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The Underlying Mechanism of Lead-induced Placental Injury

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ABSTRACT: Lead (Pb+) is a toxic heavy metal with high affinity to neurons and placenta. This review mainly focused on the lead placental toxicity. Placental lead exposure in rats leads to placenta weight loss, trophoblast hyperplasia, vascular congestion, increase of intracellular spaces and deposits of hyaline material of perivascular predominance, accompanied with the distention of rough endoplasmic reticula and reduction of the ribosomal number on membranes. All pathological presentations are consistent with molecular chemistry changes. NO and NOS levels were significantly higher in rats with lead exposure. However, extensive period of lead exposure may decompensate the host regulation, subsequently increase fetal-placental vascular resistance, leading to decrease in placental perfusion and then fetal hypoxia-ischemia; MDA level was significantly higher in the placenta of lead exposed than the control group ($P<0.05$). Thus, it is believed that imbalance of oxidation and antioxidant in placenta following lead exposure contributed to placenta damage. The expression of MMP-9 in the placentas was significantly higher in the lead exposed group than the control. We also found the positive correlation between lead poisoning and the expression of TIMP-1 on placental trophoblasts, suggesting that lead exposure during pregnancy causes imbalance of MMP-9/TIMP-1 expression, and subsequently resulting in reduced trophoblast invasion, shallow implantation, vascular remodeling barriers. The expression of nuclear factor NF-κB and thrombomodulin were significantly higher in the lead exposed placen- ta. than the controls. The activation of NF-κB can trans-activate the epidermal growth factor, platelet derived growth factor expression, and promote smooth muscle cell proliferation, vascular remodeling in membrane signal transduction, thereby increase the thickness of the collagen fibers of small arteries and vascular wall, enhance vascular spasm contraction, increase mean arterial pressure, and finally result in pregnancy hypertension. The abnormal, expression of thrombomodulin suggests lead exposure during pregnancy causing placental vascular endothelial injury, causes placental vascular endothelial injury. In a word, lead exposure during pregnancy causes pathological presentations and molecular chemistry changes. Thus may lead to premature birth, low birth weight, and mental retardation.

Key words: Lead poison; Placenta; Maternal; Reproductive Outcome

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Introduction

Lead (Pb+) is a toxic heavy metal with high affinity to neurons and placenta. It does not serve any physiological function. Lead accumulation in the body beyond a certain threshold will lead to multisystem organ damage. Lead exposure during pregnancy may lead to fetal premature birth, low birth weight, and mental retardation[1]. The placenta is important for fetus to obtain nutrients during gestation. Any modification of placental structure/function, caused by exposure to harmful environment, may compromise the health status of the newborn with long-term consequences. Placenta does not provide strong maternal-fetal barrier to lead during pregnancy. Subsequently 90% of lead in the maternal blood circulation will pass to the fetus, supported by the finding that there is correlation between fetal and maternal lead levels. Furthermore, there is also a negative correlation between cord blood lead level and neonatal birth weight, height and head circumference. Lead can be transported through placenta during the whole gestation period[2], The placental structural changes during pregnancy and toxicological analysis have been used to evaluate the effect of environmental pollution to human[3]. Most of published data focused on the biochemical effects of lead poison in mothers and children, but few on lead placental toxicity. This review will give an on the effect of lead toxicity in the reproductive system, the embryo, and subsequent teratogenic.

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1 Placen tal macroscopic and microscopic changes during lead poisonin

Material exchange thro ugh placenta depends on molecular weight, placental permeability, blood flow and the size of placenta Placental lead exposure led to reduced placenta weight, and trophoblast hyperplasia, vascular congestion, increase of intracellular spaces and deposits of hyaline material of perivascular predominance in pregnant mice[4]. In previous study, it was found that lead exposure reduced placental weight and size, causing a dark red appearance[5]. Lead exposure over entire pregnancy in rat caused macroscopic placentas swelling and congestion, fetal membrane surface opacity and dimness. Furthermore, the baseband swelling, focal necrosis, lymphocytes/plasma cells infiltration was observed. On trophospongium there was increased trophoblast giant cells and vacuolated cells; labyrinth expansion and congestion, degenerative the trophospongium Trophoblast and some trophoblastic cells, irregular-shaped nucleus of nuclear, deeper staining, the nu-

cleolus disappearance, intranuclear inclusions with some nuclei; Swelling, thickening and degenerative changes with the villi, the gap narrowing, increased fibrin deposition around the villi, increased ischemic necrosis on the maternal side of placenta. In addition, by using electronic microscope, it has been detected that there was shortened microvilli around the trophoblast with reduced but swollen mitochondrion, as well as decreased rough endoplasmic reticulum and ribosomal number on membrane. It was suggested that lead might impair placental function, particularly in trophoblast cells. Such changes may affect the blood circulation and material exchange in the placenta between mother and fetus, subsequently leading to compromised growth and development of fetus.

2 In vitro experiments addressing the molecular changes of lead-exposed placenta

2.1 Nitric oxide (NO)Nitric oxide synthase (NOS) change of lead-exposed placenta

NOS, the most important coenzyme to produce NO, catalyses arginine and oxygen to generate guanidine acid and NO. NO is an important factor in enhancing hemodynamic exchanges during pregnancy to meet the demand of fetal blood supply for nutrition and oxygen. NO increases significantly in normal pregnancy. Increased NOS activity and NO production reduce resistance between fetal-placental circulation, and it ensured sufficient nutrient supply to fetus. It has been demonstrated that NO and balance of the NO/NOS are important to embryonic development and differentiation in early stage of pregnancy [6]. Placenta contains mostly endothelial NOS (eNOS), which is presented in the trophoblasts and endothelial cells, especially syncytiotrophoblast. Secretion of eNOS in the placenta increases in the presence of umbilical cord inflammation to reduce vascular resistance as well as platelet accumulation and leucocyte infiltration around the placental villi and chorionic space[7].

Li demonstrated that NO and NOS levels were significantly higher in rats with lead exposure at either early or late pregnancy than controls group ($P < 0.01$), but not in the rats with lead exposure throughout pregnancy compared to the control ($P > 0.05$) [8]. These data suggest that increased NO and NOS provide compensatory mechanism to resist the invasion of harmful factors, might protect tissue damage from lead exposure. However, extensive period of lead exposure may decompensate the host regulation. It can be speculated that decreased NOS activity in the placenta from lead poisoning reduced NO production, subsequently increasing fetal-placental vascular resistance, leading to decrease in placental perfusion and then fetal hypoxia-ischemia. This study in the animals was supported by the finding in human. Li and coworker found that there was no significant difference of distribution of inducible nitric oxide synthase (iNOS) and eNOS in the placentas between high and low lead exposed pregnant women [9]. Interestingly, there was significantly higher expression of the iNOS and eNOS in the placentas from the high level blood lead women than that of low blood lead group ($P < 0.05$). It is suggested that iNOS and eNOS play an important compensatory role in the placentas during pregnancy. De-compensation of iNOS and eNOS in the placenta might lead to fetal maldevelopment via ischemia and hypoxia.

2.2 Lead induced oxidative damage to placental tissue

It is well know that Lipid peroxidation (LPO) causes tissue damage. Maternal hypoxia during pregnancy causes imbalance of placental oxidation environment, apoptosis, TNF and increased production of vasoconstrictor substances. LPO and antioxidant activity is upregulated during normal pregnancy, duing to high metabolism which in turn increases the production of more peroxidized lipid. Malondialdehyde (MDA), a product of LPO, reflects directly the level of LPO and indirectly the severity of tissue/organ damage in the body caused by free radicals. Thus the level of MAD correlates tissue free radicals injury [10]. Superoxide dismutase (SOD), another free radical scavenging enzymes, scavenges superoxide anion radicals to protect cells from damages. SOD provides vital role in balancing of oxidation and antioxidant. SOD compensates LPO production in response to different challenges. The activity of SOD reflects indirectly the ability to eliminate oxygen free radicals and endogenous antioxidant capacity.

Li discovered that MDA level was significantly higher in the placenta of lead exposed than the control group ($P<0.05$)[11], with trend of increased SOD level ($P>0.05$), which is consistent with the findings by Villeda-Hemandez J that low-level lead exposure induced vascular congestion and lipid fluorescent substances increase in the placenta of rats [12]. Thus, it is believed that imbalance of oxidation and antioxidant in placenta following lead exposure contributed to placenta damage. It is important to emphasize that there is more severe placenta injury in lead exposure at early pregnancy.

2.3 The expression of matrix metalloproteinase (MMP) -9 and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) in lead-exposed placenta

MMPs is a group of zinc dependent proteolytic enzymes with the property of degrading extracellular matrix (ECM). MMP is an important enzyme in endometrial stromal degradation by degrading nearly all the matrix components for embryo implantation, placenta formation and remodeling of uterine spiral arteries during early pregnancy. It has been reported that extravillous trophoblasts showed similar characteristics with cancer cells, by secreting substantial amounts of MMP-9 to selectively hydrolyze endometrial stromal and basement membrane components [13]. MMP-9 is a rate-limiting enzyme in the process of embryonic trophoblasts invasion and is the most important MMPs secreted by trophoblasts.

The expression of MMP-9 in the placentas is significantly higher in the lead exposed group than the control, particularly at the third trimester of pregnancy. Interestingly, the lowest MMP-9 is detected in placenta from the group with lead exposure throughout pregnancy among all the lead exposed groups. The findings invite speculation that host upregulated the expression of MMP-9 in

the placenta from lead exposure may promote trophoblasts invasion to reduce abortion. There is a correlation between blood lead level and placenta destruction. The morphological change in the de-compensated placenta included that trophoblasts necrosis, swelling or drop off microvilli; swelling and decrease mitochondrial, roughing endoplasmic reticulum pool expansion, and reduction of the expression of MMP-9[14].

Decreased MMP-9 placental expression can directly lead to shallow implantation, uterine spiral arteries remodeling disorder, which in return result in inadequate blood, thereby affecting fetal growth and development. The dynamic balance of their expression is physiologically important to pregnancy. TIMP-1 has a higher affinity with MMP- 9, it can combine with MMP-9 in 1:1 ratio and forms MMP-9/TIMP-1 complex, which in result prevent MMP-9 from binding the substrate[15]. In previous study[16] on lead-exposed rats in different stage of pregnancy, it was found the positive correlation between lead poisoning and the expression of TIMP-1 on placental trophoblasts, suggesting that lead exposure during pregnancy causes imbalance of MMP-9/TIMP-1 expression, and subsequently resulting in reduced trophoblast invasion, shallow implantation, vascular remodeling barriers.

2.4 The expression of nuclear factor NF-κB in the lead-exposed placenta

NF-κ B is a key transcription factor It is confirmed that it is widespread in eukaryotic cells and can bind with a variety of gene promoter or enhancer sequence-specific sites specifically to promote transcription and expression, and is closely related to inflammation, immune response, cell proliferation, transformation and apoptosis and other important pathophysiologic response. A study confirmed that NF-κ B can regulate the release of inflammatory factor of uterine tissue: TNF, IL-6, IL-8. NF-kB activation is particularly critical to the damage caused by acute inflammation and serious illness caused by the damage [17]. When inflammatory diseases occur, the NF-κ B can be activated to induce the expression of cytokine, chemotactic factor, adherence factor, matrix metalloproteinases (MMP), cyclooxygenase 2 (cox2) and iNos. In addition, the activation of NF-κ B can trans-activate the epidermal growth factor, platelet derived growth factor expression, and promote smooth muscle cell proliferation, vascular remodeling in membrane signal transduction, thereby increase the thickness of the collagen fibers of small arteries and vascular wall, enhance vascular spasm contraction, increase mean arterial pressure, and finally result in pregnancy hypertension[18].

Wang and coworker reported placental expression of NF-κ B in lead-exposed rats was significantly higher than the controls, which is supported by the findings that the expression of NF-κ B in human placenta was positively correlated with maternal blood lead levels ($r = 0.663$, $p < 0.01$)[19]. It was concluded that lead exposure at various gestational periods produced varied effects, with NF-κ B activation following lead exposure. Injury to cytoplasmic organelles of placental may interfere with the nutrition and oxygen exchange between mother and fetus, which may contribute to abnormal pregnancy outcomes, such as preterm delivery.

More recent studies [20-22] showed that pregnant women with blood lead levels ≥ 10 μg/dl was at a greater risk for pregnancy hypertension, premature delivery and intrauterine growth retardation than those with lower blood lead level, and their filial generation was at risk for nervous system damage, because the NF-κ B was an important signal transcription factor in regulating cellular response when nervous system was damaged.

2.5 The expression of thrombomodulin in the lead exposed placenta

Thrombomodulin (TM) is a glycoprotein in vascular endothelial cell surface and is considered as one of the specific molecular markers of endothelial cell injury. Lung tissue has the highest content of TM, followed by placental tissue. The study in Ma's group showed that TM expression in rat placentas exposed to lead in early and late pregnancy was lower than that in the control group[23], while the TM expression in placentas exposed to lead throughout pregnancy was significantly higher than that in the control. Thus it was believed that lead exposure during pregnancy causes placental vascular endothelial injury, making the TM drop off the surface of the vascular endothelial cells and trophoblasts, resulting in consumptive intravascular coagulation, thereby affecting placental function and fetal development. This is in line with the findings that there were high TM expression, decreased placental trophoblastic cells, damaged vascular endothelial cells, and vascular thrombosis in pregnant women with intrauterine growth retardation [24] that there were high TM expression, decreased placental trophoblastic cells, damaged vascular endothelial cells, and vascular thrombosis in pregnant women with intrauterine growth retardation. In summary, collection of placentas is non-invasive, and determination of lead content in placenta is a convenient method to recognize fetal-maternal body burden. Understand lead toxicity to placenta contributes to prevent lead pollution and mother-child health care.

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铅致胎盘损伤机制的研究进展

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摘要：铅是一种嗜神经和嗜胎盘的毒性重金属,本综述主要是关于铅的胎盘毒性。孕期铅暴露可以导致胎盘重量减轻,滋养层增生,血管堵塞,细胞间隙增宽,血管周围大量的纤维蛋白沉积,以及粗面内质网扩张,膜上核糖体数量减少。当孕期铅暴露在一定范围内时,一氧化氮(NO),一氧化氮合酶(NOS)水平升高,以保证胎盘组织器官的正常结构和功能;进一步加重时,NO 及 NOS 反而降低,导致胎儿 - 胎盘循环阻力增高,胎盘灌注量下降;孕期铅暴露时,丙二醛(MDA)升高,说明存在胎盘氧化与抗氧化系统平衡失调;基质金属蛋白酶 -9(MMP-9)表达降低,而基质蛋白酶组织抑制因子 -1(TIMP-1)表达增强,胎盘 MMP-9/ TIMP-1 的表达失衡,导致滋养细胞浸润能力减弱,胎盘着床过浅,血管重铸障碍,从而影响胎盘发育及胎儿生长;染铅胎盘 NF-kB 的表达及血栓调节蛋白(TM)的表达明显高于对照组。NF-kB 的激活又可以反式激活表皮生长因子,血小板生长因子等的表达,促进血管平滑肌细胞的增殖,细小动脉胶原纤维增加,血管痉挛性收缩;TM 表达异常,说明孕期铅暴露可致胎盘血管内皮细胞损伤。总之,孕期铅暴露可引起胎盘病理及一系列的分子化学改变,从而影响胎盘功能和胎儿发育,可引起子代早产、出生体重低、智力障碍等。

关键词：铅;母体;胎盘;子代结局

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