The influence of hydrogen sulfide on the structural characteristics of leukocytes mitochondrial apparatus in patients with arterial hypertension

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ARTICLE INFO
Received: 1 September, 2019
Accepted: 5 October, 2019

UDC: 612.816.3:547.416,.616.12-008.331.1

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One of the leading causes of arterial hypertension (AH) is mitochondrial dysfunction (MD) - moreover, disorders in regulation of blood pressure occur on the background of progressive energy deficiency. At the same time, the cardioprotective effect of $H_2S$ has been proven. In particular, the inhibition of mitochondrial pore opening by hydrogen sulfide plays an important role, and $H_2S$ should affect the structural component of MD, namely, the ultrastructure of mitochondria. However, at present, the question of structural changes in the mitochondrial apparatus in patients with hypertension is extremely insufficiently studied. For the study of MD in healthy people and patients with pathology, almost the only objects (in the absence of surgical intervention) are blood cells, in particular leukocytes. Based on the above, the aim of the study was to investigate the effect of a hydrogen sulfide donor on the ultrastructure of the mitochondrial apparatus of leukocytes in patients with arterial hypertension. The effect of a hydrogen sulfide donor on some ultrastructural characteristics of the leukocyte mitochondrial apparatus in patients with hypertension was studied. The examination involved patients (men) with arterial hypertension aged 30-60 years, who were divided into 2 age groups: 30-40 and 40-60 years. Control groups (healthy men without signs of hypertension) were randomized by age. An electron microscopic and morphometric assessment of the structure of mitochondria revealed that under hypertension it undergoes significant changes that depend on the age of the patients. It has been established that the addition of a hydrogen sulfide donor (Full Spectrum Garlic phytopreparation (Swanson Health Products, USA) at a dose of 400 mg per day) to the traditional therapy of hypertension leads to positive changes in the mitochondrial ultrastructure of the studied cells aimed at increasing the energy capacity of the mitochondrial apparatus - the quantity reduction of structurally damaged mitochondria, and with an increase in the duration of treatment - the increase their total number in people of the younger age group (by 57.5%), and in the older age group - by 53.7%. Thus, the indicated effect of $H_2S$ significantly depends both on the age of the patients (young people respond more intensively) and on the duration of the hydrogen sulfide donor using (long-term use is accompanied by a more pronounced positive dynamics of changes).

Keywords: arterial hypertension, hydrogen sulfide, mitochondrial ultrastructure, leukocytes.

Introduction

In the scientific literature of recent years, much attention has been paid to low molecular weight signaling molecules, which are generally referred to as NO and CO. Much later, it was found that $H_2S$ also belongs to them [2, 6, 28]. Currently, it is believed that the main biological effects of $H_2S$ include the regulation of vascular tone, contractile activity of the myocardium, participation in long-term synaptic potentiation, pro- and anti-inflammatory reactions, regulation of insulin secretion, etc. [3, 4, 29]. Hydrogen sulfide has also been shown to have antioxidant and anti-apoptotic properties [26].

The cardiovascular system is an important object of $H_2S$ action. Like NO and CO, it relaxes the smooth muscles of the blood vessels, decreases blood pressure and heart rate [21, 22, 23]. There is a direct correlation between the severity of the disease and the level of hydrogen sulfide in
the blood: the more severe hypertension is, the greater is the deficit of hydrogen sulfide [2].

It is now considered established - one of the leading causes of cardiovascular pathology is mitochondrial dysfunction (MD) - especially since the violation of normal regulation of blood pressure occurs against the background of progressive energy deficit [10, 17]. In recent years, cardioprotective effect of H₂S, in particular in the case of arterial hypertension (AH), has been proven, and inhibition of mitochondrial pore opening by hydrogen sulfide plays an important role in the mechanism of the protective effect [9, 24, 25]. In addition, the development of MD leads to damage to the membranes of organelles, a decrease in ATP synthesis, which is accompanied by a decrease in contractile activity and functional reserves of the heart and, as a consequence, a decrease in its pumping function. Based on the available data on the cardioprotective effect of hydrogen sulfide associated to some extent with its effect on mitochondria, namely Ca²⁺-induced mitochondrial pore opening [24, 25], it can be assumed that H₂S should affect the structural component of MD, and namely, the ultrastructure of mitochondria. However, at present, the question of structural changes of the mitochondrial apparatus in patients with hypertension has not been sufficiently studied.

For the study of MD in healthy people and patients with pathology, virtually the only objects (in the absence of surgery) are blood cells, in particular leukocytes. They are involved in many processes in the body, both related and not directly related to hypertension: in the regeneration of tissues, the development of inflammatory and immune responses, the provision of primary homeostasis. Leukocytes contain enough mitochondria to study the structure and function of these organelles in the development of pathological conditions of different genesis, including hypertension [11, 12].

Based on the above, the aim of the study was to study the effect of hydrogen sulfide donor on the ultrastructure of the mitochondrial leukocyte apparatus in patients with hypertension.

Materials and methods

Investigation of the ultrastructure of the mitochondrial apparatus of leukocytes was performed in patients (men) with newly diagnosed hypertension aged 30-60 years, which were divided into 2 age groups: 1) 30-40 years (mean age was 33.9±1.1 years); 2) 40-60 years (mean age - 50.6±1.7 years). The average blood pressure in persons in group 1 was 140/90 mm Hg and in patients of group 2 - 160/100 mm Hg. Control groups (C1 and C2 were healthy men without signs of hypertension) were randomized by age.

The examination of each patient was conducted in 4 stages: before the study; after receiving the course of treatment of the underlying disease; after a 2-week course of specific treatment; after a month of specific treatment. The latter consisted of the exogenous (per os) addition of hydrogen sulfide donor to the therapy of 400 mg/day, which was contained in a herbal drug Full Spectrum Garlic (garlic in capsules) (Swanson Health Products, USA). The course of administration of the phytopreparation was 30 days, during which the patients were examined twice - after 2 weeks (relatively short course of treatment) and at the end of treatment (conditionally long course of treatment).

Blood plasma enriched with leukocytes was obtained by centrifugation of whole blood at room temperature for 15 minutes at 120 g on a laboratory T-30 centrifuge (Ukraine). The plasma was gently separated from the precipitated cells and centrifuged at 2000 g for 20 minutes using a Vortecs Combispin FVL-2400N mini-centrifuge (Latvia) [5].

Samples for electron microscopic examination were prepared according to conventional methods for blood cells with double fixation of glutaraldehyde and OsO₄, dehydration in alcohols of increasing concentration and pouring into Epon-Araldite (reagents of firm Fluka, Switzerland) [8]. Ultrathin sections 40-60 nm thick were counterstained with solutions of uranyl acetate and lead citrate (Sigma reagents, USA) and examined in a PEM-124c electron microscope (Ukraine).

Morphometric calculations were performed using the Image Tool (USA) computer program in 130-150 fields for each group of subjects.

The statistical processing of the obtained data was performed using the programs "Microsoft Excel" and "OriginPro" using the Student's t test, due to the coherence of the samples of the surveyed. Because, due to the considerable mass of digital material, according to the Shapiro-Wilk criterion, the data obtained were within the normal distribution law, the data are presented as mean ± error of mean (M±m) [14]. The differences between the averages were considered statistically significant at p<0.05.

Results

We found significant differences in the structural organization of leukocytes in individuals with hypertension in different age groups, examined before the start of the standard treatment process.

In the analysis of some structural features of the mitochondrial apparatus of leukocytes in patients with hypertension, it was found that they have a significant number of mitochondria was structurally altered (especially in the older age group), but their total number was not significantly changed relative to the control values (Table 1).

It should be noted that in addition to the swelling and vacuolization of organelles, which is a nonspecific response to most negative endo- or exogenous effects on cells, there was a marked activation of mitochondrial autophagy (Fig. 1).

In hypertension, mitochondrial swelling led to an increase in their average diameter (see Table 1). However, in patients of age group 1 such increase was 27.8%, in patients of group 2 it was 44.3%.

Conducting traditional 2-week therapy was more effective...
for younger patients. Its most important results include the fact that the number of structurally damaged mitochondria in leukocytes is reduced by 35.1%. These changes affect only 1 group of people with hypertension (see Table 1). It can be assumed that the absence of a pronounced response of the studied indicators to the traditional treatment is associated with both the duration of the disease and the short period of treatment (due to the absence in the persons of previous therapy with hypertension).

As a result of 2-week specific treatment, positive changes were observed in the mitochondrial apparatus of leukocytes (see Table 1). In both groups of subjects with hypertension, the number of structurally altered mitochondria decreased significantly, not only with respect to the condition before treatment, but also with respect to the determined number of such mitochondria after standard treatment (by 40.9% in the first and by 29.1% in the second group). Such changes should be seen as an opportunity to increase the capacity of energy metabolism. With regard to other leukocyte morphometric parameters, the addition of hydrogen sulfide donor to standard treatment increased (in group 1) or showed a tendency (in group 2) to approach the studied indicators to levels that are characteristic of healthy individuals of the appropriate age (see Table 1).

The most optimal, in view of the obtained results, was the treatment of patients with hypertension with a monthly course of specific treatment. Long-term addition to the traditional therapy of Full Spectrum Garlic herbal medicine improved all our investigated indicators of ultrastructure of leukocyte mitochondria in both groups of persons (see Table 1). Only with such a regimen was observed a significant increase in the total number of mitochondria (see Table 1): in persons of the younger age group - by 57.5%, and in the older age group - by 53.7%.

A further decrease in the number of structurally damaged mitochondria was also detected (see Table 1). In group 1 patients, the reduction was 2-fold relative to treatment status and 51.6% relative to traditional treatment; in group 2 patients, by 61.4% relative to the condition before treatment, and by 39.6% relative to traditional treatment. In younger patients, the changes were more pronounced, probably due to the greater adaptive capacity associated with age, the number of altered mitochondria (including in the state of autophagy) was significantly reduced, which, with the increase in their total, had to significantly optimize energy metabolism.

Discussion

As noted above, in recent decades, energy deficiency at the cellular level has been considered as the main cause of the primary increase in blood pressure. A source of impaired energy metabolism of tissues is considered to be a decrease in the energy-forming function of

<table>
<thead>
<tr>
<th>Groups of surveyed</th>
<th>Number of mitochondria, (\text{un.}/10 , \mu\text{m}^2)</th>
<th>Number of structurally damaged mitochondria,%</th>
<th>The average diameter of mitochondria, (\mu\text{m})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (the average age 30.40±2.80 years)</td>
<td>11.30±0.50</td>
<td>2.400±0.090</td>
<td>0.540±0.070</td>
</tr>
<tr>
<td>Control (the average age 54.20±1.90 years)</td>
<td>9.500±0.60</td>
<td>3.600±0.070</td>
<td>0.610±0.040</td>
</tr>
<tr>
<td>Patients with hypertension aged 33.90±1.10 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>12.20±0.80</td>
<td>26.20±2.40**</td>
<td>0.690±0.050*</td>
</tr>
<tr>
<td>After 2 weeks of standard treatment</td>
<td>12.70±0.90</td>
<td>19.40±3.90**o</td>
<td>0.630±0.060*</td>
</tr>
<tr>
<td>After a 2-week course of specific treatment</td>
<td>13.10±0.60*</td>
<td>18.60±2.60**o#</td>
<td>0.580±0.030o</td>
</tr>
<tr>
<td>After a month of specific treatment</td>
<td>17.80±0.70**o#</td>
<td>12.80±1.40**o#</td>
<td>0.470±0.040o#</td>
</tr>
<tr>
<td>Patients with hypertension aged 50.60±1.70 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>10.30±0.70</td>
<td>32.60±4.80**</td>
<td>0.880±0.060*</td>
</tr>
<tr>
<td>After 2 weeks of standard treatment</td>
<td>11.20±0.80</td>
<td>28.80±5.30**</td>
<td>0.790±0.050*</td>
</tr>
<tr>
<td>After a 2-week course of specific treatment</td>
<td>11.90±0.60</td>
<td>22.30±3.70**o</td>
<td>0.770±0.060*</td>
</tr>
<tr>
<td>After a month of specific treatment</td>
<td>14.60±0.50*o#</td>
<td>20.20±1.60**o#</td>
<td>0.700±0.030o#</td>
</tr>
</tbody>
</table>

Notes: * - \(p<0.05\) relative to the control group; ** - \(p<0.01\) relative to the control group; o - \(p<0.05\) relative to the group before treatment; # - \(p<0.05\) relative to the standard treatment group.
mitochondria due to disruption of the structure of the mitochondrial apparatus [20]. At the same time, it has been shown experimentally and clinically in 2000 that hypertension decreases the level of H$_2$S in blood plasma, and the introduction of an exogenous hydrogen sulfide donor can cause a significant therapeutic effect [7, 27]. There is little data available today about the effect of H$_2$S on the total number of mitochondria in cells. However, H$_2$S is thought to exert different effects on the number, function and dynamics of mitochondria depending on the dose used [18]. Probably the concentration we use is effective for increasing the number of organelles.

One of the damaging factors for the myocardium and blood vessels is the increase in the amount of reactive oxygen species, which provokes the development of oxidative stress. At the same time, tissue respiration and ATP synthesis by mitochondria are impaired, which leads to a deterioration of cardiac contractile function due to negative structural changes in the mitochondria, which cause their functional energy failure. That is why the antioxidant properties of hydrogen sulfide improve the condition of ischemic myocardium and prevent its further damage [2]. The data obtained by us regarding the increase in the total number of mitochondria and the decrease in the percentage of structurally damaged organelles can be considered as evidence of such changes of the mitochondrial apparatus in leukocytes, which should contribute to the optimization of energy metabolism.

Positive changes in the morphometric characteristics of mitochondria include the decrease in the average diameter of organelles. This difference is important given that the increase in mitochondrial diameter within 25-30% is considered to be an adaptive response aimed at enhancing the energy capacity of organelles caused by the activation of the ATP-dependent K+ channel. More growth in the diameter of mitochondria indicates the possibility of their necrotic death and is often irreversible [16, 23]. The exact mechanism of action of H$_2$S on KATP-channels remains unclear, but it is suggested that hydrogen sulfide exerts its influence through the K+ conductivity feature, and specific KATP-channel inhibitors completely inhibit H$_2$S effects on it [1]. We are inclined to take this view, since it is precisely such dynamics of changes of the mitochondrial apparatus in different tissues of the body that promotes not only the ultrastructure but also the function of the mitochondria [16, 19].

Another of our findings is the activation of autophagy in hypertension. As autophagy is thought to be a process that contributes not only to the death of damaged cells but also to their preservation (by preventing apoptosis by mitochondrial pathways) [4, 19], it can be assumed that in hypertension regardless of patients’ age, such a mechanism is activated at least in blood cells. Addition to the traditional therapy of AH donor H$_2$S did not lead to significant changes in the intensity of mitochondrial autophagy, although in the literature there are isolated data on the reduction of the process under the influence of endogenous H$_2$S [15, 22]. It can be assumed that under such conditions, the increase in the concentration of hydrogen sulfide does not significantly affect the process of autophagy, which we (given its physiological role) tend to consider as an additional positive fact in favor of the therapy of hypertension.

The results indicate a positive effect of hydrogen sulfide on the ultrastructure and, consequently, the function of mitochondria in leukocytes, which requires a further search for effective ways to increase the content of H$_2$S in patients with hypertension.

Conclusions
1. Hypertension is accompanied by significant changes in the ultrastructure of the mitochondrial apparatus of leukocytes, the severity of which depends on the age of the patients.
2. Addition to the traditional therapy of hypertension of a donor of hydrogen sulfide leads to positive changes in the ultrastructure of mitochondria of leukocytes aimed at increasing the energy capacity of the mitochondrial apparatus.
3. The established positive effect depends significantly on the age of the patients (more young people react more intensively) and the duration of use of the hydrogen sulfide donor (long-term use is accompanied by more pronounced positive dynamics of change).

References
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Одной из ведущих причин развития артериальной гипертензии (АГ) является митохондриальная дисфункция (МД) - тем более, что нарушение нормальной регуляции артериального давления происходит на фоне прогрессирования энергетического дефицита. В то же время, доказано кардиопротекторное действие H₂S. В частности, важную роль играет торможение сероводородом открываия митохондриальной поры, и H₂S должен влиять на структурную составляющую МД, а именно - на ультраструктуру митохондрий. Однако, в настоящее время вопросы о структурных изменениях митохондрионального аппарата у пациентов с АГ исследованы крайне недостаточно. Для изучения МД у здоровых людей и пациентов с патологией практически единственными объектами (при отсутствии оперативного вмешательства) являются клетки крови, в частности, лейкоциты. Исходя из указанного, целью исследования было изучение влияния донора сероводорода на ультраструктуру митохондрионального аппарата лейкоцитов у пациентов с артериальной гипертензиеей. Проведено изучение влияния донора сероводорода на некоторые ультраструктурные характеристики митохондрионального аппарата лейкоцитов у пациентов с АГ. В обследовании принимали участие пациенты (мужчины) с артериальной гипертензией в возрасте 30-60 лет, которые были разделены на 2 возрастные группы: 30-40 и 40-60 лет. Контрольные группы (здоровые мужчины без признаков АГ) были рандомизированы по возрасту. Электронно-микроскопическая и морфометрическая оценка структуры митохондрий выявили, что при АГ она претерпевает значительные изменения, которые зависят от возраста пациентов. Установлено, что добавление к традиционной терапии АГ донора сероводорода (фитопрепарат Full Spectrum Garlic (Swanson Health Products, США) в дозе 400 мг в сутки) приводит к позитивным изменениям ультраструктуры митохондриональных исследуемых клеток, направленным на увеличение энергетической мощности митохондрионального аппарата - уменьшается количество структурно поврежденных митохондрий, а при увеличении продолжительности лечения - их общего количества у лиц молодшей возрастной группы (на 57,5%), а в старшей возрастной группе - на 53,7%. Таким образом, указанный эффект H₂S существенно зависит как от возраста пациента (интенсивнее реагируют молодые люди), так и от продолжительности использования донора сероводорода (длительное применение сопровождается более выраженной положительной динамикой изменений).

Ключевые слова: артериальная гипертензия, сероводород, ультраструктура митохондрий, лейкоциты.