5 years; suspected malignant degeneration, based on FNAB findings.

The study did not involve the patients with hyperthyroidism, those manifesting hypothyroidism, hypertension and cardio-vascular diseases, as well as with severe somatic pathology and after the onset of menopause.

The patients of the 1^{st} and 2^{nd} groups were comparable in age (34,2 ± 10,33 and 38,0 ± 10,62 years respectively, p = 0.12), anthropometric data (body mass index - BMI under 23 5 ± 2, 71 and 24,3 ± 4,88 kg / m2, p = 0.43) and the level of free T3 (4,4 ± 0,91 and 4,4 ± 0,93 ng / L, p = 0 93) but differed in terms of free T4 (16,6 ± 2,02 and 12,9 ± 3,42 mmol / L, p

<0.0001), TSH (1,9 ± 0,76 and 4,9 3 ± 51 mU / L, p <0.0001) and AT-TPO (11,9 ± 13,92 and 255,7 ± 340,58 mU / L, p = 0.0009). In general, the differences between the groups were naturally determined and confirmed autoimmune destruction and tendency towards depression of the function against the background of AIT in patients from the 2nd group.

All the patients underwent a surgery. The range of surgical intervention - from hemithyroidectomy to thyroidectomy. After the intervention the tissue was taken in the operating room no later than 30 minutes after the operation. In patients from the 1st group we isolated separately macroscopically unchanged (paranodular) tissue, which served as the control one for both comparison groups, and adenomatous tissue. In patients with AIT we took the tissue from the left, right lobes and from the isthmus. The pieces of tissue weighing 100-300 mg were transported to the laboratory on ice and immediately cut into 4-6 pieces weighing an average of 50-70 mg each. After the partition they were closed in a special plastic container and stored at -70 ° C before performing basic research with them.

Besides, we investigated pro- and antioxidant activity in 5% of thyroid tissue homogenates by determining the activity of glutathione peroxidase (GP mmol / min • g tissue), glutathione-S-transferase (GST, umol / min • g tissue) and degree oxidative modification of proteins (OMP, optical density unit / g protein) by means of accepted methods.

To study the activity of caspases 3 and 8 we crushed the thyroid tissue in homogenizer «WiseTis» HG-15 series («Daihan Scientific», South Korea) with a rotor 8mm at a speed of 4500 rev. / min. For this purpose we used the isolating medium (20 mM HEPES, pH 7,5, 10 mM KCl, 1,5 mM MgCl2, 1 mM DTT), to which a cocktail of protease inhibitors (104 mM AEBSF, 0,08 mM aprotinin, 1.5 A pepstatin mM, 2 mM leptin 4 bestatin mM, 1.4 mM E-64) was added at a ratio of 100: 1 (all reagents were manufactured by «Sigma», USA). The homogenates were centrifuged at microcentrifuge «Heraeus fresco 17» («Thermo Electron LED GmbH», Germany) at 1500 revolutions for 30 minutes at a temperature of +4 ° C. The resulting supernatant was used to assess the activity of caspase-3 and caspase-8. Specific activity of the effectors' caspase-3 and initiator caspase-8 in the tissue was studied using colorimetric method with enzyme-linked immunosorbent (ELISA) «Sanrise ™ -Tecan» (Austria), at a wavelength of 405 nm, with the speed of splitting the synthetic substrate N-acetyl-Asp-Glu -Val-Asp-nitroanilin (Ac-DEVD-NHA) and N-acetyl-Ili-Glu-Asp-Tre-nitroaniline (Al-IETD-PNA) respectively. All reagents used in this study were made by the company «Sigma» (USA). Caspase activity was assessed in (mmol of paranitro-aniline/ [h • mg of protein]).

Discussing the study results

In the study of peroxidation processes in the thyroid tissue it was established that there is a significant increase of OMP parameters in the modified tissue at the same time, the activity of antioxidant enzymes (AOE) is significantly reduced, especially in patients with AIT (Table. 1).

Table 1

Values of oxidant and antioxidant state in the thyroid tissue M±м

Values	Unchanged tissue of the TG (n=25) **control**	Thyroid adenoma (n = 25) **1st group**	Tissue of the patients with AIT (n = 50) **2nd group**
	1	2	3
OMP	46,19±2,75	60,68±2,92 P 1-2*	68,41±3,07 P 1-3*
GP	191,55±14,55	166,65±15,85	139,57±13,72
GST	24,65±1,82	12,72±1,43 P 1-2*	10,64±1,36 P 1-3*

*(P< 0,001) difference compared to the activity in the paranodular unchanged tissue.

For instance, the activity of GP in patients from 1st group decreased by almost 15% compared to the paranodular tissue and in patients with 2nd group - 18%. ST-T level in patients from the first group decreased by 49.5%, and in patients from 2nd group by 56.8%. POM degree was 24% higher in patients from 1st group and by 33.4% in those from 2nd group.

In the course of the study we found out that caspase-3 activity in the tissue having signs of AIT was twice higher than in the unchanged thyroid tissue (Table. 2), indicating the activation of caspase-dependent way of apoptosis under these conditions. In this case, the activity of caspase-8 both as compared to that of intact thyroid tissue, and to patients from 1st group increases significantly.

Table 2

The activity of caspase-3 and caspase-8 in the thyroid tissue M±м

Tissue	caspase-3	caspase-8
Unchanged tissue (n = 25) – **control**	0,098+0,026	0,89±0,14
Thyroid adenoma (n = 25) – **1st group**	0,123+0,036	0,93±0,15
Tissue of the patients with AIT (n = 50) – **2nd group**	0,186+0,028*	1,39±0,11*#

* - (P<0,05) difference compared to the activity in the paranodular unchanged tissue

- (P<0,05) difference compared to the activity of the caspase in the thyroid tissue with adenoma

Such an imbalance between the activity of peroxidation processes and antioxidant defense systems creates the conditions for damaging action of peroxidation processes on the thyroid structures and for the impact of ROS on pro- and antiapoptic targets and mechanisms directly or indirectly through the intracellular redox-dependent signal-conveying systems. In our opinion, these structures can be the elements of the thyrocytes - cell membrane, intracellular structures, which cause launching an apoptotic signal, the indication of which is likely activation of both initiator and effectors' caspases.

At the same time, several molecular paths interacting with each other can be activated in the cell. The findings of different sources confirm the role of antiapoptotic protein Bcl-2 under the condition of AIT development and this protein is among the main factors in the regulation of apoptotic function of mitochondria [6,7,9,21].

Oxidative stress leads to the formation of cell membranes of oxidated lipids which are also apoptotic factors. Caspase oxidative modification (including caspases-3), which are sensitive to the redox status of cells, depending on the type and location of such changes may cause their activation.

Considering this, as well as the findings obtained while performing the work we can assume that in patients with AIT basic mechanisms of apoptosis are triggered due to excessive activation of peroxidation aimed at attracting external receptor mechanism of initiation and increased activity of caspase-3, and can also occur as a result of caspase-8, the indicative of which is probable high increase in the activity of this index both as compared to that in unchanged tissue in the thyroid gland and in patients with thyroid adenoma.

The research of caspase signaling pathways in apoptosis of thyroid cells started not long ago and requires further study. Discovery of physiological regulators of apoptosis in

caspase activity shows the inexhaustible possibilities of cells to maintain homeostasis and the natural end of life cycle. Tracking ways that cause cell death may contribute to the development of new approaches to the prevention and treatment of autoimmune thyroid disease.

Conclusions

1. Processes of protein peroxidation are sure to get activated and the systems of antioxidant defense become weaker in the thyroid tissue of patients with autoimmune thyroiditis (AIT).

2. Induction of thyrocytes apoptosis in patients with AIT on external mechanism is associated with increased activity of caspase-8, which significantly predominates that in patients with thyroid adenoma and in practically healthy people by 56.18% and 49.46%, with an implementation through effectors' caspase-3, whose activity grew almost twice.

Prospects for further research

Detection of pathogenic factors and mechanisms of apoptosis deregulation in case of AIT will allow determining the additional causes of their onset and formulate pathogenetically grounded methods of correction of immunopathological changes.

References

1. Андреев А.Ю. Метаболизм активных форм кислорода в митохондриях / А.Ю. Андреев, Ю.Е Кушнарева, А.А Старков // Биохимия. — 2005. Т. 70, №.2. - С.246-264.

2. Апоптоз, роль в патологии и значимость его оценки при клинико-иммунологическом обследовании больных / А. А. Ярилин, М. Ф. Никонова, А. А. Ярилина и соавт. // Медицинская иммунология. 2000. - Т. 2, № 1. -1. С. 7-16.

3. Белушкина Н.Н. Молекулярные основы патологии апоптоза /Н.Н. Белушкина, С.Е. Северин // Арх. патол. 2001. №1. С. 51-60.

4. Болдырев, А.А. Дискриминация между апоптозом м некрозом под влиянием окислительного стресса / А.А. Болдырев // Биохимия. - 2000. -Т. 5, № 7. С.981-991.

5. Власенко М.В. Аутоиммунный тиреоидит у подростков / М.В. Власенко // В кн.: 100 избранных лекций по эндокринологии / Под ред. Ю.И. Караченцев, А.В. Казакова, Н.А. Кравчуна, И.М. Ильиной. - Харьков, 2009. - С.372-378.

6. Мишуніна Т.М., Калініченко О.В., Тронько М.Д. Механізми апоптозу клітин щитоподібної залози за умов її патології //Фізіол. журн., 2009, Т. 55, № 6. С. 90-102.

7. Мишуніна Т. М., Калініченко О. В., Тронько М. Д. та ін. Мітохондріальні та постмітохондріальні механізми апоптозу у тканині щитоподібної залози з ознаками лімфоїдної інфільтрації чи хронічного тироїдиту // Ендокринологія. — 2009. — 14, № 1. — С. 48-56.

8. Москалева Е. Ю. Возможные механизмы адаптации клетки к повреждениям, индуцирующим программированную гибель. Связь с патологией / Е. Ю. Москалева, С. Е. Северин // Патологическая физиология и экспериментальная терапия. - 2006. - № 2. - С. 2-14.

9. Недосекова Ю.В., Уразова О.И., Кравец Е.Б., Чайковский А.В. Роль апоптоза в развитии аутоиммунных заболеваний щитовидной железы// Бюллетень сибирской медицины. - 2009. - № 4 (2).- С. 64 – 71.

10. Некрасова Т.А., Щербатюк Т.Г., Давыденко Д.В. и др. Особенности перекисного окисления липидов и белков при аутоиммунном тиреоидите без и с минимальной тиреоидной дисфункцией //Клиническая и экспериментальная тиреоидология. 2011, 7, N 4, 38-43.

11. Новицкий, В.В., Рязанцева, Н.В., Часовских, Н.Ю. и др. Модуляция апоптоза мононуклеаров в условиях окислительного стресса // Бюл. эксперим. биол. и мед. 2008. №3. С. 251-254.

12. Олійник В.А. Хронічний лімфоцитарний тироїдит (тиреоїдит Хашимото): сучасний стан проблеми / В.А. Олійник // Ендокринологія. - 2006. - № 1. - С. 71-79.

13. Пальцев М.А., Иванов А.А., Северин С.Е. Межклеточные взаимодействия. М.: Медицина, 2003.

14. Сарвилина И.В. Междисциплинарные исследования в медицине / Сарвилина И.В., Каркищенко В.Н., Горшкова Ю.В. М.: Техносфера, 2007. С. 112–122.

15. Ai J., Leonhardt J.M., Heymann W.R. Autoimmune thyroid diseases: Etiology, pathogenesis, and dermatologic manifestations. J Am Acad. Dermatol., 2003, V.48. P.641-659.

16. Bretz J.D., Baker J.R. Apoptosis and autoimmune thyroid disease: following a TRAIL to thyroid destruction? // Clin. Endocrinol.2001. V. 55. N1. P. 1–11.

17. Bretz J.D., Rymaszeweski M. TRAIL death pathway expression and induction in thyroid follicular cells // J. Biolog. Chemistr. 1999. V. 274. P. 23627–23632.

18. Hancock J.T., Desikan R., Neill S.J. Role of reactive oxygen species in cell signaling pathways. // Biochem. Soc. Trans. – 2001. – Vol. 29. – P. 345-350.

19. Giordano C., Stassi G., De Maria R. et al. Potential involvement of Fas and its

ligand in the pathogenesis of Hashimoto's thyroiditis // Science. 1997. V. 275. P. 960–963.

20. Erdamar H., Demirci H., Yaman H.et al.The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status // Clin. Chem. Lab. Med. - 2008. – V.46, № 7. - P. 1004-1010.

21. Łącka K., Maciejewski M., Współczesne poglądy na temat etiopatogenezy autoimmunologicznego zapalenia tarczycy, (choroby Hashimoto) //Pol. Merk. Lek., - 2011. V.30. N.176. P. 132-138.

22. Łącka K., Maciejewski A. Rola procesu apoptozy w etiopatogenezie autoimmunologicznego zapalenia tarczycy // Pol. Merk. Lek.,- 2012. V.32, N. 188. P. 87-92.

23. Mitsiades N., Poulaki V., Kotoula V. et. al. Fas/Fas ligand up -regulation and bcl-2 down regulation may be significant in the pathogenesis of Hashimoto's thyroiditis. J Clin Endocrinol Metab. 1998. V.83. P. 2199-2203.

24. Nakahara M., Nagayama Y., Saitoh O. et al. Expression of Immunoregulatory Molecules by Thyrocytes Protects NonobeseDiabetic-H2h4 Mice from Developing Autoimmune Thyroiditis // Endocrinol. 2009. V. 150. P. 1545–1551.

25. Shen H.M., Yang C.F., Ding W.X. et al. Superoxide radical-initiated apoptotic signaling pathway in selenite-treated HepG(2) cell: mitochondria serve as main target. // Free Radic. Biol. Med. 2001, N 30, 9-21.

26. Yanagisawa J., Takahashi M., Kanki H. et al. The molecular interaction of Fas and FAP-1: a tripeptide blocker of human Fas interaction with FAP-1 promotes Fas-induced apoptosis // J. Biol. Chem. 1997. V. 272. P. 8539–8545.