Original Research

Reproduction Parameters in Low Dose Chronic Exposure with Heavy Metals in Rats

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Abstract

The aim of this study was to evaluate the effects of low-dose chronic lead, mercury and cadmium exposure on the reproductive potency of Wistar rats and on their progeny. The number of litters and the number of newborns in rats exposed to Pb and Hg were significantly higher compared to controls, whereas between Cd exposure group and controls there was almost no difference. In contrast, the survival rate of the weanlings decreased in the order Cd>Pb>Hg and in all exposed groups was higher than in controls. The results suggest that an increase in reproduction rate early after intoxication onset may disclose certain reactive adaptation mechanisms. The number of weanlings appears to be a sensitive marker in reprotoxicity tests.

Keywords: heavy metals, reprotoxicity, low dose, chronic intoxication

Introduction

In spite of recent intense attempts at legislative regulations, the issue of environmental pollution and the restriction of existing freshwater resources is far from solved. Clean air, drinking water and food are basic requirements for a healthy life and therefore health professionals and biomedical researchers are in charge to draw the public attention to the possible health risks and consequences of industrial and agricultural pollution of air, soil and water resources [1-4]. Frequent cases of contamination of groundwater reservoirs, surface irrigation and water resources with excessive concentrations of metals call for more precisely defined safety precautions and require adequate practical steps for their elimination [5].

Many aspects of acute intoxication with heavy metals including their genotoxic, terato-genic and metabolic effects are relatively well known and are the subject of several in-depth papers and reviews [6-10]. However, there is a scarcity of available data about chronic exposition by low doses of various metals in multigeneration studies. Chronic lifelong contamination with acutely nontoxic concentrations may produce many unpredictable consequences not only for directly exposed individuals, but also in their offsprings [11, 12].

The main purpose of the present study was to evaluate changes in basic physiological and reproductive parameters due to lifelong exposure to low doses of heavy metals (lead, cadmium, mercury) in Wistar rats. This is an important prerequisite to find the most suitable and precise indicators for estimation of the risks of the chronic low-dose exposure with various harmful substances on healthy human population.

Experimental Procedures

Animals

Experiments were carried out on 80 Wistar albino rats of both sexes (40 females and 40 males, age at the beginning of study 4 weeks, average weight 120±19 g) and their

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28-day-old newborns. The animals were kept in male – female couples in separate cages with free access to water and food and day–night regime 12:12 hours. The experiment was terminated at 3 years of age.

Experiments were performed in central animal facility of the medical faculty with accreditation for laboratory animal-breeding. The experiments were approved by local ethical commission and the state veterinary and food agency (ŠVPS SR Č.k. Ro-7879/04-220/3).

Arrangement of Experiment

The animals were divided randomly into 4 groups (10 couples each group). Group I (C, control rats) did not receive any additives into the drinking water. The experimental groups were received metal-containing compounds diluted in drinking water in 200-times higher concentration than the maximal allowable concentration in drinking water (MAC). Group II (Pb) received water with 100 μmol/l of lead acetate in alkaline solution, group III (Cd;) 20 μmol/l of cadmium chloride dihydrate and group IV (Hg) 1 μmol/l of mercury chloride ($HgCl_2$).

Parameters of reprotoxicity such as number of litters, total number of newborns (assigned in the birth day), number of newborns per litter and number of weanlings (raised youngs that reached the 28th day of life) were measured.

Statistical Methods

For statistical evaluations of significant differences pair- and unpaired t-tests were used in combination with Wilcox-Mann-Whitney' U-test (Statgraphic) or one-way ANOVA supplemented by Newman-Keuls post-hoc test. The dates were considered significant if $P<0.05$.

Table 1. Values of selected reproduction indices after the end of experiment on 78th week.

Parameter	C	Pb	Cd	Hg
Number of litters	98	106	90	128
Total number of newborns	754	853	706	1015
Average number of newborns in litter	7.69	8.05	7.84	7.93
Total number of weanlings	686	599	606	574
% of weanlings from total number of newborns	91	70.2	85.8	56.6

Table 2. P values of number of litters (right, up) and number of newborns (left, down).

Group	C	Pb	Cd	Hg
C		0.0008	0.0878	0.0006
Pb	0.0003		0.0008	0.0421
Cd	0.0366	0.0004		0.0027
Hg	0.0006	0.0172	0.0013	

Table 3. P values of total number of weanlings (right, up) and % of weanlings from total number of newborns (left, down).

Group	C	Pb	Cd	Hg
C		0.0669	0.0386	0.0569
Pb	0.0217		0.4444	0.3000
Cd	0.1660	0.0022		0.2864
Hg	0.0039	0.0204	0.0018	

Results

The female rats brought forth from the 13th to 78th week of the experiment. The number of lit-ters were higher in rats exposed to Pb and Hg than in the healthy unexposed animals (Table 1). Significantly lower values were observed in animals exposed to Cd, which did not show significant difference from the control group (Table 2).

Similar to the number of litters, the highest number of newborns was observed in Hg-group (n=1015) followed by the group exposed to lead (n=853) (Table 2). In both these groups the number of surviving newborn was much higher compared to control group (754). The Cd-group showed a markedly lower number of newborns (n=706) than the Hg and Pb-groups, but significantly higher values when compared to control group (Table 2).

From the 13th to 39th week of exposure the average number of newborns per litter was higher in all intoxicated groups compared to control in the order Hg>Pb>Cd>C.

Differences between the groups treated by different metals were also observed in the dynamics of reproductive response, i.e. time of the onset, time course of reproduction rate and the offset of the reproduction burst over the experimental period (78 weeks). Hg and Pb groups showed steep and continuous rise in the number of litters and newborns early after the beginning of the experiment, but this was maintained only until the 26th week and was followed by a fast decline (Fig. 1). On the other side, both the Cd and the control group revealed a low-grade sustained reproduction rate over the initial 39 weeks. On the 52nd week the number of newborns in all groups was approximately the same and gradually declined until the end of week 78. The sharpest decline was noted in Pb group.

The number of newborns in a litter (Fig. 2) is a useful indicator of the reprotoxicity in general, particularly in longitudinal studies. Surprisingly, from the beginning until week 39 of the trial the lowest values of newborns per litter were recorded regularly in the control females. On the 52nd week the counts from different groups were quite similar and a marked decline in females exposed to metals appeared only in the last stage of the study.

The numbers of weanlings (Tables 1 and 3), in particular when expressed as a ratio of the total number of newborns), were always lower in the all intoxicated groups compared to healthy control (Tables 1 and 3). The lowest total number of weanlings was observed in Hg group (n=574), followed by Pb and Cd group. (n=599 and 606 respectively; controls 686). Similar results were obtained when comparing relative numbers of survivors to total numbers of newborns (Fig. 3, Table 3).

Discussion of Results

There is good knowledge that gonads are one of the primary target for several environmental toxins [13]. Cadmium is well known for its prominent inhibitory action on testosterone production by interference with hypothalamic-hypophyseal-testis axis [14]. Though many studies have repeatedly demonstrated the inhibitory effects of the various toxins on the gonadal functions, one has to consider that the timing and doses reported were much higher than those subtoxic ones in the present work. The present work demonstrated that the very low concentrations of heavy metals given in a chronic life-long manner may provide in the rats at least temporarily stimulatory effect on reproductivity rates. Negative effects likely began only after the achieving threshold cumulative dose of metals. The nature of this response is little known, but one can speculate about several adaptive mechanisms. As reported with many other noxious agents chronic administration of very low doses of metals might induce the adaptive preconditioning effect, which attenuates the toxic impact on physiological processes. This may certainly explain weakening and/or lack of response over time but can only hardly explain the immediate bursts in reproductivity after exposure. This may suggest involvement of the stress–related self–protecting biological mechanism aimed at preservation of species similar to what can be observed in other life-threatening situations.

Among useful parameters for any definitive conclusions when viewed in the context of other selected parameters, they provide good prediction value for reprotoxicity evaluation after heavy metal exposure. The belongs mainly to the proportion of weanlings from the total number of newborns. The numbers of weanlings and increases in the numbers of newborns was also found by the other authors experimenting with chronic exposure to heavy metals, which increased mortality throughout the first 2 weeks at much higher doses [11, 12].

Even though this parameter itself may be of partial value for any definitive conclusions when viewed in the context of other selected parameters, they provide good prediction value for the reprotoxicity evaluation after heavy metal exposure.

Conclusions

There is a wealth of data on the morphological and physiological alterations after acute and chronic exposure by toxic levels of heavy metals as an occurrence of tumours, embryonic developmental abnormalities, growth retardation, loss of tail, skin pathology (including loss of hairiness or even alopecia), bleeding into inner organs and cavities, diarrhoea, etc. In comparison with these traditional views the present work disclosed new, unexpected and in some aspects contradictory data about the reprotoxicity effects of chronic subtoxic exposure to heavy metals.

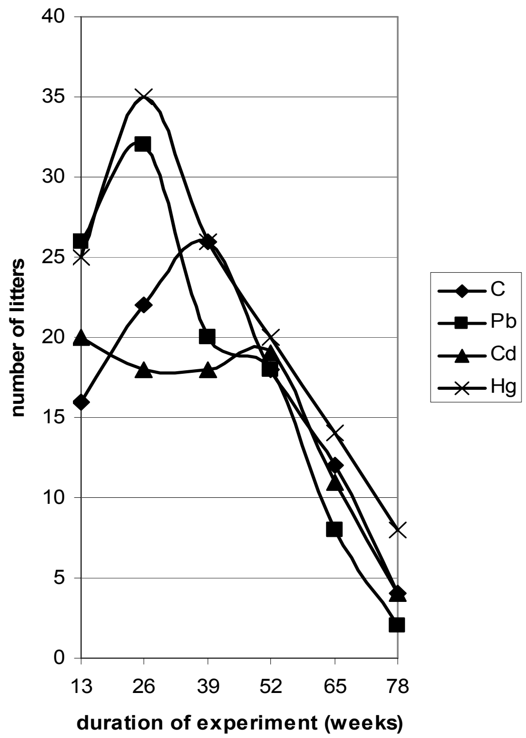

Fig. 1. Number of litters during of reproduction period.

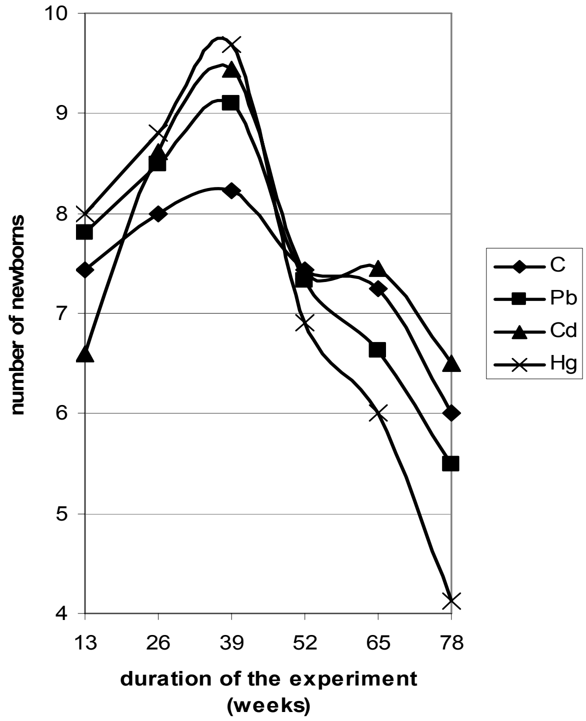

Fig. 2. Number of newborns per litter.

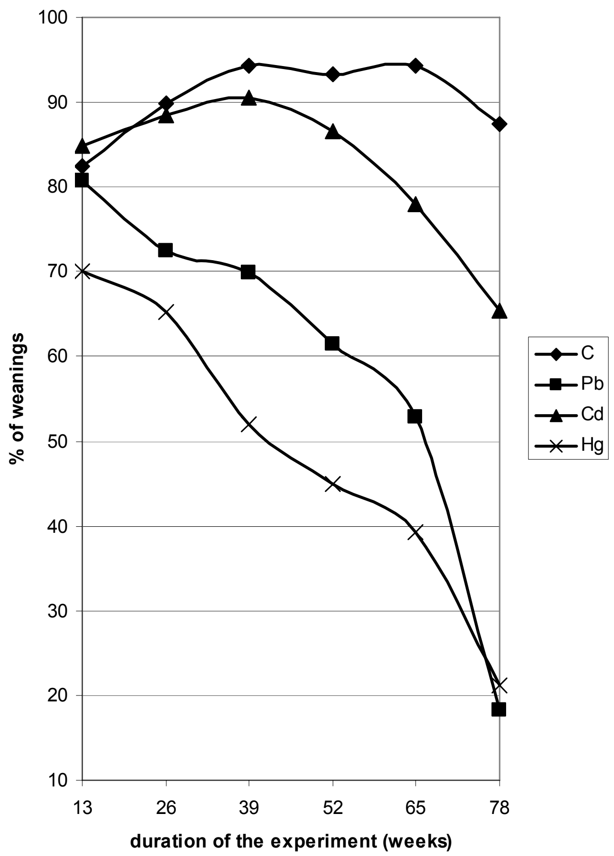

Fig. 3. Relative number of weanlings from total number of newborns.

The surprising observation was the transient but considerable rise in the total reproduction rate in groups exposed to all heavy metals. The order of the survival rate of newborns in these groups (Cd>Pb>Hg) was near inversely proportional to the absolute reproduction rate given by numbers of litters and young (Hg>Pb>Cd). Exposure to mercury thus induced the highest reproduction rate on account of lowest survival rate of offsprings. In the group intoxicated with cadmium the reproduction parameters were closest to the healthy control. The number of weanlings in absolute values or expressed as a ratio of the total number of newborns appears to be most practical marker as to the outcome of progeny in reprotoxicity tests.

In order to revaluate current data and to elucidate the mechanism involved in low-dose action of heavy metals on the reproduction capacity the present measurements have to be extended by additional biochemical, immunological and genetic analyses [15-20]. Detailed comparisons are planned in several directions, e.g.:

(a) the role of reactive forms of oxygen and metallothionein in genetic damage,
(b) the role and/or alterations of inherent and acquired immunity in response to chronic metal exposure or
(c) the tumorigenesis and carcinogenesis under longterm application of nontoxic doses of heavy metals.

We believe this initial study may provide a fertile ground for more detailed studies of the longterm effects of sublimit exposure to heavy metals and other toxins for the estimation of possible risks and preparation of the effective measures for their prevention.

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