

A COMPARATIVE STUDY ON THE EFFECT OF MONO- AND DITHIOL ANTIDOTES ON CELL DIVISION AND NUCLEOLUS FORMATION IN ACUTE MERCURY BICHLORIDE INTOXICATION

L. Vassileva, E. Boshnakova, A. Belcheva, M. Mangarova, D. Zhelyazkov

The influence of mercury and mercuric compounds on cell division and hereditary structures of the organism is a problem not sufficiently clarified, despite available literature data along this line concerning embryocide and teratogenic effect of organic mercury compounds (Mei-Quey Su et al, 1976; F. Olson et al, 1977; Yamaguchi et al, 1974; T. Gale, 1974), increased amount of aneuploid cells in subjects exposed to occupational contact with metal mercury and organic mercury compounds (Z. Verschaeve et al, 1976) etc. Still more limited is our knowledge of the efficacy of the classical heavy metal antidotes in terms of this particular aspect of the toxic action of mercury and its compounds.

The work submitted summarizes the results of comparative assessment of the influence exerted by monothiol antidote-D-Penicillamine (Cuprenil-Polfa), and the dithiol antidotes unithiol and 2,3-dimercaptopropanol (BAL) on cell division and nucleolus formation in rats, exposed to acute mercury bichloride intoxication.

Material and methods

The study was conducted on a series of 25 male white rats, distributed in five groups, as follows: group I — control, injected subcutaneously with physiological saline; group II — treated with mercury bichloride, subcutaneously at dose 7.5 mg/kg. The dose represents LD_{50} of the compound for this animal species and mode of introduction (Zhelyazkov et al, 1978); group III — treated with mercury bichloride as the animals in group II, and given D-penicillamine per os, at dose 16.5 mg/kg; group IV — treated with mercury bichloride and injected i. m. with unithiol at dose 12 mg/kg; group V — treated with mercury bichloride and injected i. m. with BAL at dose 7 mg/kg. All antidotes were introduced twice (at 30 min and 8 hours) after mercury bichloride treatment. The doses were selected in a fashion that a single introduction of each antidote would secure the introduction of sulfhydryl groups at a 4:1 ratio relative to the amount of mercury ions administered.

The animals were killed within 24 hrs of mercury bichloride intoxication. From all animals preparations from the femoral bone marrow were worked out after the modified method of Fox and Zeiss (1961). The number of mature, blast, and mitotically and amitotically dividing cells (through constriction and budding) was determined on a total of 125 000 cells. The percentage of blasts without, or with one and more nucleoli was calculated per 50,000 cells. The data underwent statistical elaboration according to the alternative analysis method.

Results and discussion

Upon application of mercury bichloride alone, a change was noted in the correlation between cells multiplying amitotically through constriction and budding. A reduction of the number of budding cells was established,

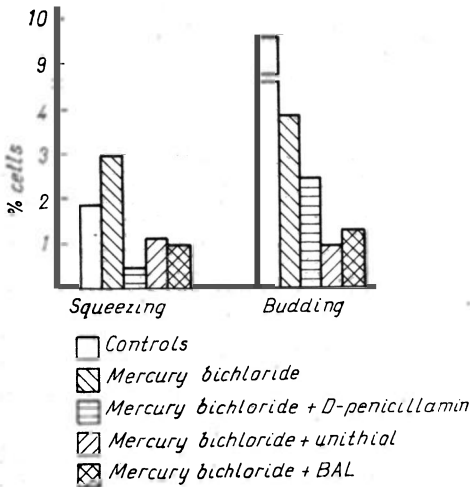


Fig. 1: Effect of mercury bichloride administered alone and in combination with mono- and dithiol antidotes on amitotic division of rat bone marrow cells through constriction and budding.

whilst the percentage of dividing cells through constriction showed a 1.5 times increase by comparison with the controls. The combination of mercury bichloride with mono- and dithiol compounds led to an inhibition of both types of amitotic division (Fig. 1). The inhibition of division through constriction was mostly pronounced in penicillamine treatment (by 80 per cent), and of that through budding — in unithiol treatment (by 90 per cent). Mitotic activity of the bone marrow cells among mercury bichloride treated animals was preserved, and the number of blasts showed a reliable increase ($p < 0.001$) (Fig. 2). This may be due to the blast cells' functional inferiority, or to an impairment of their transition to division. The fall of the percentage of mitotically dividing cells among the mercury bichloride + unithiol treated animals was not statistically reliable, unlike the increase in blasts observed. Among

animals treated with 2,3-dimercaptopropanol, the amount of mitoses was decreased ($p < 0.002$) whilst the percentage of blast cells was augmented. The mitotic activity of cells upon combined treatment with mercury bichloride + penicillamine was unchanged, whereas the quantity of blasts displayed a significant increase by comparison with controls ($p < 0.001$).

These results show that upon treatment with single toxic doses mercury bichloride, certain aspects of the cell division processes are inhibited; combined application of the inorganic mercury compound with dithiol antidotes enhances the inhibitory effect (rather marked in BAL), whereas monothiol-penicillamine impairs only the amitotic activity of cells.

On the other hand, proceeding from literature data claiming a parallelism between quantity of blasts and mitotically dividing cells in the lymphocyte cultures (L. Vassileva, 1976; M. T. Tzoneva-Maneva, 1970), the assumption is warranted that upon independent application of mercury bichloride and its combination with penicillamine, a certain degree of impairment in the G_2M transition from vital cycle to cultivated cells occurs, leading to an increase in the amount of blasts without a respective change in mitoses.

In support of the above statement are also the results of the other indicator under study, e. g. nucleolus formation in the bone marrow cells. Compared to controls, the percentage of blasts free of nucleoli augments in all expe-

rimental setups. The latter effect is comparatively the least manifested among penicillamine treated animals. The percentage of blast cells with a single nucleolus is close to that in the control setup using penicillamine, and reduced in independent and combined application of mercury bichloride with unithiol and 2,3-dimercaptopropanol (Fig. 3). The inhibition of nucleolus-for-

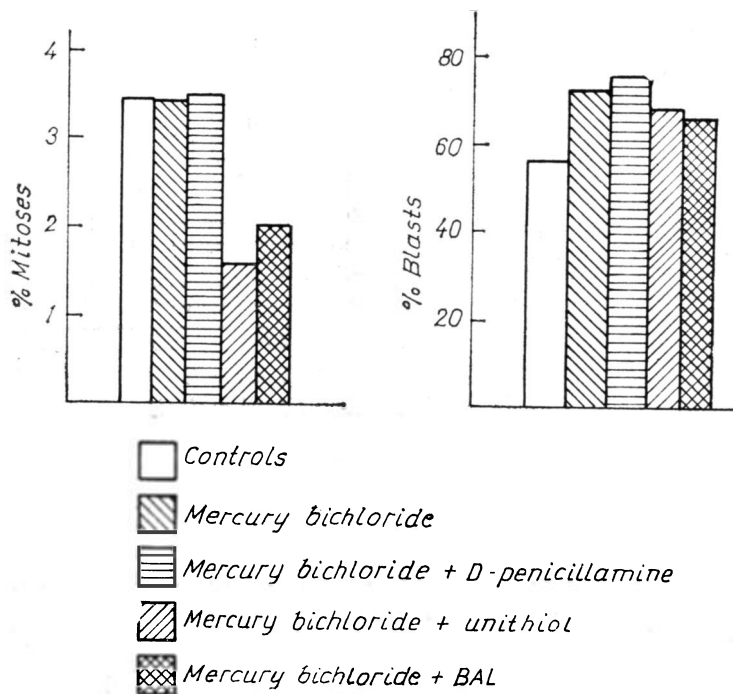


Fig. 2: Effect of mercury bichloride administered alone and in combination with mono- and dithiol antidotes on mitotic division and blasts from the bone marrow cells of rats.

mation processes is manifested with a statistically reliable ($p < 0.01$) reduction of the quantity of blasts by one and three nucleoli in any one of the experimental groups. The latter effect is expressed through a quantitative difference. While with penicillamine the decrease is very close to that caused by the administration of mercury bichloride alone, in the combination with unithiol, and particularly with BAL, the quantity of blasts with two and more nucleoli is 2—2½ times lower. It is possible that inhibition of the nucleolus-forming processes referred to may be the result of a functional inferiority of blast cells. The noted increase in the percentage of cells with a single nucleolus under the effect of combined mercury bichloride + penicillamine application, most likely mirrors the RNA-synthesis preservation under the influence of the monothiol antidote. Such data corroborate indirectly the statement made about the viability of cells treated in the fashion described, and indicate the level of their functional activity (A. A. Prokofieva-Belgovskaya, 1969).

Our results demonstrate that the dithiol antidotes — unithiol and BAL — not only fail to exert a favourable effect on mercury-bichloride induced changes in the indicators under study, but on the contrary, they account for their intensification. Only the monothiol antidote — penicillamine — exhibits a

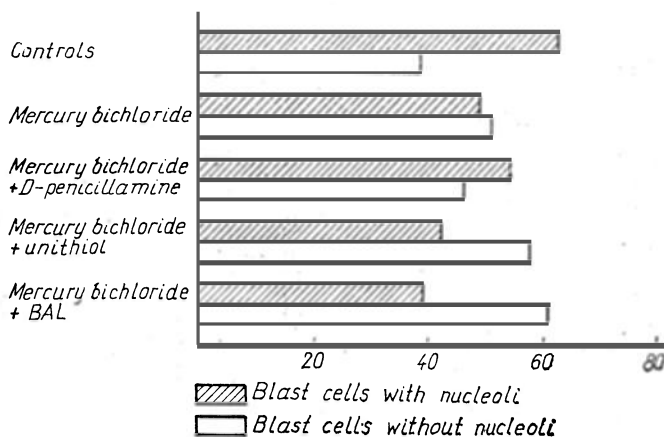


Fig. 3: Percentage values of blast cells without and with one and more nucleoli in animals treated independently or in combination with mono- and dithiol antidotes.

tendency to stimulate nucleolus formation in the blast cells, which might be considered as an indirect indicator of an enhancement of the restoration processes in them.

Having in mind the powerful antidote action of unithiol also in terms of lethality, renal lesions, and other manifestations of the acute mercuric intoxication, the observed intensification of the mutagenic action of mercury bichloride becomes a fact of utmost interest. It is difficult to answer the question why penicillamine, introduced at doses securing a molar ratio between SH-groups and mercury ions, analogical to that of dithiol antidotes, fails to intensify, but rather tends to compensate the effect of mercury bichloride. On the basis of the present research it seems reasonable to suggest the significance of the molecule — carrier of SH groups — as well as of the number and activity of thiol groups in the molecule of antidotes.

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**СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ВЛИЯНИЯ МОНО- И ДИТИОЛОВЫХ
АНТИДОТОВ НА КЛЕТОЧНОЕ ДЕЛЕНИЕ
И ОБРАЗОВАНИЕ ЯДРЫШКА ПРИ ОСТРОМ ОТРАВЛЕНИИ
РТУТЬЮ ДВУХЛОРИСТОЙ**

Л. Василева, Е. Бошнакова, А. Белчева, М. Мангырова, Д. Желязков

Р Е З Ю М Е

У крыс, третируемых однократно 7,5 мг/кг ртутью двухлористой, изучено сравнительно действие моноитолового антидота D-пенициллина и дитиоловых антидотов унитиола и 2,3-димеркаптопропанола (BAL) на деление и образование ядрышка в костно-мозговых клетках. Антидоты применялись в дозах, обеспечивающих двукратное введение сульфгидрильных групп в соотношении 4:1 в отношении ртутного иона.

Как при самостоятельном, так и при комбинированном с антидотами введении ртути двухлористой наблюдалось подавление амитотического деления. Обнаружено уменьшение митотической активности при самостоятельном и в комбинации с дитиоловыми антидотами применением ртути двухлористой, с одной стороны, и, с другой — увеличение митозов при третировании животных ртутью двухлористой и D-пенициллинамом.

Самостоятельное применение ртути двухлористой связано со слабым уменьшением числа ядрышек. Ее комбинация с дитиоловыми соединениями ингибирует образование ядрышек (более подчеркнуто при применении BAL), в то время как при третировании ртутью двухлористой и D-пенициллинамом, общее число ядрышек оказывается ближе к контролю. Обсуждена роль молекулы-носителя сульфгидрильной группы в осуществлении антидотного эффекта в отношении деления и образования ядрышка в костно-мозговых клетках у опытных животных.