INFLUENCE OF UNITHIOL UPON THE PROGRESS OF RENAL REGENERATIVE PROCESS OF SUBCHRONICAL MERCURY INTOXICATION

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The authors investigate the influence of Unithiol (U) on renal structural alterations and serum urea and creatinine levels of subchronical mercury bichloride intoxication with doses of 0.25 mg/kg and 1 mg/kg. U is applied in a double molar ratio to the injected amounts of mercury bichloride. In these conditions mercury bichloride induces as well severe dystrophic alterations of the cells in proximal tubules, as a necrotic nephrosis which are not attended by significant changes of serum urea and creatinine levels. After U application the morphological changes are gradually slighter expressed and certain regenerative signs occur, too.

In the course of our complex investigations of the influence of recognized mercury antidotes Unithiol (U), BAL and Penicillamine and of other substances with potential prophylactic and therapeutic activity, a special attention was paid to the dithiol antidote U. Concerning the manifestations of acute mercury bichloride intoxication in rats U displayed the most expressed antidote activity for our experimental conditions (4, 7). However, as regards the indices of subchronical poisoning there are not always favourable effects only. Concerning the changes of brain ATPase and 5'-nucleotidase activity and serum AP activity, the effects of U are moderate and completely comparable with those of other substances (phenobarbital and protein hydrolysate) (5, 8, 12). U action is unfavourable on the changes of cell division of acute and subchronical mercury bichloride intoxication (1, 2, 9, 10). On the other hand, an increase of suppressed renal ATPase activity is observed (6).

In the present work we followed-up U effect on the progress of renal structural alterations of subchronical mercury bichloride intoxication. U was applied in a double molar ratio to the injected quantities of mercury bichloride in order to provide the introduction of sulfhydril groups in a significant superiority over the mercury ions (4:1). Serum urea and creatinine levels were also determined with a view to an eventual correlation between these tradition indexes of renal failure and the corresponding morphologic changes of the kidneys.

Material and methods

The study covered 162 white male rats divided into 6 groups. The animals were treated with mercury bichloride, U, or with combination of them for a period of 45 days: 1st — controls; 2nd — treated with 0.25 mg/kg mercury bichloride s. c. (1/30 LD₃₀); 3rd — treated with 1 mg/kg mercury bichloride (1/7.5 LD₃₀); 4th — regularly injected with 0.38 mg/kg U i. m.; 5th — treated with 0.25 mg/kg mercury dichloride and with 0.38 mg/kg U i. m. to supply
a double molar ratio of U to the mercury bichloride; VI$^{th}$—treated with 1 mg/kg mercury bichloride s. c. and 1,55 mg/kg U i. m.

The investigations were carried out periodically on the 7$^{th}$, 15$^{th}$, 30$^{th}$ and 45$^{th}$ day of treatment, and on the 30$^{th}$ day after its cessation for the groups of animals given 0,25 mg/kg mercury bichloride, and on the 15$^{th}$, 30$^{th}$ and 45$^{th}$ day of treatment and on the 60$^{th}$ day after its cessation for the groups of animals given 1 mg/kg mercury bichloride. 6 animals of each group were killed on every mentioned interval. The material for histologic investigation was taken from the kidneys and prepared after the paraffin method. Preparations were stained with hemalaun-eosin. PAS-reaction was done under the control of alpha-amylase to detect the glycoproteins and Blacheau-reaction to establish RNA. Urea level was determined after the urease method (urea test N$_{4}$) and creatinine levels after the method of Jaffe-Folin.

Results and discussion

Seventh day:
Clearly expressed dystrophic changes (parenchymatous and partially vacuolar dystrophy) of the epithelial cells in proximal tubules predominate in animals injected with 0,25 mg/kg mercury bichloride. The cytoplasm in apical part of these cells shows an increased content of PAS (+) non-glycogenic substances. A certain reduction of pironinophilic granules in the cytoplasm of the epithelial cells of both proximal and distal tubules is found out. The lumens of the latter are dilated and filled with protein substance. The interstitial blood vessels are also dilated, with perivascular swell around some of them.

After U application only slightly expressed dystrophic alterations of the epithelial cells of some contort tubules can be observed. However, the haemodynamic disorders still persist.

Fourteenth day after mercury intoxication:
Besides the dystrophic changes there is a necrosis of the epithelial cells in contort tubules. Most of them are ripped off into the lumens and basal membrane is naked. In the cytoplasm of intact cells in proximal tubules RNA content is strongly reduced. The quantity of PAS (+) non-glycogenic substances in the apical part of the cells is irregularly expressed—from moderately to considerably diminished. Basal membranes of glomerular capillaries and arteriolar walls are thickened and soaked with PAS (+) materia. The blood vessels are dilated and filled with erythrocytes. There is a perivascular swell and haemorrhages at certain places. Although after U application the complete restitution is absent, for all that the dystrophic alterations are gradually less expressed. There are also regenerative signs. RNA contents is increased in the cytoplasm and nuclei of the new-formed cells. The haemodynamic disorders persist.

Thirteenth and forty-fifth day:
The changes of the epithelial cells of contort tubules are most manifestedly expressed. Most of them are necrotic and ripped off into the lumens and the basal membrane is naked (fig. 1). The PAS-quantity (+) non-glycogenic substance
is reduced in the apical part of the cytoplasm of intact cells. There are cylinders and protein substances in the lumens of most tubules. The blood vessels are dilated and filled with erythrocytes, especially in the medulla. One can also see some interstitial haemorrhages. After U application some regenerative signs are ascertained in most cells. In proximal tubules binuclear cells with an increased PNA content are observed. The naked basal membrane is covered with low cells with a pale cytoplasm. The quantity of protein materia in tubular lumens is diminished. Only in a few of them some cylinders can be observed. The walls of some arterioles are thickened and soaked with PAS (+) materia. Most venous blood vessels are dilated with surrounding tissue swell. When the animals were given a higher dose (1 mg/kg mercury bichloride) the renal alterations are analogous to those described above but they are more expressed. The necrosis of the epithelial cells in contort tubules predominates (fig. 2). U application causes regenerative signs with a tendency to restitution of damaged structures but the disturbances don’t disappear completely. In the restoration period (30 days after injection with 0,25 mg/kg and 60 days after injection with 1 mg/kg mercury bichloride) the developed dystrophic and necrotic changes of the cells of contort tubules at the higher dose don’t disappear although in this case the survival term is longer. In most lumens there are protein cylinders. The haemodynamic disturbances in the interstitium persist. They are comparatively less expressed in the glomerules. U application induces some expressed regenerative changes in most affected tubules. There is an increase of the number of binuclear cells and of RNA content in them. PAS (+) substances are comparatively more regularly distributed in the cytoplasm of the epithelial cells of prox-
mal tubules. The haemodynamic disorders are less expressed but the vessels are still dilated and show a marked hypocaemia.

The mean urea level in all studied intervals doesn't exceed 45 mg % (a level considered normal for the animal species used) in all animals as well treated with both doses mercury bichloride, as given U alone or in combination. The serum creatinine level is quite stable and doesn't alter considerably under the influence of doses of mercury bichloride and U used alone or in combination (its levels vary about 0,8—0,9 mg % in all groups of animals).

Our studies show that subchronical mercury bichloride intoxication causes renal changes which argue for an expressed lesion of tissues and cell structures. Together with that, U application induces a certain diminution of ascertained toxic alterations — the quantity of RNA and partially of RAS (+) non-glycogenic substances in the cytoplasm of the epithelial cells in contort tubules increases and the protein materia in their lumens decreases. However, certain events of pathologic regeneration are also established as argued by the presence of epithelial cells with more than one nucleus. The intensified pironinophilia in them is probably an expression of a developing intracellular regeneration (3). Besides the regenerative processes the haemodynamic disorders still persist. The irregular manifestation of PAS (+) materia in the cytoplasm of the epithelial cells of proximal tubules argues for still disturbed processes of resorption which are going on in them (11). The higher the applied dose of mercury bichloride and the longer the intoxication term, the more expressed the observed alterations. In such cases U application doesn't cause a complete restitution.
The considerable morphologic renal alterations of animals treated with mercury bichloride doesn’t correlate with the stability of serum urea and creatinine levels. These data allow us to assume that these indexes are not informative enough in cases of damaged renal parenchyma after a subchronical mercury intoxication.

In conclusion, we have also to note that the U application as antidote in these conditions causes a certain restitution of damaged structures which, however, is not complete and even accompanied by symptoms of pathologic regeneration.

REFERENCES


ВЛИЯНИЕ УНИТИОЛА ПРОТЕКАНИЕ РЕГЕНЕРАТОРНЫХ ПРОЦЕССОВ В ПОЧКАХ ПРИ СУБХРОНИЧЕСКОЙ ИНТОКСИКАЦИИ РТУТЬЮ

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РЕЗЮМЕ

В работе прослеживается влияние унитиола на структурные изменения почек и на уровень сыворотки мочевины и креатинина при субхронической интоксикации хлористой ртутью в дозах 0,25 мг/кг и 1 мг/кг.

Унитиол применялся в двойном молярном соотношении по сравнению с введенным количеством хлористой ртути. При указанных условиях хлористая ртуть вызывала тяжелые дистрофические изменения в клетках проксимальных канальцев и некротический нефроз, что не сопровождалось значительными изменениями уровня сыворотки мочевины и креатинина. При применении унитиола морфологические изменения выражены в более низкой степени, причем наблюдаются и процессы регенерации.