STUDY OF THIOL GROUP AMOUNT IN AORTA OF RATS INFLUENCED BY MERCURIC CHLORIDE AND TREATED BY ASCORBIC ACID

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According to literature data thiol groups play an important role in numerous physiological and biochemical processes as well as in cases of application of certain toxic substances.

The effect of thiol agents is determined by the inhibition of the thiol groups of enzymes, other proteins and low-molecular functioning compounds (2). It is known that mercuric compounds realize their connection with proteins through thiol groups to which they demonstrate a high specificity and affinity as well (W. L. Hughes, 1950, cited after 2).

A series of investigations show that the degree of toxic damages caused by numerous mercuric compounds is determined by the blockade of the thiol enzymes (11). There are studies of the interaction of mercuric compounds with serum proteins (7), liver proteins (7) and renal ones (9). However, no data concerning aortic proteins are reported. It is known that ascorbic acid possesses a detoxication ability. It was used in cases of intoxication with heavy metals including mercury, too (B. W. Vilter, cited after 9).

C. D. Sharma et al. (10) established a detoxication effect of the ascorbic acid in mercuric chloride poisoning of fishes of the species Carrassius auratus as expressed by the higher percentage of survived fishes. A similar effect concerning inhibition of the toxicity of organic mercuric compounds by the ascorbic acid is observed by H. C. Hill (cited after 10) in chickens. These investigations allow us to suppose that ascorbic acid could have a preventive effect on mercuric intoxications.

This presumption and the lack of data about the action of the ascorbic acid on aortic thiol groups in cases of mercuric poisoning is the reason to perform both single and combined influence of the ascorbic acid and mercuric chloride on thiol groups of aortic proteins in rats.

Material and methods

Our study covered 36 white male rats with body weight between 150 and 170 g divided into four groups : Ist — treated with mercuric chloride (8 animals); IInd — controls — treated with saline (8 ones); IIIrd — treated with ascorbic acid (10 ones), and IVth — treated with both mercuric chloride and ascorbic acid.

The ascorbic acid was i. m. injected in a dose of 5 mg/100 g b. w. every day for 7 days. The mercuric chloride was s. c. injected in a dose of 7,5 mg/kg b. w. only once 24 hours before killing the animals. Experimental and control animals were fed a standard food. They were killed at one and the same hour (9 h. a. m.). The aorta was extracted immediately and cleansed from the periaortic tissue.

It was weighed by using balance and homogenized in Potter's homogenizer with bidistilled water in a ratio 1:160. Then the homogenate was centrifugalized at 5000 revolutions per minute for 5 min. 1 ml of the surfactant was then used in our examination. The amount of thiol groups was determined in this surfactant after the method of amperometric titration of Torchinskij in Ivanov's modification (5). The protein was estimated after Lowry's method. The results are shown as $\mu \times 10^{-7}$ groups/mg protein. They were statistically processed after the method of variation analysis by using t-criterion of Student-Fischer.

Results and discussion

The results obtained are demonstrated on fig. 1.

There is a statistically reliable decrease of the amount of thiol groups in mercuric chloride treated animals. They are reduced from $1,7 \times 10^{-7}$ SH groups/mg protein in control animals down to $0,9 \times 10^{-7}$ SH groups/mg protein in mercuric

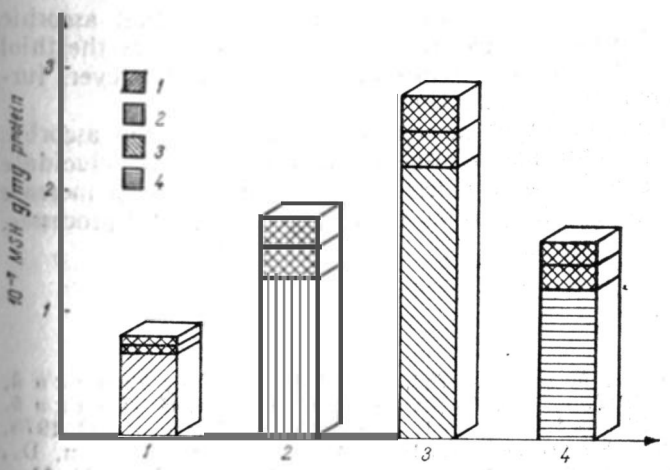

Fig. 1. The results are presented as $\bar{x} + S\bar{x}$:
Legend: 1) treated with $HgCl_2$; 2) controls; 3) treated with ascorbic acid; 4) treated with ascorbic acid and $HgCl_2$
1:2 $p < 0,05$
2:3 $p < 0,05$
2:4 $p < 0,05$

chloride treated ones, or by 47 per cent ($t = 3,3$, $p < 0,05$). It is most probably due to their inhibition by the mercuric chloride that shows a strong affinity towards them and is able to form links with one or even two thiol groups thus forming compounds of two types: protein-SHgCl, and (protein S)$_2$Hg.

Concerning the animals of the 3rd group it is to be noted that there is a statistically significant thiol group increase in comparison with the control values (2nd group), namely from $1,7 \times 10^{-7}$ SH groups/mg protein up to $2,7 \times 10^{-7}$ SH groups/mg protein — with 59 per cent ($t = 2,2$, $p < 0,05$). An increase of thiol groups in cataractal lens homogenate as a result from the action of ascorbic acid is already established by D. Kalitzin et al. (6). This increase can be explained with the reducing properties of ascorbic acid towards disulphide ties of the proteins and its ability to prevent the oxidation of protein thiol groups (1, 3).

There is a statistically significant increase of thiol groups in the animals from the 4th group as compared with the levels in those from the 1st one — with 66 per cent ($t=2,4$, $p<0,05$). Simultaneously to that a tendency towards normalization is found out. There are no statistically significant differences between the 2nd and 4th group. This is an evidence of the effect of the ascorbic acid on thiol groups in cases of mercuric chloride intoxication.

The investigations of some authors (10) are related with our results. These authors established a surprising great biotransformation of mercuric chloride into methyl mercury and dimethyl mercury resulting in a considerable toxicity reduction demonstrated by the high percentage of survived fishes of the species Carrassius auratus.

J. Gage (4) found out in in vitro experiments that soluble protein fractions from liver homogenate possessed a biodegradation effect on methyl mercury and other organic compounds which was due to the presence of active thiol groups in their molecule.

Having in mind the aforementioned studies we could suggest that ascorbic acid biotransforms mercuric chloride into methyl mercury and activates the thiol groups of proteins thus inducing methyl mercury biodegradation. However, further investigations are required in this direction.

Our studies cannot explain the exact mechanism of action of the ascorbic acid in animals under mercuric chloride treatment. Nevertheless, they elucidate the detoxication effect of the ascorbic acid which can be interpreted as an increase of protein thiol groups well-known to play an important role in vital processes.

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ИССЛЕДОВАНИЕ КОЛИЧЕСТВА ТИОЛОВЫХ ГРУПП В АОРТЕ КРЫС, ТРЕТИРОВАННЫХ МЕРКУРОХЛОРОМ И АСКОРБИНОВОЙ КИСЛОТОЙ

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РЕЗЮМЕ

Проведены исследования 36 белых крыс — самцов. Они распределены в четыре группы: первая группа (8 животных) подвергалась действию меркурохлора; вторая группа — контрольная (8 животных) — с применением физиологического раствора; третья группа (10 животных) — с примемением аскорбиновой кислоты; четвертая группа (10 животных) подвергалась действию меркурохлора и аскорбиновой кислоты.

Аскорбиновая кислота вводилась интрамышечно в виде водного раствора в дозе 5 мг/100 г веса ежедневно в течение 7 дней. Меркурохлор вводился подкожно в дозе 1,7 мг/кг веса 24 часа до смерти животного. Контрольные животные принимали физиологический раствор. Тиоловые группы определялись по отношению к содержанию белка.

Статистическая обработка данных показывает понижение количества тиоловых групп при применении меркурохлора (группа первая) по сравнению с контрольными животными (вторая группа).

У животных четвертой группы (с применением меркурохлора и аскорбиновой кислоты) устанавливается статистически достоверное повышение тиоловых групп по сравнению с животными первой группы и близкие стоимости с второй контрольной группой.

Полученные данные говорят о детоксикирующем действии аскорбиновой кислоты при интоксикации меркурохлором.