RESEARCH PAPER:

Effects of maternal lead acetate exposure on prenatal development of swiss albino mice

SHEETAL MOGRA, RAGINI SHARMA AND NAZERA QURESHI

Asian Journal of Environmental Science (December, 2009 to May, 2010) Vol. 4 No. 2 : 216-220

See end of the article for authors' affiliations

Correspondence to :
SHEETAL MOGRA
Environmental Toxicology Research Lab, Department of Zoology, University College of Science, Mohanlal Sukhadia University, UDAIPUR (RAJASTHAN) INDIA

SUMMARY

In the present study the teratogenic effects of lead acetate on prenatal development of mice were investigated. Females aged 9-10 weeks weighing 25-30 g. and showing vaginal plug were selected and divided into two groups. These groups were (i) control (ii) lead exposed groups at different days of gestation- (a) from the 1st day of gestation (dg) (b) from the 8th dg and (c) from the 10th dg. In all, these groups lead acetate was administered orally in the dose of 8mg, 16mg, and 32mg/animal/day. At the end of 17th dg, their uteri were excised out for the examination. Result showed that implantation rate was approximately nil in the uteri of females which were exposed from 1st dg. Dual results were obtained when exposure was began from 8th dg .Most of ther female uteri showed only implantation site while some female uteri contained developed fetus but in very few number. The administration of lead on 10th dg did not affect the ability to conceive, to carry a normal litter. The percentage of malformed fetuses, resorptions were unaffected/ less affected on this day. Results suggest that exposure of lead acetate from 10th dg and there after, fetal toxicity (resorption) sharply declined. In conclusion, it is inferred that gestational lead exposure has an adverse effect on development, with an effect that may be most pronounced during the first trimester.

Key words :
Lead, Mice, Fetus, Prenatal, Gestation, Uteri, Implantation

Accepted :
November, 2009

Environmental lead toxicity is an old but persistent public health problem throughout the world and children are more susceptible to lead than adults (Ahamed and Siddiqui, 2007). Lead poisoning among pregnant women is a significant public health probleam, as it effects development(Katharina Weizsaecker, 2003). The development of a child begins in utero and continues following birth, thus, both of these time frames must be examined as possible periods of lead intoxication. During development, the fetus is at the mercy of its mother. If the mother has high blood lead levels during pregnancy, the developing fetus will have the same. This is due to the lack of a transplacental barrier to lead (Goyer *et al.*, 1990). Lead freely crosses the placenta consequently, gestational lead poisoning is not only harmful to the woman but also to the developing fetus (Shannon *et al.*, 2003).

The presence of lead in placenta indicates that lead moves from mother's blood erythrocytes in the intervillous space released and received by the villous syncytiotrophoblast. This finding enriches relation between mother's erythrocytes, lead, calcium that is a lead carrier and syncytiotrophoblast (Foltinova *et al.*, 2007). Lead can be incorporated into the bone structure of the mother as a result of previous lead exposure, up to thirty years before in some cases. Thus, whenever, net bone resorption occurs to increase blood calcium levels, lead may also be released into the circulation. During gestation, there are two such periods. The first is in the first trimester when maternal blood volume increases, thus increasing the need for calcium to hold a constant concentration. The second is the third trimester when fetal ossification begins, thus increasing the fetal requirement for calcium (Silbergeld *et al.*, 1991). Both cases can result in higher lead concentrations in the fetal blood. Maternal exposure to lead is more important during fetal development than during breast feeding (Dorea and Donangelo, 2006).Early gestational exposure of lead slightly delays the development of the embryo and inhibits its implantation (Jacquet, 1976) .Implantation is an intricately timed event necessary in the process of viviparous birth that allows mammals to nourish and protect their young during early development (Kevin and Franceso, 2004). Implantation is the process that leads from blastocyst attachment to its

embedding in the uterine wall. It is widely believed that failure of implantation is a common cause of pregnancy loss. Toxic agents can interfere directly with the process of implantation and therefore may account for unexplained implantation failures (Genbacev *et al.*, 1993). Classical signs of lead poisoning for pregnant women are spontaneous abortion. Manifestation in the fetus and newborn include prematurity, fetal hypotrophy and malformation (Klein *et al.*, 1994).

MATERIALS AND METHODS

The animals were bred in animal house under optimum conditions. The animal room was maintained at 22-25º C. with relative humidity of 50-60% and 12 hours light and dark cycles. Sexually mature (9-10 week) male and female mice, weighing 25-30g were kept in breeding cages in the ratio of 1:4 (one male and four females) and were provided standard diet and water *ad libitum*. The cages were checked everyday in the morning and females showing vaginal plug were isolated and duration of their gestation periods were recorded. The selected pregnant females divided in the following groups.

(1) Control group (2) Experimental (lead treated) groups.

Control group contained 6 animals while experimental group contained 27 animals. Experimental group was further divided into 9 different sub groups for 3 different days of pregnancy at 3 different doses. Each sub group contained 3 animals.

Group- I: Served as a control group (Treated with only distilled water)

Group-II: Served as experimental group. It was treated orally with freshly prepared solution of lead acetate with the help of canula.

Sub group 1: Exposure of 8 mg/ animal/ day lead acetate from 1st day of gestation (dg).

Sub group 2: Exposure of 8 mg/ animal/ day lead acetate from 8th dg.

Sub group 3: Exposure of 8 mg/ animal/ day lead acetate from 10th dg.

Sub group 4: Exposure of 16 mg/ animal/ day lead acetate from 1st dg.

Sub group 5: Exposure of 16 mg/ animal/ day lead acetate from 8th dg.

Sub group 6: Exposure of 16 mg/ animal/ day lead acetate from 10th dg.

Sub group 7: Exposure of 32 mg/ animal/ day lead acetate from 1st dg.

Sub group 8: Exposure of 32 mg/ animal/ day lead acetate from 8th dg.

Sub group 9: Exposure of 32 mg/ animal/ day lead acetate from 10th dg.

Control females were sacrificed on 6th dg, 9th dg and17th dg while females of experimental group were sacrificed at the termination of 17th dg. The existence of pregnancy was noted and the uterus was opened by a longitudinal incision. Their uteri were quickly dissected out and immersed in 2% NaOH solution for the examination. The following observations were taken which were considered as prenatal developmental toxicity (1) Pre-implantation loss (2) Post-implantation loss(3) Resorption (4) Fetal death (5) Retarded growth (6) Growth alteration (7) Structural abnormalities and these were compared with controls.

In all lead treated animals which were exposed from 10th dg at different doses, development of fetus take place. Viable fetuses were examined carefully at the termination of 17th dg for many different types of malformation, which are commonly classified into two malformation categories: (1) External (2) Visceral.

External malformations are those affecting morphological changes in the organism such as abnormal development, growth retardation etc.

Visceral malformations are those affecting internal organs such as the liver and kidney and are detectable only after histopathological studies.

RESULTS AND DISCUSSION

In control groups, normal development was observed without any alteration (Fig. i, iii and iv).

In lead exposed groups, prenatal development process altered as the day of exposure changed. In experimental groups lead acetate administered at equal doses, but at different phases of gestational period may produced essentially different types of abnormalities or functional deficits. Disturbances in the normal developmental cycle can lead to the early loss of the conceptus and embryo-fetal damage. The administration of lead acetate to female mice on 1st dg caused pregnancy failure (Fig. v). Most of female uteri showed no implantation site when dissected on day 17th of gestation while only 10% female uteri showed implantation site but they are not replica of the controlled animal. It indicates that exposure to lead not only alters the time of implantation but it inhibits the process of implantation.

If the treatment of lead started from the 8th dg, at the dose of 8, 16, 32 mg. / animal /day, their uteri showed certain implants but further development after implantation was inhibited (Fig. vi). In contrast to these findings, in few exposed females one or two viable fetuses were developed from implantation sites (Fig. vii).

When the females exposed from 10th dg in all females

complete fetal development was observed with few resorption sites (Fig. viii and Fig. ix).These results suggest that mice exposed to lead acetate at an early stage of gestation cause preimplantation loss, as the preimplantation process after fertilization does not take place. While exposure to lead acetate at a time of early organogenesis caused histological alteration but does not affect fetal development process.

The results of histopathological study of fetal kidney and liver indicate that due to toxic effects of lead, there

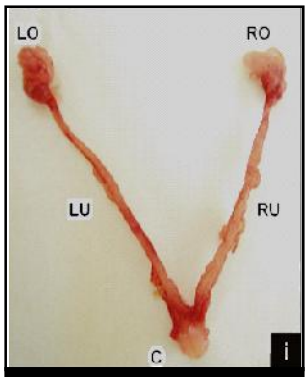

Fig. (i): Uterus of non pregnent female

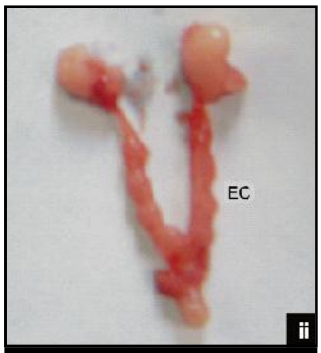

Fig.(ii): Control uterus on 6th day of gestation with implatation sites (IM)

Fig.(iii): Control uterus on 9th day gestation with embrayo (ES) fixed

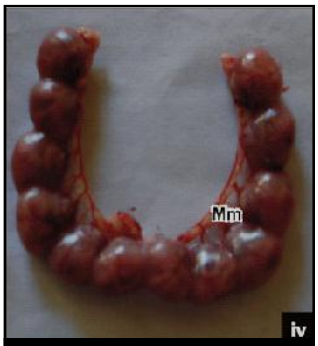

Fig.(iv): Control uterus day gestation

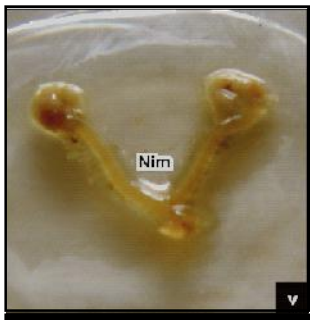

Fig.(v): Uterus of lead treated mouse exposed from 1st day of gestation immersed in NaOH. Both uterine hornswith-outimplants

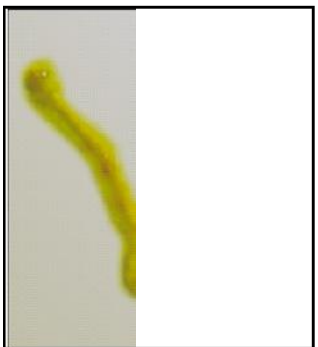

Fig.(vi): Uterus of lead acetate treated mouse exposed from 8th day gestation. Both uterine horn show implatation

Fig. (vii) : Uterus of lead treated mouse exposed from 8th day of gastation. Utrine horns with two viable fetuses (VF) and resorption (R) [in inset a : early b : late]

Fig.(viii) :Uterus on 17th day of gestation exposed with (16 mg/animal/day) lead acetate from 10th day of gestation. Both uterine horns contain developed fetus

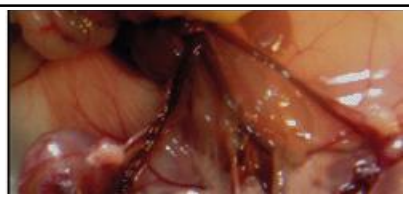

Fig.(ix) :Uterus on 17th day of gestation exposed with (32 mg/ animal/day) lead acetate from 10th day of gestation. Both uterine horn contain developed fetus and resorption [in inset]

are apparent alterations in the histological structure of these organs, although these results are not included in the present paper.

There is an accumulation of evidence, which indicates that maternal exposure prior to conception can play an important role in fetal development during pregnancy. Preterm delivery, congenital abnormalities and decrease in growth stature have all been associated with prenatal lead exposure.

Lead poisoning is a global public health problem as it effects developmental period (Ambrus *et al.*, 2001). The present study shows that exposure of lead from 1st dg caused preimplantation loss in female because it might be possible that metal are directly bind to critical membrane sites and/ or intracellular ligands, including protein and nucleic acid, may trigger inhibition of development and death prior to metal-associated oxidative

damage.

The administration of lead from the 1st day of gestation slightly delays the development of the embryo and inhibits its implantation. It seems that a deficiency in the progesterone levels is directly implied in this inhibition (Jacquet, 1976). The following developmental stages: the attachment of the blastocyst, the invasion of the trophoblast and the formation of the primitive streak were studied after intravenous administration of inorganic lead during preimplantation period of embryonic development in the mouse .Result indicates that all three stages were affected but the stage of invasion was found to be the most susceptible to lead (Wide and Nilsson,1977). Female mice, which displayed a vaginal plug after mating, were given lead acetate in diet and were dissected 16 to 18 days later. Lead treatment was found to reduce significantly the incidence of pregnancies and increase the post implantation loss in the pregnant females (Jacquet *et al.*, 1975). Disproportionately high plasma lead levels were also observed at early times after the injection of lead and may act as a significant factor in placental lead transfer and subsequent malformation or fetal mortality (Hackelt *et al.*, 1982).

In the present studies post implantation loss and only one or two viable fetus recorded when exposure was given on the 8th dg. The period of organogenesis is considered to be the most sensitive developmental phase to exogenous toxicants which can induce malformation. The results also demonstrate that the short-term exposure of female mice to heavy metal lead, during the early stage of gestation, would cause failure of pregnancy and in some cases produce fetotoxic or fetal resorptive potentials.

While in other case, when exposure was given from the 10th dg, no preimplantation and postimplantation loss were observed as recorded earlier on 8th dg exposure but at the higher dose level (32mg/animal/day) fetal resorption and general retardation of development were encountered. Fetuses derived from lead exposed females were examined grossly and no morphological alteration was evident but lead can cause visceral malformation in fetal liver and kidney which were observed in histopathological studies. It was assumed that the fetal stage is characterized by growth and functional maturation, thus the teratogen do not cause morphological defects but may cause functional abnormalities.

A normal diet containing 1.1% of ca or ca-deficient were given in female mice from the first day of pregnancy. Animals of two groups were injected intraperitoneal lead acetate at different times of the fetal organogenesis (8th, 9th, 10th, and 12th day of pregnancy). In the normal diet group, injection of lead increases the post implantation mortality and rate of skeletal anomalies among the fetuses (Jacquet and Gerber, 1979).

The agent applied; even at high dose during the predifferentiation period (from the time of fertilization through formation of the blastocyst) typically produce no teratogenic response (Generoso *et al.*,1988).

The results of present investigation show that in the embryonic stage, the cells undergo differentiation, mobilization and organization including organogenesis. As a result, the embryo becomes more susceptible to teratogen (10-14 days of gestation in rodents and 14th week in human). However, not all organs are susceptible in the same period of pregnancy.

Other studies also support that not only do embryos themselves have a sudden onset of susceptibility to teratogenesis but also each organ of an embryo has a sensitive period of teratogenesis (Wilson, 1973 and Desesso and Harris, 1996). In general, we can summarise that the susceptibility to teratogenesis decreases as differentiation and organ processed. This is because the proliferative and morphogenetic activities that characterize the early stages of the formation of tissues and organs become less prominent as the organ develops.

From present study it is clear that both the time of exposure and amount of teratogen affect the development process. But the mechanism through teratogen causing teratogencity is very important. Lead toxicity leads to free radical damage via two separate, although related pathways: (1) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide and (2) the direct depletion of antioxidant reserves.In any biological system, oxidative stress (OS) can arise as result of excessive production of free radicals .The oxidant status can influence early embryo development by modifying the key transcription factors (Dennery, 2004).

Another aspect which affects the development may be hormonal imbalance. The reproductive axis is particularly sensitive to lead during specific developmental period. The mechanisms underlying this appear to involve lead actions on both LH release and gonadal function (Ronis *et al.*, 1998). The inhibition of implantation caused by lead seems to be due to mainly an action of this metal on the hormonal balance of the exposed mother (Jacquet, 1978). The implantation failure may be due to an effect of lead on uterine responsiveness to ovarian steroids (Wide, 1980).

The present experimental results suggest that during pre-differentiation stage, the embryonic cells multiply and differentiate at high rates so that the embryo is more susceptible to teratogenic agents, which will either cause

death of the embryo or produce no apparent effect on the embryo.

Acknowledgement:

Authors are thankful to the Department of Zoology, College of Science, M.L.S. University, Udaipur for providing necessary facilities for the present piece of research work.

Figure: Abbreviations:

RU: Right uterus, Mm: Mesometrial membrane E: Embryo, LU: Left uterus, Bv: Blood vessel R: Resorption, RO: Right ovary. Nim: No implantation P: Placenta, LO: Left ovary, IM: Implantation site VF: Viable fetus, C: Cervix, ES: Embryo sac AS: Amniotic sac

Authors' affiliations

RAGINI SHARMA AND NAZERA QURESHI, Environmental Toxicology Research Lab, Department of Zoology, University College of Science, Mohanlal Sukhadia University, UDAIPUR (RAJASTHAN) INDIA

REFERENCES

Ahamed, M. and Siddiqui, M.K. (2007) Environmental lead toxicity and nutritional factors. *Clin. Nut.,* **26** : 400-408

Ambrus, C.M., Anthone, S., Stadler, A., Cameron, M.S., Wells, K., Stadler, I. and Ambrus, J.L. (2001). Treatment of lead poisoning with an immobilized chelator comparison with conventional therapy. *Res. Commun. Pathol. Pharmacol*, **10** : 253-263.

Dennery, P.A. (2004). Role of redox in fetal development and neonatal diseases. *Antioxid Redox Signal*, **6** : 147-153.

Desesso, J.M. and Harris, S.B. (1996). Principles underlying developmental toxicity. In: Fan, A.M., and Chang, P., (ed) *Toxicology and risk assessment*, Markel Dekker, New York.

Dorea, J.G. and Donangelo, C.M. (2006). Early (in uterus and infant) exposure to mercury and lead. *Clin. Nut.,* **25**: 369-376.

Foltinová, J., Foltin, V. and Neu, E. (2007). Occurrence of lead in placenta—important information for prenatal and postnatal development of child**.** *Neuro. Endocrinol. Lett.,* **28** : 335-340.

Genbacev, O., White, T.E., Gavin, C.E. and Miller, R.K. (1993). Human trophoblast cultures: Models for implantation and peri-implantation toxicology. *Reprod Toxicol*, **7**: 75-94.

Generoso, W.M., Rutledge, J.C., Cain, K.T., Hughes, L.A. and Downing, D.J. (1998). Mutagen induced fetal anomalies and death following treatment of female within hours after mating. *Mutat. Res.,* **199** : 175.

Goyer, R.A. (1990). Transplacental Transport of Lead. *Environ. Health Perspect*, **89**: 101-105

Hackelt, P.L., Hess, J. and Sikov, M.R. (1982). Effect of dose level and pregnancy on the distribution and toxicity of intravenous lead in rat. *J. Toxicol Environ Health*, **9** : 1007-1020

Jacquet, P. (1976). Effect of lead administered during pregnancy to C57BL mice. *C R Seances Soc. Biol. Fil.,* **170** :1319-1322.

Jacquet, P. (1978). Effect of exogenous progesterone and estradiol on the process of embryonic implantation in lead intoxicated female mice. *C.R. Seanes Soc Biol Fil.,* **172** : 1037-1040.

Jacquet, P. and Gerber, G.B. (1979). Teratogenic effects of lead in the mouse. *Biomedicine*, **30** : 223-229.

Jacquet, P., Leonard, A. and Gerber, G.B. (1975). Embryonic death in mouse due to lead exposure. *Experientia*, **31**: 1312-1313.

Katharina Weizsaecker, M.D. (2003). Lead toxicity during pregnancy. *Primary care update for OB/GYNS*, 10: 304-309.

Kevin, Y.L. and Francesco, J.D. (2004). Animal models of implantation. *Reproduction*, **128** : 679-695.

Klein, M., Kaminsky, P., Barbe, F. and Due, M. (1994). Lead poisoning in pregnancy. *Press Med.,* **23**: 576-580.

Ronis, M.J., Gandy, J. and Badger, T. (1998). Endocrine mechanisms underlying reproductive toxicity in the developing rat chronically exposed to dietary lead. *J. Toxicol. Environ. Health A.,* **54** : 77-99.

Shannon, M. (2003). Severe lead poisoning in pregnancy. *Ambul. Pediatr.,* **3** : 37-39.

Silbergeld, E.K. (1991). Lead in bone: Implications for toxicology during pregnancy and lactation. *Environ. Health Perspect.,* **91**: 63-70.

Wide, M. (1980). Interference of lead with implantation in the mouse: Effect of exogenous oestradiol and progesterone. *Teratology*, **21**:187-191.

Wide, M. and Nilsson, O. (1977). Differential susceptibility of the embryo to inorganic lead during periimplantation in the mouse. *Teratology*, **16** : 273-276.

Wilson, J.G. (1973). *Environment and birth defects*. Academic Press, New York.