Character and features of morphofunctional reactions of renal vessels in rats at chronic poisoning with acetylsalicylic acid

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The prevalence and availability of aspirin-containing analgesic products makes them sources of unintentional overdose, and even suicidal admission. Therefore, the toxic effect of salicylates is an important cause of morbidity and mortality. In this case, as a rule, the target body is the kidneys. The purpose of the study was to identify the structural changes in the kidneys in the process of chronic poisoning with acetylsalicylic acid. In an experiment on rats using the histological methods of the study, it was found that chronic poisoning with acetylsalicylic acid leads to severe organ disorders in the kidneys, which are manifested in the form of congestive venous plethora with reactive ascending vasoconstriction of the arterial part of the blood route of the organ with a decrease in its throughput. At the same time there was a thickening of the walls of the vessels, which occurred both at the expense of increasing their tone, and due to edema, which was confirmed by the enlightenment of the cytoplasm of the leiomyocytes of the medial cover and an increase in the distance between the nuclei of adjacent layers of the smooth muscle cells. The endothelium of these arteries looked swollen as evidenced by the protrusion of its nuclei into the lumen of the vessels in the form of a “palisade”. Such changes can be considered as a protective reaction that partially prevents hemodynamic overload of the hemomicrocirculatory channel, known in the scientific literature as the reflex of Kitaev. However, in spite of this, in the process of chronic poisoning with acetylsalicylic acid, the complete compensation of blood circulation in the kidneys does not occur. Prolonged vasoconstriction leads to the tissue ischemia, which may be the cause of the development and progression of functional and dystrophic changes in the structural components of the organ parenchyma as a morphological substrate of renal insufficiency. Confirmation of this can be considered the changes in the glomerular apparatus found during the study, which consisted in the gradual reduction of the total area of nephrons with the simultaneous decrease in the area of capillary glomeruli. Moreover, the decrease in the area of capillary glomeruli occurred more intensively, which led to the expansion of urinary spaces. In the final stage of the experiment, the capillary glomeruli were often in collaptoid condition, their size significantly decreased, and the contours became uneven-hilly. Thus, because of the chronic poisoning by acetylsalicylic acid in the kidneys appears stagnant disturbances of the organ circulation, which cause ascending vasoconstriction in the arterial part of the blood stream of the organ, which subsequently leads to ischemia and dystrophic changes in its parenchyma.

Keywords: acetylsalicylic acid, arteries, plethora, nephrons, capillary glomeruli.

Introduction

Salicylates are the most commonly used pharmacologically active substances found in hundreds of prescription and over-the-counter forms of medicines. They are used with an analgesic purpose for the treatment of mild to moderate pain. As an antipyretic drug and as an anti-inflammatory agent, they are used in the treatment of inflammatory processes of soft tissues, joints, and also in vasculitis. In low doses, acetylsalicylic acid preparations are also used to prevent thrombosis. At the same time, the prevalence of aspirin-containing analgesic products makes these agents available in almost every household, the public sources of unintentional and suicidal admission [5,
Drugs containing acetylsalicylic acid, as the main active compound, are considered safe at low therapeutic doses, but at the same time have life-threatening side-effects when applied at high doses. Long-term administration of therapeutic doses of the drug is associated with the risk of hepatotoxicity, nephrotoxicity, the appearance of ulcerative processes in the gastrointestinal tract and the development of malignant tumors in the kidneys [7, 10]. Also reported is the negative effects of acetylsalicylic acid in pregnancy [4]. In vitro and in vivo studies have shown that aspirin at high doses has a destructive effect on the tissues of the blood vessels [18].

That is why the toxic effect of salicylates is an important cause of morbidity and mortality. In this case, often the target organ is the kidney as it is known that acetylsalicylic acid is rapidly absorbed from the stomach and small intestine, primarily through passive diffusion and rapidly hydrolyzed to salicylic acid with esterase in the mucosa and blood plasma. The salicylic acid itself is metabolized by conjugation in the liver to form salicylic acid and several other metabolites. The drug is spread throughout the body, with its highest concentration contained in plasma, kidney cortex, liver, heart and lung [13].

The purpose of the study: to establish the features of structural changes in the structural components of rat's kidney in the process of chronic poisoning with acetylsalicylic acid.

Materials and methods

Experiments were performed on 42 white laboratory mature male rats weighing 160-180 g and age 2.5-3 months. Of these, 12 animals were a control group; another 30 rats received pre-gastric acetylsalicylic acid every day at a rate of 150 mg per 1 kg of body weight for the simulation of chronic intoxication [3, 19]. Slaughter of animals was carried out at 1, 3, 7, 14 and 28 days from the beginning of the experiment by rapid decapitation under ketamine anesthesia. All experimental studies were carried out in accordance with the principles of bioethics set forth in the Helsinki Declaration and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (№1759-VI of 15.12.2009).

The material for the histological examination (kidneys slices) was fixed after collection in the alcohol and in 10% neutral formalin. Histological sections were stained by H&E, Weigert's and Van Gieson’s. Morphometric studies included determination of: area of glomeruli with Bowman’s capsule (µm²), the area of the capillary loops of the glomeruli (µm²), and area of the urinary space in the renal corpuscle (µm²). Morphometry was performed using an eyepiece micrometer MOB-1-15x, with measurements performed in 5 visual fields on each histological cut.

The digital material obtained during the research was statistically processed using Microsoft Exel for Windows 98 with the definition of averages and their standard errors. Reliability was estimated by the Student’s coefficient (t) at p<0.05.

Results

According to the results of the study, it was found that 1 day after pre-gastric administration of acetylsalicylic acid in the structural components of the kidney of rats there were certain morphofunctional changes, the basis of which were disorders of organ blood circulation. The latter manifested itself in the form of moderate venous and partly arterial hypertrophy, capillary stasis. On the background of congestive plethora there was an increase in the tone of the walls of small arteries, which was confirmed by the strengthening of the tortuosity of their internal elastic membranes with the simultaneous decrease in the throughput of arterioles due to the narrowing of their lumen (Fig. 1).

At the same time, there was a slight increase in the area of the section of the glomeruli capillary loops with a slight narrowing of the area of the urinary spaces of the renal corpuscles, which was also a consequence and manifestation of stagnant phenomena in the bloodstream of the organ (Table 1).

Such hemodynamic changes were accompanied by moderate edema of the epithelium of the renal tubules, especially in the subcapsular regions of the kidneys, which was manifested by the enlightenment of the epithelial cytoplasm with a simultaneous increase in their cross-sectional area.

After 3 days of the experiment there was partial compensation for the detected pre-changes with signs of normalization of organ blood flow. Slightly decreased the tone of the walls of small arteries and arterioles, signs of hyperhydration of the epithelial cells of the walls of the renal tubules disappeared. However, in the lumen as the arteries and especially the veins, various concentrations of...
erythrocytes continued to manifest themselves.

However, 7 days after experimental observation, the recovery and progression of detected pre-vascular reactions, which consisted in the repeated increase in the tone of arterioles and small arteries, with the simultaneous thickening of their walls and narrowing of lumen and decrease in bandwidth, was characteristic. Moreover, thickening of the walls of the vessels occurred both by increasing their tone, and due to edema, which was confirmed by the enlightenment of the leiomyocytes cytoplasm of the middle mucosa and increasing the distance between the nuclei of adjacent layers of the smooth muscle cells. The endothelium of these arteries looked swollen as evidenced by the protrusion of its nuclei into the lumen of the vessels in the form of "palisade" (Fig. 2).

The arteries of the middle and larger caliber, as well as the veins, were expanded and full-blooded. At the same time, enlightenment of the urinary spaces of the kidney cells became moderately enlarged. In the epithelium of the renal tubules, signs of hydropic dystrophy were renewed.

After 14 days from the beginning of the experiment, changes that were detected in the 7-day period, progressively increased. As a result of ascending vasoconstriction, the tone increased not only small but also arteries of medium caliber (Fig. 3).

The veins and arteries of large caliber remained elevated and full-blooded. As a result of violation of hemomicrocirculation developed perivascular and interstitial edema.

Changes in the glomerular apparatus during this period of observation were to reduce the total area of nephrons with a simultaneous decrease in the area of capillary glomeruli. Moreover, the decrease in the area of capillary glomeruli occurred more intensively, which led to a reliable \( p<0.05 \) expansion of the Bowman's capsule urinary spaces (Fig. 4).

The epithelium of the proximal and distal tubules was in

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Area of glomerulus with Bowman's capsule (( \mu m^2 ))</th>
<th>Area of glomerulus capillary loops (( \mu m^2 ))</th>
<th>Area of urinary space in the renal corpuscle (( \mu m^2 ))</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>5596±215</td>
<td>3961±253</td>
<td>1635±73</td>
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<tr>
<td>Chronic poisoning</td>
<td></td>
<td></td>
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<tr>
<td>with acetylsalicylic acid</td>
<td>5641±217</td>
<td>4095±272</td>
<td>1546±62</td>
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<td>1 day</td>
<td>5485±193</td>
<td>3872±271</td>
<td>1612±86</td>
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<td>3 days</td>
<td>5280±197</td>
<td>3583±250</td>
<td>1707±60</td>
</tr>
<tr>
<td>7 days</td>
<td>5187±234</td>
<td>3179±177</td>
<td>2008±67</td>
</tr>
<tr>
<td>14 days</td>
<td>5250±218</td>
<td>2892±203</td>
<td>2357±32</td>
</tr>
<tr>
<td>28 days</td>
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Note: * - \( p<0.05 \); ** - \( p<0.01 \) in comparison with control.

Table 1. Quantitative characteristic of changes in parameters of structural components of rat’s renal corpuscle in chronic poisoning with acetylsalicylic acid (M±m).

Fig. 2. The structural organization of the rat’s kidney 7 days after the introduction of acetylsalicylic acid. H&E stain. \( \times 140 \). Artery with thickened wall, narrowed lumen and swollen endothelium - 1, erythrocytes in the lumen of the vein - 2, renal corpuscles - 3.

Fig. 3. The structural organization of the rat’s kidney 14 days after the introduction of acetylsalicylic acid. H&E stain. \( \times 100 \). Intra-organ artery with thickened wall - 1, full-blooded vein - 2, renal corpuscle - 3.

The veins and arteries of large caliber remained elevated and full-blooded. As a result of violation of hemomicrocirculation developed perivascular and interstitial edema.

Changes in the glomerular apparatus during this period of observation were to reduce the total area of nephrons with a simultaneous decrease in the area of capillary glomeruli. Moreover, the decrease in the area of capillary glomeruli occurred more intensively, which led to a reliable \( p<0.05 \) expansion of the Bowman's capsule urinary spaces (Fig. 4).

The epithelium of the proximal and distal tubules was in
a state of hydropic dystrophy, the intensity of which increased. Tubular lumen was unevenly enlarged, some of them containing cell detritus and cylinders. In the renal medulla, there were also focuses of dystrophy, somewhere with polymorphic-cellular infiltrates.

The 28-day observation period was characterized by signs of further progression of organ-hemodynamics disorders of ischemic orientation. The tonus increased, the wall thickness increased and the throughput of the intraorganic arteries was already decreasing in all investigated types. The veins remained expressed full-blooded.

In the renal corpuscle, due to the decrease in the blood flow to the capillary glomerular loops, there was a pronounced (p<0,05) increase in the Bowman's capsule urinary space, while the capillary glomeruli were often collaptoid: their size decreased significantly (by 25-30%), and contours became uneven-hilly (Fig. 5).

At the same time, for this observation period, signs of perivascular and interstitial sclerosis that could have been due to ischemia, as well as the result of tissue infiltration in the pre-term period (Fig. 6).

**Discussion**

Thus, the results of our study suggest that chronic poisoning with acetylsalicylic acid leads to severe organ disorders in the kidneys that manifest themselves in the form of congestive venous hypertension with the development of reactive plethora of the arterial department of the blood stream of the organ and the decrease in its throughput, the morphological manifestation of which is the thickening of the arterial walls, the foldiness of their internal elastic membranes and the narrowing of the lumen.

Objective confirmation of morphological changes in the structural components of kidney parenchyma was the results of morphometric studies according to which a significant decrease in the area of capillary loops of glomeruli in the long term (28 days of experiment) was established with a simultaneous significant increase in the area of urinary spaces in the renal corpuscles (14-28 days of experiment).

The results obtained agree with other studies carried out in this area, according to which the main link in the pathogenesis of acute renal failure due to acute poisoning by substances that cause the state of toxic shock and accompanied by changes in tissue metabolism, is precisely the violation of renal blood circulation [9, 20, 21].

The structural changes we have established in the renal vessels can be considered as a protective reaction that partially prevents hemodynamic overload of the hemomicrocirculatory bed during venous congestion, known in the scientific literature as the veno-arterial reaction, or the reflex of Kitaev [6].

However, in the process of chronic poisoning with acetylsalicylic acid, this mechanism does not provide complete compensation for organ renal blood circulation. In addition, prolonged vasoconstriction leads to tissue ischemia, which may be the cause of the development and progression of functional and dystrophic changes in the structural components of the organ parenchyma as a morphological substrate of renal failure, which is also fully consistent with modern notions [17, 21].

One of the links in the morphogenesis of structural changes in the kidneys may be the development of acidosis, which is also common in overdose, in particular, salicylic preparations [1, 11]. Products of metabolism, causing acidosis, are a real danger, as they are able not only to break the functions, but also lead to morphological changes in various organs and tissues [2]. The negative effect of acidosis on the state of the vessels is manifested by an increase in the permeability of the capillary wall and a change in the reaction of vascular sphincters, arterioles, and venules, which also contributes to the development of tissue hypoxia and their degenerative transformation [8, 22]. Therefore, further detailed study of structural changes in the kidneys and their bloodstream after the application of toxic doses of...
acetylsalicylic acid and its preparations can contribute to an understanding of the pathogenesis of poisoning and to be important in the development of new complex methods for their correction.

Further research in this direction will allow the development of correction methods for chronic poisoning with acetylsalicylic acid and give them a morpho-functional justification.

Conclusions

1. Chronic poisoning with acetylsalicylic acid leads to stagnant organ-hemodynamic disorders in the kidneys, which is manifested by capillary stasis, venous and arterial plethora.

2. At the venous stasis, the arterial section of the bloodstream of the kidneys reacts with ascending vasoconstriction with increased tone (increased folding of the internal elastic membranes), thickening of the walls (due to swelling and hypertrophy of the smooth muscle cells of the middle membrane), and narrowing of the lumen initially of the arterioles and small arteries, and in the subsequent - intraorganic arteries medium and large diameter.

3. As a result of prolonged vasoconstriction, ischemia of the organ develops, which leads to dystrophic changes in the structural components of the parenchyma: hydroptic dystrophy of the epithelium of the proximal and distal tubules, plasma permeation of tissues with the subsequent development of perivascular and interstitial sclerosis.

4. A quantitative confirmation of the reorganization of the renal parenchyma in the long term is a significant decrease in the area of the capillary loops of the glomeruli by 25-30% with a simultaneous significant increase in the area of the urinary space in the renal corpuscles to 44%.

References


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