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The effects of selenium on fetal growth and development in CD-1 mice exposed with mercury for the gestation period

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임신 중 수은을 섭취한 CD-1 마우스 태아의 성장발육과 기형발생에 미친 셀레늄의 효과

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초 록 : 기형발생을 나타내는 수은의 독성에 대하여는 이미 보고된 바 있으나 임신중에 폭로된 수은의 독작용을 완화시키거나 치료할 수 있는 제재들에 대하여는 별로 알려진 바가 없다. 수은에 대한 셀레늄의 치료적인 상호작용에 대하여는 이미 보고된바 있으나 태아에 대한 효과를 평가하기 위하여 이 연구는 임신한 CD-1 마우스에 20ppm 농도의 유기수은을 음수를 통하여 투여한후 동시에 sodium selenite(체중 kg당 0.1mg, 0.2mg, 0.3mg, 피하주사) 또는 BAL(체중 kg당 5.0mg, 피하주사)을 임신 6일째부터 15일 사이에 단독 또는 병용 투여한 후 임신 18일째에 제왕절개술을 실시하였다. 수은단독 투여군에서는 태아의 체중 및 두부-둔부의 길이 그리고 태반의 무게가 투여를 하지 않은 대조군에 비하여 유의성있게 감소하였고 태아의 사망/재흡수 발생과 기형발생(특히 구개열)이 현저히 증가하였다. 셀레늄과 BAL의 단독 또는 병용투여는 수은에 의하여 발생된 태아에 대한 독작용을 억제하거나 감소시키고 기형발생 역시 유의성있게 감소시켰으나 투여용량 그리고 투여계획과는 특이한 상관관계가 발견되지 않았다. 모체의 수은중독 결과로 보이는 모체의 음수, 사료섭취량과 모체의 상대적 기관(간장, 신장, 비장) 무게의 감소도 셀레늄과 BAL의 투여로 증가되었다.

이 연구 결과는 세레늄을 단독 또는 BAL과 같은 기존치료제와 함께 투여할 경우 수은에 의한 기형발생이나 모체의 아급성 중독 증세를 감소시키거나 완화시킬수 있는 효과가 있음을 보여주고 있다.

Key words: mercury, selenium, teratogenecity, embryotoxicity, therapeutic effects

Introduction

Mercury and organomercurials have been used extensively throughout the world. Major mercury-consuming industries are those which manufacture electrical apparatus and general laboratory instruments, as well as those which use mercury as a catalyst in the chemical precessing and synthesis of various organomercurials.[1]

A significant proportion of the mercury found in

foods, especially in fish, is in the form of methylmecury complexes.[2] On the other hand, several reports indicated a high accumulation of inorganic mercury in animals given methylmercury, due to biotransformation.[3-8]

The toxicity of methylmercury has been well documented. Methylmercury causes a neurologic disease affecting many species, including man.[9-11] Neonates have absorbed significant amounts of mercury after the breakage of elemental mercury switches in their incubators.[12] The children congenitally affected after maternal ingestion of methylmercury-contaminated foods develope severe neurological symptoms.[13,14] Although the effects of methylmercury on fetuses have been well documented[15-17], little is known about their effects on early mammalian embryos.

Although the use of BAL(British Antilewisite; dimercaprol), a conventional antidote for mercury poisoning, is indicated in severe inorganic mercury poisoning, the effectiveness of BAL in organic and elemental mercury poisoning is highly questionable. Dimercaprol is also ineffective in reversing chronic organic toxic effects.[18]

Selenium(Se) is an essential trace metal that facilitates the lowering of tissue peroxide levels in the body by destorying hydrogen peroxide through the action of the selenium-containing enzyme, glutathione peroxidase. Biological effects of selenium under the dificiency or replenishment condition were studies in both animals and human.[19] Although animal toxicity of selenium is common, human toxicity is rare. Chronic elemental seleniun exposure can cause dental caries, but adverse effects are unusual.[20] Selenious acid is the most toxic form of selenium and usually causes fatalities.[21] However, no information is available concerning the effects of selenium on the teratological changes induced by mercury.

In the present experiment, therapeutic effects of selenium and differences depended upon treatment regimen were investigated following treatment with methylmercury with/without conventional antidotes on the pregnant mice.

Materials and Methods

Six to 8 week-old virgin female CD-1 mice (Laboratory Animal Center, Seoul National University), weighing 25-30 gm, were housed under conditions of constant temperature and humidity and a 12 hr light/dark cycle. After a 7 day acclimatization feamle mice were mated with male mice of the same stock for approximately 2 house (8 a.m. to 10 a.m). The presence of a vaginal plug was considered evidence of copulation and represented day 0 of pregnancy. Water and lab feed were available ad *libitum*.

Pregnant mice were exposed to 20ppm of commercial-grade methylmercuric chloride(MeHg) (Wako Pure Chemical Industry Ltd., Osaka, Japan) Purity:98%) in the drinking water from day 6 to 15 of gestation period with/without therapeutic agents (selenium, BAL or combined). The dosages of sodium selenite(Na_2SeO_3) (Sigma, PO Box 14508, St Louis, Mo 63178, USA) administered subcutaneously are 0.1, 0.2 and 0.3mg/body weight(b.w.), kg and 5.0 mg/kg b.w. for BAL(Sigma, PO Box 14508, St Louis, Mo 63178, USA). Combined treatements were consisted of BAL(5.0mg/kg) and sodium selenite(0.2 and 0.3mg/kg). The mice were weighed on day 0,6,10,15, and 18 of gestation. Water and feed consumptions were also recorded throughout gestation.

On day 18 of gestation the fetuses were removed by caesarian section, examined for external malformations, sexed, weighed, and crown-rump lengths determined. Two-thirds of the fetuses were fixed in Bouin's solution in preparation for visceral examination by the Wilson razor blade sectioning technique.[22] The remaining one-third of the fetuses were fixed in 95% ethanol, cleared, and stained in alcian blue and alizarin red S in a modification of Staples[23] and Schnell. In addition, placental weights and maternal major organ weight were determined. Relative maternal organ weights were calculated based on terminal body weight minus the contribution of the gravid uterus.

The percentage of malformed fetuses was analyzed by Kruskal-Wallis test with multiple comparisons.[24]

The relative organ weights were analyzed by one-way analysis of variance of arcin-tranformed data[25] and Scheffe's test.[26] All remaining dta were analyzed by one-way analysis of variance.[25] The statistical unit was the litter. Statistical significance was $p<0.05$.

Results

The effects of gestational exposure to mercury with/without therapeutic agents (BAL, sodium selenite or combined) on reproductive parameters are shown in Table 1. The number of live fetuses per litter was reduced following gestational exposure at all treatment groups although there were significantly differences among experimental groups depend on the regimen of treatment compared to no treatment animal. The number of implantation was not affected by mercury exposure and administration of chemicals. Exposure to mercury during gestational period increased number of dead fetuses and reabsorption approximately to 110% of no treatment animals. Even though all therapeutic agents reduced number of dead fetuses and reabsorption, higher doses were less effective. Especially, groups treated with combined agent(BAL+selenium) showed higher incidence rate around 200% than that from sodium selenite alone treatment.

As indicated in Table 1, exposure of mercury significantly decreased fetal growth parameters(fetal weight and crown-rump length) compared to the control. Most of treatment groups with selenium or BAL were effective to recover fetal growth parameters to normal levels, however, a group treated selenium alone(0.3mg/kg) showed no difference after treatment. Similar effects following treatment of therapeutic agents were observed from changes of plancental weight.

Table 2. Effects of gestational treatment with selenium on the incidence of malformed fetuses in CD-1 mice exposed with mercury

Treatment	%Malformed fetuses[a]	Incidence of malformation	
		Cleft palate	Other
Control	1.0±1.0(1)	0	1
Hg	15.7±2.4[d]	8	7
Hg+Se0.10	5.5±1.3(5)[c,f]	2	4
Hg+Se0.20	8.0±3.0(4)[d,f]	1	4
Hg+Se0.30	11.0±3.0(10)[c,f]	4	8
Hg+BAL5.0	6.8±2.0(7)[c,f]	2	7
Hg+BAL+Se0.2	9.1±1.8(8)[d,e]	2	8
Hg+BAL+Se0.3	8.5±2.5(7)[c,f]	2	5

Se0.1= sodium selenite 0.1mg/kg bw, Se0.2=sodium selenite 0.2mg/kg bw,
Se0.3= sodium selenite 0.3mg/kg bw, BAL5.0=BAL 5.0mg/kg bw
[a]Values expressed as mean±SEM (# of fetuses); n=8-10 litter
[b]Significantly different from controls ($p<0.01$).
[c]Significantly different from controls ($p<0.05$).
[d]Significantly different from controls ($p<0.001$).
[e]Significantly different from mercury only treatment ($p<0.01$).
[f]Significantly different from mercury only treatment ($p<0.05$).

Table 1. Effects of gestational treatment with selenium on reproductive parameters in CD-1 mice exposed with mercury

Treatment	# of implantation[a]	# of live fetuses	# of dead fetuses & reabsorption	Fetal weight (g)	Crown-rump length (mm)	Placenta weight (g)
Control	10.4±0.6(104)	10.1±0.4(101)	0.3±0.1(3)	1.53±0.05	25.1±0.2	0.110±0.009
Hg	10.6±0.4(95)	7.1±1.3(64)[d]	3.5±1.0(31)[d]	0.83±0.06[d]	19.9±0.7[d]	0.078±0.004[d]
Hg+Se0.1	10.0±0.5(90)	8.9±0.8(80)[c]	1.1±0.3(10)[c,f]	1.27±0.05[c]	22.3±0.9[c]	0.101±0.010[c]
Hg+Se0.2	10.1±0.7(101)	8.6±1.5(87)[c]	1.4±0.2(14)[c,f]	1.30±0.07[c,e]	22.0±0.8[c,e]	0.103±0.007[c]
Hg+Se0.3	10.4±0.6(94)	8.0±0.7(72)[c]	2.4±0.5(22)[d,f]	0.93±0.09[c,f]	20.2±0.7[c,f]	0.094±0.008[c]
Hg+BAL5.0	10.5±0.3(105)	9.9±1.0(99)[f]	0.6±0.3(6)[f]	1.41±0.05[f]	23.2±1.2[f]	0.102±0.909[c]
Hg+BAL+Se0.2	11.0±1.0(88)	8.7±0.3(70)[c]	2.3±0.1(18)[c,e]	1.14±0.06[c]	22.2±0.7[c]	0.110±0.006[c]
Hg+BAL+Se0.3	10.2±0.3(82)	8.2±0.7(66)[c]	2.0±0.5(16)[c,e]	1.02±0.08[c,f]	20.9±0.9[c,f]	0.100±0.007[c]

Se0.1= sodium selenite 0.1mg/kg bw, Se0.2=sodium selenite 0.2mg/kg bw,
Se0.3= sodium selenite 0.3mg/kg bw, BAL5.0=BAL 5.0mg/kg bw
[a]Values expressed as mean±SEM (# of fetuses); n=8-10 litter
[b]Significantly different from controls ($p<0.01$).
[c]Significantly different from controls ($p<0.05$).
[d]Significantly different from controls ($p<0.001$).
[e]Significantly different from mercury only treatment ($p<0.01$).
[f]Significantly different from mercury only treatment ($p<0.05$).

Table 3. Effects of gestational treatment with selenium on maternal organ weights in CD-1 mice exposed with mercury

Treatment	Relative weight (%)			
	Liver	Kidney	Brain	Spleen
Control	7.91±0.32(10)	1.32±0.03	2.08±0.12	0.37±0.03
Hg	9.91±0.45(9)[d]	1.75±0.05[d]	1.84±0.27[c]	0.45±0.04[c]
Hg+Se0.10	8.44±0.37(9)[c]	1.39±0.04[c]	2.15±0.13[c]	0.41±0.03
Hg+Se0.20	8.73±0.52(8)[b,e]	1.41±0.02[c]	2.00±0.10[c]	0.39±0.04
Hg+Se0.30	9.01±0.66(9)[b,e]	1.36±0.06[c]	2.04±0.22[c]	0.38±0.03[c]
Hg+BAL5.0	8.22±0.33(10)[c]	1.44±0.04[c]	2.12±0.15[c]	0.41±0.02
Hg+BAL+Se0.20	9.57±0.76(8)	1.38±0.03[f]	2.02±0.23[c]	0.39±0.06[c]
Hg+BAL+Se0.30	9.23±0.98(8)[c]	1.51±0.11[c]	2.16±0.51[c,f]	0.35±0.10[c]

Se0.1= sodium selenite 0.1mg/kg bw, Se0.2=sodium selenite 0.2mg/kg bw,
Se0.3= sodium selenite 0.3mg/kg bw, BAL5.0=BAL 5.0mg/kg bw
[a]Values expressed as mean±SEM (# of fetuses); n=8-10 litter
[b]Significantly different from controls ($p<0.01$).
[c]Significantly different from controls ($p<0.05$).
[d]Significantly different from controls ($p<0.001$).
[e]Significantly different from mercury only treatment ($p<0.01$).
[f]Significantly different from mercury only treatment ($p<0.05$).

Table 4. Effects of gestational treatment with selenium on maternal weight gain and water and feed consumption in CD-1 mice exposed with mercury

Treatment	Maternal weight gain[a] (g)	Water consumption (ml/day)	Feed consumption (g/day)
Control	7.4±0.4(10)	8.2±0.3	7.0±0.2
Hg	5.1±0.6(9)[d]	4.5±0.2[d]	5.6±0.2[d]
Hg+Se0.10	6.9±0.4(9)[c]	5.3±0.2[c]	6.2±0.1[c,e]
Hg+Se0.20	6.1±0.4(8)[c]	5.1±0.3[c,e]	6.5±0.5[f]
Hg+Se0.30	6.0±0.3(9)[c,e]	5.5±0.2[c,e]	6.3±0.4[c]
Hg+BAL5.0	7.0±0.4(10)[f]	7.1±0.6[f]	6.7±0.5[f]
Hg+BAL+Se0.20	7.0±0.3(8)[f]	6.9±0.4[c]	6.9±0.4[f]
Hg+BAL+Se0.30	6.3±0.4(8)[c,e]	7.0±0.4[f]	6.4±0.4[c]

Se0.1= sodium selenite 0.1mg/kg bw, Se0.2=sodium selenite 0.2mg/kg bw,
Se0.3= sodium selenite 0.3mg/kg bw, BAL5.0=BAL 5.0mg/kg bw
Values expressed as mean± SEM (# of mice).
[a]Maternal weight gain calculated minus contribution of gravid uterus.
[b]Significantly different from controls ($p<0.01$).
[c]Significantly different from controls ($p<0.05$).
[d]Significantly different from controls ($p<0.001$).
[e]Significantly different from mercury only treatment ($p<0.01$).
[f]Significantly different from mercury only treatment ($p<0.05$).

The effect of methylmercuric cholride with/without therapeutic agents on the incidence of abnormal fetuses is presented in Table 2. Abnormal fetuses included those with malformations and those with developmental variants. The percentage of abnormal and malformed fetuses increased dramatically in mice treated with mercury only. Although selenium affected positively reduction of the the percentage of abnormal and malformed alterations induced by exposure of mercury but was ineffective in a dose-dependent manner. In addition, there was similar reduction effect in mice treated with combination of Se and BAL when compared to the control. The malformations observed included cleft palate, hydronephrosis, microcephaly and umbilical hernia. A significant number of fetuses from dams with Se alone treatment exhibited developmental deviations including incomplete ossification of the cranial bones, pelvic bones and misplaced gonads as well as an extra pair of ribs.

The effects of subacute exposure to mercury with/without therapeutic agents on relative maternal organ weights are indicated in Table 3. Relative maternal liver, kidney and spleen weights were significantly increased following mercury exposure. In the other hand, exposure of mercury decreased relative maternal brain weight. Se alone treatment demonstrated the beneficial effect to prevent the increase (liver, kidney and spleen)/reduction(brain) of organ weight possibly producred by mercury treatment but reversly in a dose-dependent manner.

The effects of gestational exposure to methylmercuric chloride with/without some drugs on maternal water and feed consumption are shown in Table 4. Water and feed consumption after mercury without any treatment were reduced. However, treatment with Se, BAL or combined showed preventive effects against the reductive effects on water and feed consumption possibly produced by mercury in the regardless with a dose/regimen-dependent manner. Mater weight gain was also affected to reduction after exposure of mercury alone but was improved to normal values after therapeutic treatment without any specific manner.

Discussion

Gestational exposure to methylmercuric chloride with/without antidotes adversely affected fetal growth and development in CD-1 mice although there was no definitive dose-response pattern. The number of live fetuses was reduced following expousre of mercury alone but the reduction of number possibly influenced by mercury was prevented or inhibited by some therapeutic agents such as selenium, BAL and their combination. No specific relationship between the reproductive parameters and treatment regimen was established. Fetal growth parameters, including fetal weight and crown-rump length, were reduced by maternal subacute conceptional exposure to the mercury. The reduced fetal growth was reflected in decreased maternal gestational weight gain. These results are in aggrement with Inouye[15] and Murakami, who reported decreased fetal weight and number of liver fetuses following gestational exposure in rats.

The teratogenicity of mercury was evident in this study as reported already by other researchers. When dams were treated with 2.0mg Hg/kg mercury, about half the embryos were abnormal[1]. Several reports showed that large doses(i,e., 10 mg Hg/kg of methylmercuric chloride) produced a small number of malformed fetuses(i,e., cleft palate).[16,17] Therefore, the embryos in the early preimplantation period are more sensitive to mercury toxicities than in the fetal stage *in vivo*. The embryos were arrested at various stages but not at a specific stage.[1] The highly sensitive developmental stage could not be observed clearly, though there was a higher rate of abnormal embryos in the one to eight-cell stage than in the other stages. Matsumoto and Spindle[27] showed that the acute effect of methylmercuric chloride was most severe on blastocysts and least severe on morulae. Therefore, it is considered that the sensitive developmental stages are different, *in vivo* and *in vitro*.

The percentage of abnormal and malformed fetuses with variants was not followed to designed factors such as dose and kinds of treatment. The types of malformations observed in the offspring of treated mice were not observed in the offspring of the control group. The variants observed in the treated fetuses were primarily associated with developmental delay and paralleled the reduction in fetal growth. The present study clearly indicates that mercury is a developmental toxicant in mice. The question remains, however, whether the presence of selenium increases their individual toxicities.

The discussion of selenium and mercury interaction can be found in several review articles on selenium and heavy metal interactions.[28-31] Selenium compounds were found to be protective against mercury toxicity in a manner analogous to the interactions of selenium and cadmium.[28] The kidney and small intestine of rats injected with mercuric chloride were severely damaged; this effect was completely abolished by the simultaneous administration of selenite. The physiological significance of these injection experiments with mercury and selenium was probably not fully relaized until Ganther et al.[32] found the mercury in tuna fish

to be less toxic, possibley because of its selenium content.[33] When selenium was added to the corn-soya diet to equal the amount in the tuna, the mortality of the quail was markedly reduced. Similar protective effects of selenium against methylmercury toxicity were found with rats fed casein-based diets. A number of other researchers have shown that selenium will alleviate the toxicity of both organic and inorganic mercury.[31]

A summary of the influence of selenium on deposition of mercury in tissues has been presented.[31] In injection experiments, selenium caused a reduction in mercury deposition in kidney when given with either inorganic or organic mercury. In contrast, selenium usually resulted in an increased deposition of mercury in other tissues, especially increases of mercury deposition of mercury in the brain when methylmercury was used. When included in the diet, selenium also causes an increased deposition of hepatic mercury, but in contrast, the pattern in the kidney may depend upon the levels in the diet as well as upon the selenium status of animals.[34] At least on a report is available in the interaction of selenium with mercury in humans.[35]

In the present study there was no difference in maternal mortality and a decrease in the net conceptional weight gain in all of the treated groups. Another potential cause for the teratogenicity is maternal toxicity. Khera[36] reported a correlation between embryotoxicity and maternal toxicity. Decreased water and feed consumption may have contributed to the decrease weight gain. In addition relative liver and kidney weights were increased by mercury exposure. Such increases are suggestive of subclinical toxicity. Maternal toxicity is unlikely, however, to be the sole cause of the embryotoxicity. Teratogenesis and fetal growth depression were apparent at dosages of which overt no maternal toxicity was not a factor. The biological significance of these results in terms of actual human and animal exposure risk in unknown. The present study suggests, however, that embryotoxicity and teratogenicity occur with methylmercuric chloride in CD-1 mice when administered during organogenesis as demonstrated already by several researchers and that sodium selenite administration may have therapeutic application for the treatment or prevention against of harmful effects produced by mercury during gestation period.

Summary

Teratogenic and embryotoxic effects of mercury have been reported, however, there is little information about possible antidotes against mercury exposure during gestation. In order to evaluate therapeutic effects of selenium as an antidote against mercury poisoning, pregnant CD-1 mice were exposed to methylmercury chloride(20ppm) through the drinking water with treatment of sodium selenite (1.0mg, 2.0mg or 3.0mg/kg b.w., subcutaneously) or BAL(5.0mg/kg b.w., subcutaneously) under the single or combination base as the therapeutic agents from day 6 to 15 of gestation. Fetal growth parameters such as body weight and crown-rump length in the mice exposed to mercury, were reduced as was placental weight compared to those in the control. Treatment of selenium(alone, combination with BAL) reduced the harmful effects induced by mercury on the fetal growth parameters even though no specific relationship between dose and therapeutic effect. The incidence of dead fetuses/resorptions and malformed fetuses(especially cleft palate) was also increased in the mercury only treated group. Selenium treatment demonostrated reduced the incidence of abnormal fetuses under the exposure of mercury. Relative maternal organ weights(liver, kidney, spleen) were increased significantly but relative brain weight was decreased as evidenced by decreased in the mercury treated mice compared to that in the control. A subtle indication of maternal mercury toxicity evidenced by changes of relative maternal organ weights, decreased water and feed consumption were also prevented efficiently by selenium treatment. The present study suggests that methylmercuric chloride is embrytoxic and teratogenic in CD-1 mice when exposured during organogenesis and that selenium administration may have therapeutic application for the treatment of mercury poisoning although more applicable study in

human should be performed with caution in the future.

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