REPRODUCTION PARAMETERS IN CHRONIC INTOXICATION WITH HEAVY METALS IN RATS

Agnesa Lukačínová¹, Roman Beňačka², Eva Lovásová², Emília Hijová³, František Ništiar²

¹Institute of Physiology, ²Institute of Pathological Physiology, ³Institute of experimental medicine, Medical Faculty, University of P.J.Šafárik, Košice, Slovak Republic

INTRODUCTION

In spite of many ecological regulations in recent years the environmental pollution and the containment of existing resources of fresh water become a live issue today more that ever before and this tendency cannot be accepted as a rule of future socio-economical reality require. Because the clean drinkwater is the basic requirement for the healthy life it is necessity that the health professionals and bioresearchers have to draw the public attention to the possible health risks and consequences of industrial and agricultural pollution of the air, soil and water resources (Bencko et al., 1995). Frequent cases of contaminations of groundwater reservoirs, surface irrigations and water resources with excessive concentrations of metals call for more precisely defined safety precautions and require adequate practical steps for their elimination.

Many aspects of acute intoxication by heavy metals including the genotoxic, teratogenic, metabolic and physiological effects are relatively well known and were the subject of several in depth papers and reviews. Surprisingly little data, however, is available about chronic exposition by low doses of various metals in multigeneration studies, even more, that this environmental condition may have an real health impact on population may have (Sutou et al., 1980; Paksy et al., 1996). Chronic life-long non-toxic contamination may produce many unpredictable consequences not only for those individuals which are directly exposed, but for their offsprings in next generations. Effective solution of the above problems focused our attention on the possible long-term reproductive consequences of the chronic sub-toxic pollution by heavy metals in mutigenerational experimental design. We believe this initial study may provide a fertile ground for further more detailed studies of the long-term effects of sub-limit expositions to metals and other toxins for the estimation of possible risks and preparation of the effective measures for their prevention.

The main purpose of the present study was to evaluate the selected physiological, reproductive and biochemical parameters during chronic (lifetime) exposition to low-doses of heavy metals (lead, cadmium, mercury) in Wistar rats...
in order to find the most suitable and precise indicators for the estimations of the risks of the chronic low-dose intoxications with various substances on healthy state of population.

**MATERIALS AND METHODS**

**Animals:** Experiments were on 80 Wistar albino-rats of either sex (40 females and 40 males, age 4 weeks, average weight 120±19 g) and their 28-days old newborns (older youngs were transferred into another experiment). The females and males were kept in couples in separate cages with free access to water and food and day-night regime 12:12 hours. The experiment was terminated on 56th week. 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 agency (ŠVPS SR Č.k. Ro-7879/04-220/3).

**Arrangement of experiment:** The animals were divided randomly into 4 groups (10 females and 10 males in each) Always 1 female and 1 male was kept in separate cage. While control rats (group I, n=10) did not received any additives into the drinking water 3 other groups were given metal-containing compounds diluted in drinking water in amounts such that the metal content was 200 – times higher than the maximal allowable concentration in drinking water (MAC). Group II (signed as Pb; n=10) received the water with 100 µmol/l of the lead acetate in alkaline solution (20,0 mg of lead/l), group III (Cd; n=10) was added 20 µmol/l of cadmium dichloride dihydrate (CdCl₂) (2,0 mg of cadmium/l) and group IV (Hg; n=10) was given the water with 1 µmol/l of mercurous bichloride (HgCl₂) (0,2 mg of mercury/l). 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 28th day of life) were measured in 13-week intervals.

**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 date were considered significant if P<0.05.

**RESULTS AND DISCUSSION**

In the all groups the female rats brought forth obviously from 13th to 78th week of experiment. When comparing the number of litters we found that the values were higher in the rats exposed either to Pb or Hg than those in the healthy unexposed animals (C) (C vs Pb, P = 0.0008; C vs Pb, P = 0.0006; (Tab. 1). Significantly lower values than those in Pb and Hg – groups were observed in animals exposed to Cd (Pb vs Cd (P = 0.0008); Cd vs Hg (P = 0.0027) which did not show significant difference from the control group (C vs Cd ; P = 0.0878)
Similar to the number of litters (Pb vs Hg, \( P = 0.0421 \)), the highest number of newborns was observed in Hg- group (n= 1015) followed by the group exposed to lead (n=853) (Pb vs Hg; \( P = 0.0172 \)) (Tab.1). In both these groups number of surviving newborn was much higher compared to control 754 (Pb vs C, \( P = 0.0003 \); Hg vs C, \( P = 0.0006 \)). The Cd-group showed markedly lower number of newborns (n=706) than Hg- and Pb- groups (Cd vs Hg, \( P = 0.0013 \); Cd vs Pb, \( P = 0.0004 \)) but significantly higher values when compared to control group (C vs Cd , \( P = 0.0366 \)). From 13\(^{th}\) to 39\(^{th}\) week of exposition the average number of newborns per litter was higher in all intoxicated groups compared to control in the following order Hg> Pb> Cd> C. Increases in the numbers of newborns were also found by the other authors experimenting with chronic exposition to heavy metals increased mortality throughout the first 2 weeks much higher doses (Sutou et al., 1980; Paksy et al., 1996).

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 off-set of the reproduction burst over the experimental period (78 weeks). Thus while Hg- and Pb- groups showed steep and continuous rise in number of litters and number newborns early after the beginning of experiment this was maintained only until 26\(^{th}\) week when it was followed by fast decline. Both the Cd- group and control group revealed low-grade sustained reproduction rate over the initial 39 weeks. On 52nd week the number of newborns in all groups was approximately the same and gradually declined until the end of 78th week. Most profound decline was noted in Pb-group.

Even that this parameter itself may be of partial value for any definitive conclusions when viewed in the context of other selected parameters (Tab. 1) they provide good prediction value for the reprotoxicity evaluation after heavy metal exposition.

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

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

There is good knowledge that gonads are the primary target for several environmental toxins (Sokol, 1997). From among heavy metals cadmium is well known by prominent inhibitory action on the testosterone production by interference with hypothalamic-hypophyseal-testis axis (Lafuente et al., 2001). Though many studies demonstrated repeatedly the inhibitory effects of the various toxins on the gonadal functions, one has to consider that the the timing and the doses obviously reported were much higher than those sub-toxic ones in the present work. The present work demonstrated that the very low concentrations...
of heavy metals given in chronic life-long manner may provide in the rats at least temporarily stimulatory effect on the 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 adaptive preconditioning effect which attenuate 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 exposition. This may suggest involvement of the stress-related self-protecting biological mechanism aimed to preservation of species similar to what can be observed in other life threatening situations.

Number of newborns in the litter (Fig. 1) is useful indicator of the reprotoxicity in general, particularly in longitudinal studies. Surprisingly, from the beginning until 39 week 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 quite similar but later there was very marked decline particularly in females exposed to metals in drink water.

The numbers of weanlings, 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 (p<0.05 – 0.001) (Tab. 1). Lowest number of weanlings was observed in Hg group (n=574) from the beginning until the end of experiment. Considerably lower numbers than in control (n =686) were also noted in Pb-group (n=599) and Cd-group (n=606) where the counts were comparable. The significant differences throughout the entire experimental period were in the following comparisons: C vs Cd (P = 0.0386); Pb vs Cd (P = 0.0444) a Pb vs Hg (P = 0.0003). When comparing relative numbers of survivors to total numbers of newborns (Fig. 2) the significant differences were noted in between following
groups: C vs Pb (P = 0.0217); C vs Hg (P = 0.0039); Pb vs Cd (P = 0.0022); Pb vs Hg (P = 0.0204) a Cd vs Hg (P = 0.0018).

CONCLUSIONS

There is a wealth of data on the morphological and physiological alterations after acute and chronic expositions by toxic levels of heavy metals as 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 sub-toxic exposition to heavy metals. 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 youngs (Hg>Pb>Cd). The exposition to mercury, thus, induced the highest reproduction rate on account of lowest survival rate of off-springs. 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 (Sato, Kondoh, 2002). Further more detailed comparisons are planned in several directions, e.g. a) a role of reactive forms of oxygen and metalotionein in genetic damage, b) the role and/or alterations of inherent and acquired immunity in response to chronic metal expositions (Singh et al., 2003; Biser et al., 2004) or c) the tumorigenesis and carcinogenesis under long-term application of non-toxic doses of heavy metals (Waalkes, 2003; Waisberg et al., 2003).

REFERENCES


Contact address:
Prof. MVDr. František Ništiar, CSc., Phone: +421 55 640 22 79
Department of Pathological Physiology Fax: +421 55 642 33 50
Faculty of Medicine, Šafárik University E-mail: nifran@central.medic.upjs.sk
Trieda SNP 1, 040 66 Košice, Slovak republic