I. EXPERIMENTAL PROBLEMS

EFFECTS OF UNSOPORIFIC DOSES PHENOBARBITALUM UPON MITOSIS AND NUCLEIFORMING OF BONE-MARROW CELLS OF RATS WITH ACUTE MERCURY INTOXICATION

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

Though the toxic action of mercury and its compounds is thoroughly investigated, their influence upon mitosis and genetic apparatus of cells is still insufficiently studied. Almost nothing is known about the eventual effect and degree of action of those numerous classical and actual mercury antidotes upon the aforementioned influence of mercury on alive organisms. The importance of the problem is characterized with the wide application of the metalic mercury and its compounds in the industry and the constant pollution of the environment.

The present work studies the influence of a preliminary application of phenobarbitalum (PB) on the mitosis and nucleiforming of bone-marrow cells of rats treated with a single toxic dose mercury bichloride. Treating with PB (after bibliographic data) affects the tissue-distribution of some heavy metals (Hiroshi J. — 1976), decreases the acute toxicity of cadmium (Hiroshi J., Jasutomo S. — 1976) and increases the billiary excretion of methyl-mercury and cadmium in rats (Magos L. et al. — 1974; Motoyasu O. and Kasuo F. — 1976). Our own investigation proves the positive effect of PB, applied in unsoporific doses, upon LD_{50} of mercury bichloride and survival of rats received lethal dose of the same substance (Jeljazkov D. et. al. — unpublished data). Our interest in influence of PB upon eventual changes caused by mercury bichloride on cell mitosis is supported by bibliographic data of stimulation of the mitotic activity of barbiturate (Argyris T. S. — 1971).

Material and methods

The study covers 15 animals divided into three groups: 1) Controls injected s. c. with saline solution; 2) Animals injected s. c. with a single dose of 7.5 mg/kg mercury bichloride; 3) Animals injected in the same way as those of the second group, but preliminarily treated with PB (60 mg/kg) 4 times intraperitoneally. The animals were killed 24 hours after the toxic dose mercury bichloride was injected.

Bone-marrow cells were cultivated by using the method of Fox et al. (1961). A total number of 75 000 cells were counted to study the effect of mercury bichloride, or its combination with PB, on mitosis. Also, 30 000 cells were counted to analyse the nucleiforming processes. The statistical analysis was done after the alternative method.

Results and discussion

Table 1 and table 2 show the received data. A combined application of PB and mercury bichloride tends to a slight activation of amitotic mitosis (with constriction) compared to that of the controls ($p<0.001$). The number of the budding cells in mitosis is less, especially the cells with one bud, followed by those with two buds; the total number of the buds is also decreased. A suppression of the amitotic mitosis, in comparison with the controls, is simultaneously estabilished. (Table 1).

Table 1

Influence of the single and combined with PB applicication of mercury bichloride on amitotic and mitotic reproduction of bone-marrow blastic cells of rats:

Indexes / Treated with	Amitosis — %			Mitosis %	Blastic cells — %	Mature cells %	Total number of cells
	contraction	budding	totally				
Saline solution	1.86	9.54	11.40	3.49	56.02	29.09	25000
Marcury bichloride	2.98 $p>0.1$	3.80 $p<0.001$	6.78 $p<0.001$	3.45 $p>0.3$	72.55 $p<0.001$	17.22 $p<0.001$	25000
Mercury bichloride+PB	2.18 $p<0.001$	2.80 $p<0.001$	4.98 $p<0.001$	4.38 $p<0.001$	75.78 $p<0.001$	14.86 $p<0.001$	25000

The single dose of mercury bichloride also influences the quantity of the constrictive mitosa (more expressively than its combination with PB). The budding and the total number of buds are decreased, when compared to those of the controls, but not so well manifested as they are resulted of the combination with PB. The mercury bichloride inhibits the amitotic cell mitosis but this effect is weaker than that of its combination with the antidote (Table 1).

The simultaneous application of PB with mercury bichloride stimulates the mitosis and blastic transformation of cells. As for the former, it coincides with the opinion of some authors (Glinskii I. A. — 1964; Tzoneva-Maneva M. T. — 1970), concerning a parallelism of amitotic constrictionlike reproduction and mitotic activity. The number of mitosa in the controls and with the experiments with mercury bichloride is the same. The blastic cells after the single dose mercury bichloride or its combination with PB show similar quantitative values and also a statistical difference, in comparison with those of the controls ($p<0.001$). This perhaps, is a result of a comparative inmaturity of the blastic cells treated with mercury bichloride and block of the G_2M-transition of their life-cycle. It is known, that the blastic cells' quantity corresponds to that of the mitosa of the cell cultures (Vassileva L. — 1972; Tzoneva-Maneva M. T. — 1970).

Table 2

Percentage of blastic cells without or with 1 and more nucleoli in animals treated with mercury bichloride only or its combination with PB

Indexes / Treated with	Blastic cells				
	No nucleolus %	1 nucleolus %	2 nucleoli %	3 nucleoli %	Total number of blastic cels
Saline solution	37,81	29,67	17,59	14,93	10000
Mercury bichloride	51,4 $p<0,001$	23,65 $p<0,002$	14,36 $p<0,045$	10,95 $p<0,01$	10000
Mercury bichloride+PB	26,78 $p<0,001$	31,96 $p<0,1$	21,23 $p<0,045$	20,03 $p<0,002$	10000

In unison with the aforementioned view of inmaturity and inferiority of the blastic cells. after treatment with mercury bichloride, the quantity of the cells without nucleoli in our study is statistically higher than that of controls and also that of the combined effect of mercury bichloride +PB (Table 2). The single application of mercury bichloride decreases considerably the number of the blastic cells with 1, 2, 3 nucleoli, while its combination with PB tends to an increased number of the blastic cells with 1 nucleolus and statistically reliable, still higher, number of cells with 2 and 3 nucleoli ($p<0.045$). These data support the opinion of a functional insufficiency of the bone-marrow blastic cells of animals treated with a single dose mercury bichloride. Most probably, it is a result of disorders in the nucleo acid-synthesis, espesially RNA (Inamoto H. et al. — 1976). On the other hand, it is also possible that the combination: mercury bichloride + PB causes a preservation of the quantity of the vital-necessary nucleoacids for any cell.

In comparison with other antidotes (Unithiol, 2, 3-dimercaptopropanol and D-penicillamin) studied in our laboratory, the PB shows certain peculiar features: 1) It has the highest protective effect on preservation of amitotic cell activity (not to the control level, however). 2) It increases the quantity of mitosa considerably, also the number of blastic cells and the cells with nucleoli. This parallelism of the presented indexes proves the preserved actual functional sufficiency of bone-marrow cells of the experimental animals overtreated with PB.

REFERENCES

1. Василева Л. Н. І-ва национ. конф. по биол. и медиц. генетика, 1972, резюме, 43—44. — 2. Глинский И. А. Бюлл. экспер. биол., 1964, 10, 84. — 3. Желязков Д., М. Мангърова, А. Белчева. Непубликувани

данни. — 4. Цонева-Манева М. Т. Scripta scient. med., Vol. VIII, 1970, f. II, 137—139. — 5. Argyris T. S. Develop. Biol., 1971, 25, 293—309. — 6. Inamoto H., I. Yosisuko, I. Nabuko, I. Nogayumi, J. J. Med., 1976, 99, 140—142. — 7. Magos L., Mc Gregor J. T., Clarkson T. W., Toxicol. Appl. Pharmac., 1974, 3—1, 6. — 8. Motoyasu O., F. Kazuo, Ind. Health, 1976, 14, 7—14. —9. H. Yoshikawa, S. Yasumoto. Ind. Health, 1976, 14, 103—108. — 10. H. Yoshikawa. Ind. Health, 1976, 14, 45—46.

ВЛИЯНИЕ НЕСНОТВОРНЫХ ДОЗ ФЕНОБАРБИТАЛА НА ДЕЛЕНИЕ И ЯДРЫШКООБРАЗОВАНИЕ КОСТНОМОЗНОВЫХ КЛЕТОК У КРЫС С ОСТРОЙ РТУТНОЙ ИНТОКСИКАЦИЕЙ

Д. Желязков, Б. Бошнакова, Л. Василева, М. Мангарова, А. Белчева

РЕЗЮМЕ

У крыс, которым однократно вводится подкожно 7,5 мг/кг двухлористой ртути исследовано влияние предварительного четырехразового введения фенобарбитала в дозе 60 мг/кг и его влияние на деление и ядрышкообразование костномозговых клеток.

Как при самостоятельном применении двухлористой ртути, так и при предварительном введении фенобарбитала, наблюдается процесс подавления амитотического деления. Введение избыточных доз фенобарбитала приводит к стимулированию митотического деления. У животных, получающих только двухлористую ртуть, количество митоз остается неизменным по сравнению с контролями. Число бластных клеток увеличивается как при самостоятельном введении двухлористой ртути, така и при применении избыточных доз фенобарбитала.

Самостоятельное применение двухлористой ртути понижает значимое количество бластных клеток на 1, 2 и 3 ядрышка, а ее комбинация с фенобарбиталом показывает тендецию к увеличению числа бластов на 1 ядрышко и значимое увеличение числа клеток на 2 и 3 ядрышка.