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The effects of chronic mercuric chloride ingestion in female Sprague–Dawley rats on fertility and reproduction

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ABSTRACT

Thirty-days-old female rats were chronically exposed, for 60 days, to 1or 2 mg/kg/day of mercuric chloride or an equivalent volume of water, via gavage. At 90 days of age they were mated with unexposed males. At approximately day 13 of gestation necropsies were performed on the females. Data were collected on the number of implantations and non-viable implantations in the uterus. No physical signs of Hg intoxication were seen except in weight gain. There were significantly fewer implantations in the high $HgCl_2$ group, with significantly more non-viable implantations in the low and high $HgCl_2$ groups, compared to controls. Lower levels of progesterone and higher levels of pituitary luteinizing hormone (LH) were found in the high $HgCl_2$ group compared to controls, whereas pituitary follicle stimulating hormone levels (FSH), while not significant, showed a dose–response relationship to $HgCl_2$ levels. No difference was found in the number of corpora lutea. The experiment indicated low level chronic ingestion of mercuric chloride, in female rats, while not effecting ovulation, produced disruption of implantation and fetal viability. Lower progesterone levels, higher LH, and possibly FSH levels, indicate that mercuric chloride may have a disruptive effect in the corpora lutea which manifests itself after ovulation.

1. Introduction

Mercury (Hg) is a naturally occurring metallic element that is ubiquitous throughout the environment. It is present in several inorganic forms including metallic (Hg^0), mercurous (Hg^+), and mercuric (Hg^{2+}) valence states (Daintith, 1996). Metallic Hg is found in such household products as barometers, blood pressure instruments, and switches in automobiles (Davis et al., 2001). It is also found in older products such as switches in children's shoes that light up, thermometers, as well as being used frequently in dental amalgams. Mercuric Hg is found in fluorescent lights, (Davis et al., 2001), including the newer type that are replacing incandescent light bulbs.

Research on the reproductive effects of inorganic Hg is sparse although several studies in the 1980s documented reproductive problems of women working in Hg contaminated environments. These included reproductive failures, menstrual cycle disorders, primary subfecundity, and adverse pregnancy outcomes (De Rosis et al., 1985; Sikorski et al., 1985), however, a relationship with the level of Hg exposure was not always clear (De Rosis et al., 1985). A more recent study involving females in an Hg exposed work environment also revealed a higher frequency of adverse reproductive outcomes, especially congenital anomalies, (Elghany et al., 1997). Ovarian function in women can be compromised by exposure to toxic chemicals in the environment through direct effects on the ovaries or indirectly via the hypothalamus–pituitary–ovarian axis (Hoyer, 2005). Inorganic Hg in placental cord blood can be 20–65% higher than in maternal venous blood (Grandjean et al., 1992).

In animal models, exposure to inorganic Hg has been shown to cause anovulation in hamsters (Davis et al., 2001), and to show a prolongation of diestrus of up to 10 days in rats, with evidence of Hg in the ovaries (Stadnicka, 1980). A previous study in our laboratory in which both male and female rats were exposed to mercuric chloride ($HgCl_2$) and then mated together, revealed significant differences in implantation efficiency, fertility, live births, and litter size (Atkinson et al., 2001), however, because both males and females were exposed, it is hard to differentiate what proportion the males and females contributed to the differences in fertility and reproductive variables.

The evidence of fertility problems, in females, in Hg exposed work environments, together with the animal-based studies, demonstrates that inorganic Hg has adverse effects in human and

Abbreviations: FSH, follicle stimulating hormone; GD, gestation day; Hg, mercury; Hg^0, metallic; Hg^+, mercurous; Hg^{2+}, mercuric; $HgCl_2$, mercuric chloride; LH, luteinizing hormone; PD, postnatal day.

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rodent female reproduction. However, a study is needed to ascertain the effects of inorganic Hg on fertility, from chronic exposure, in concentrations that do not cause any physical signs, to see if these concentrations affect reproduction adversely.

2. Materials and methods

2.1. Subjects

Seventeen pregnant Sprague–Dawley rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN) at approximately gestation day (GD) 11. The animals were housed in individual polycarbonate cages on beta chip bedding (Northeastern Products, Warrensburg, NY), given access to Purina certified rodent chow 5001 (Purina Mills, St. Louis, MO) and water ad libitum, and allowed to come to term. Room conditions were controlled at 22°–23 °C ambient temperature, 50–60% relative humidity and 12 h light/dark cycle. All conditions conformed to the regulations of the Tuskegee University Animal Care and Use Committee.

At postnatal day (PD) 21 60 female offspring were separated and randomly assigned to one of three experimental groups, control, low Hg and high Hg. They were housed two animals per cage with cage mates belonging to the same experimental group. All subjects were given free access to food and water. At PD 30, subjects in the Hg groups started exposure to a solution of mercuric chloride ($HgCl_2$) in concentrations of 1.0 (low Hg), or 2.0 mg/kg/day (high Hg). All subjects (including controls) were weighed three times per week during exposure, which lasted for 60 days.

2.2. Preparation and administration of $HgCl_2$ solutions

Concentrations of 1.0 mg/ml and 0.5 mg/ml $HgCl_2$ were prepared. The 1.0 mg/ml solution was prepared using 1 g of mercuric chloride ($HgCl_2$, Sigma–Aldrich Inc, St. Louis, MO) added to a solution of 998.5 ml of de-ionized H_2O plus 1.5 ml of HNO_3 (nitric acid). The 0.5 mg/ml solution was similarly prepared with 0.5 g of $HgCl_2$. The amount of $HgCl_2$ administered each day, to each subject, was calculated based upon its weight and exposure group. The control group received an equivalent volume of de-ionized H_2O (with no HNO_3 added). Each subject was weighed three times per week and the dose was adjusted accordingly. The solution was administered seven days a week via gavage until the subjects reached 90 days of age.

2.3. Breeding protocol

Exposure to $HgCl_2$ ceased after 60 days, at which time 30 subjects (10 from each group) were transferred to hanging cages where they were mated with adult unexposed males. Females were examined each morning for vaginal plugs. If a plug was found, presence of sperm was confirmed by vaginal wash and the subject was transferred to its original cage. An unmated subject was transferred to the hanging cage with the same male and the procedure repeated. If no vaginal plug was observed within 10 days of mating the male was removed and replaced with a new male that had successfully mated previously with a female. This protocol was observed in order to preclude the possibility that a male may be infertile. The exposed females were euthanized at approximately gestation day (GD) 13, calculated from the day the presence of plug/sperm was confirmed.

2.4. Procedures

Subjects were anaesthetized by CO_2 inhalation, weighed and blood was collected, via cardiac puncture. Euthanasia was then completed with CO_2 inhalation. Blood samples were centrifuged at 3000 rpm for 10 min at 4 °C and plasma was stored at −70 °C until processed for progesterone levels. Ovaries were removed, fixed in 1% formaldehyde and examined for number of corpora lutea. The pituitary glands were also collected and stored in the same manner for future luteinizing hormone (LH) and follicle stimulating hormones (FSH) analysis.

2.5. Luteinizing hormone and follicle stimulating hormone analysis

After collection, the weight of each pituitary gland was recorded. At analysis each gland was placed in 1 ml homogenization buffer (phosphate-buffered saline containing 2.5 M urea; pH 7.4). It was then homogenized with 20 strokes in a dounce glass homogenizer followed by a freeze–thaw (−20 °C). Samples were sonicated for 60 s, followed by centrifugation at 16,000 rpm for 15 min at 4 °C. The supernate was decanted and frozen (−20 °C) until assayed for luteinizing hormone (LH) and follicle stimulating hormone (FSH). Pituitary gland content of LH was measured by radioimmunoassay at the Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO (primary Ab R15, standard NIDDKrLE-RP-3, National Hormone and Pituitary Programs, NIDDK), following homogenization (Tena-Sempere et al., 2004). Each sample was measured in duplicate. The lower limit of detection for this assay was 0.2 ng/ml. Pituitary gland content of FSH was also measured by the same laboratory (primary Ab NIDDK-anti-rFSH-S-11 and standard NIH-RFSH-RP2). The lower limit of this assay was 12.2 ng/ml.

2.6. Progesterone analysis

Plasma progesterone levels were measured using a Coat-A-Count kit according to manufacturer's protocol (Siemans, Tarrytown, NY). The intra-assay CV was 3% and the limit of detection was 0.1 ng/ml.

2.7. Statistical analysis

Body weight was analyzed as repeated measures ANOVA 3 ($HgCl_2$) × 8 (length of exposure) factorial design, with duration of exposure as the repeated measure. The first weight taken and every subsequent fourth weight taken were used as the repeated measure. All other variables were analyzed using ANOVA 3($HgCl_2$) × quantity or number of variable. When a significant difference was observed a post hoc analysis was performed to identify the differences between the groups. Results were considered significant at α level of 0.05.

3. Results

3.1. Body weight

Both a main effect of $HgCl_2$ ($F_{(2,56)} = 3.463$, $p = 0.038$) and an interaction between exposure concentration and exposure duration ($F_{(14,392)} = 2.887$, $p < 0.001$) revealed that the exposed groups had significantly less weight gain than controls. An analysis of weights taken on the last day of exposure also showed an overall significant difference ($F_{(2,56)} = 6.192$, $p = 0.004$) between groups. Post hoc analysis using a Bonferroni correction revealed a significant difference between the low Hg ($p = 0.015$) and high Hg ($p = 0.008$) compared to controls. Post hoc analysis using Fisher's Least-Significant-Difference test (FLSD) did reveal significantly lower weights in the high Hg group starting from day 9 of exposure ($p = 0.015$) and in the low Hg from day 30 of exposure ($p = 0.044$) compared to controls. However, it should be noted that FLSD is a less conservative test than Bonferroni. Table 1 gives a list of mean weights, the standard error, in grams, and whether significantly different from controls, for a particular day of exposure for all groups.

3.2. Implantations

A significant effect of $HgCl_2$ concentration was found for total implantations ($F_{(2,56)} = 3.404$, $p = 0.04$), and non-viable implantations, ($F_{(2,56)} = 6.46$, $p = 0.003$). Non-viable implantations were defined as sites on the uterus that showed signs of resorption or had not developed to a live fetus. Post hoc analysis (Bonferroni) indicated total implantations were significantly lower for the high Hg group ($p = 0.033$) compared to the control group, while the number of non-viable implantations in the high $HgCl_2$ group were significantly higher than the low group ($p = 0.047$) and controls ($p = 0.003$). The number of corpora lutea, a measure of ovulation rate, did not differ between controls and either of the exposed groups (data not shown).

3.3. Progesterone, luteinizing and follicle stimulating hormones levels

In the low Hg group the pituitary gland was unavailable for one subject, while a plasma sample was unavailable for a separate subject. Pituitary glands were unavailable for four subjects in the high Hg group.

A significant effect for plasma progesterone levels between exposure groups ($F_{(2,56)} = 3.924$, $p = 0.025$) was found. The mean level of progesterone in the high Hg group was 74.2 ng/ml which was significantly lower ($p = 0.019$) than the control group at 90.6 ng/ml (see Fig. 3).

Analysis of pituitary LH showed a significant difference between groups ($F_{(2,52)} = 3.244$, $p = 0.047$), with a mean concentration for the high Hg group at 2.3 μg/pituitary gland which was significantly higher than either the low Hg group at 1.9 μg/pituitary