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

Jin-suk Kim • Seok-wha Choi*

Department of Veterinary Medicine and Animal Resources Research Centre, Kon-Kuk University
*College of Veterinary Medicine, Chungbuk University**

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임신 중 수은을 섭취한 CD-1 마우스 태아의 성장발육과 기형발생에 미친 티아민의 효능 평가

김진석 • 최석화*

건국대학교 수의학과 및 동물자원연구센타
충북대학교 수의과대학*

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초 록 : 수은에 의한 기형발생은 이미 밝혀졌으나 이에 대한 치료가능 약제들의 효과에 대하여는 아직 밝혀진바 없다. 임신한 CD-1마우스에 20ppm의 수은(methylmercuic chloride)을 음수를 통해 임신 6일째 부터 15일 사이에 투여하고 이와 동시에 투여계획에 따라 기존 치료제 BAL과 티아민(thiamin)을 피하로 투여한 후 임신 18일째에 제왕절개술을 실시하였다. 티아민(200mg/체중 kg)과 BAL(5.0mg/체중kg) 그리고 티아민과 BAL의 병용치료군의 태아는 체중과 두부-둔부 길이 그리고 태반의 무게가 수은 단독 투여군에 비하여 유의성 있게 무겁거나 길어 대조군에서 보인 수치에 가까왔다. 죽은 태아/재흡수율과 기형인 태아 발생율은 티아민과 BAL 치료군에서 감소되었으며 투여용량이 높을수록 발생율이 낮았다. 또한 높은 농도의 티아민과 BAL은 어미 마우스의 사료 및 음수섭취량을 증가시켰으며 간의 상대적 무게도 증가 시켰다.

이 연구 결과는 티아민을 단독 또는 키레이트제재와 병용투여할 경우 수은에 의한 기형발생을 감소시키거나 방지할 수 있는 효과가 있음을 보여주고 있다.

Key words : mercury, thiamin, teratogenecity, therapeutic effect.

Introduction

Mercury poisoning is a common toxicosis reported in man[1] and animals.[2] In addition to biochemical, histopathological and neurobehavioral changes, the teratogenicity was also demonstrated from mercury toxicity[1,3]. All froms of mercury cross the placenta to the fetus, but most of what is known has been learned from experimental animals. Fetal uptake of elemental mercury in rats probably because of lipid solubility has been shown to be 10-40 times higher than uptake after exposure to inorganic salts[4].

The primary therapeutic goal is to reduce the total body burden of mercury. For the most severe cases, infusion of chelating agents for mercury such as cysteine or penicillamine may be the first measure. For less severe cases of inorganic mercury poisoning, chelation with BAL(British Antilewisite:dimercaprol)

may be effective[4]. However, no therapeutic agents was established yet for accidental exposure to mercury during gestation period.

Thiamin and other water-soluble vitamins have been neglected as therapeutic agents. This is largely because such disorders are considered to be dietary in orgin. Previous studies have demonstrated that thiamin alleviated the clinical manifestations and also prevents the accumulation of heavy metals in various organs[5-8]. Thiamin was also reported to have a beneficial effect on the neurological alterations observed in thiamin-related disease such as encephalomalacia of cattle[9]. However, no information is available concerning the effects of thiamin in the teratological alterations produced by mercury.

In the present study, the effects of thiamin, BAL or their combined treatments on the mercury-induced fetal maldevelopments in CD-1 mice exposed to subclinical levels of mercury were evaluated for the therapeutic effect of thiamin with/without a conventional therapeutic agent(BAL) aganist the deleterious effect induced by mercury during pregnant period.

Materials and Methods

Nulliparous female CD-1 mice(Laboratory Animals Centre, Seoul National University, Seoul), 6-8 weeks old and weighing 25-30g, were mated with male mice of the same stock for approximately 2 hours(8 a. m. to 10 a.m.). The presence of a vaginal plug was considered evidence of copulation and represented day 0 of pregnancy. The mice were maintained in a temperature-and humidity-controlled room(23±2℃ and 55±10%) with a 12 hours light/dark cycle. 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 with/without injection of therapeutic agents(thiamin, BAL or combined). The dosages of thiaminHCL(Sigma, PO Box 14508, St Louis, Mo 63178, USA) administered subcutaneously are 100, 200 and 300mg/body weight(b.w), kg and 5.0mg/kg b.w. for BAL. Combined treatments were consisted of BAL(5.0mg/kg) and thiamin(200 and 300mg/kg). The mice were weighed on day 0, 6, 10, 15 and 18 of gestation. Water and feed consuptions were recorded on day 10 and 15 of gestation.

On day 18 of gestation the fetuses were removed by caesarian section, examined for external malformation, 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[10]. 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[11] 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[12]. The relative organ weights were analyzed by one-way analysis of variance of arcin-tranformed data[13] and Scheffe's test[14]. All remaining data were analyzed by one-way analysis of variance[13]. The statistical unit was the litter. Statistical significance was $p<0.05$.

Results

No maternal death was observed at any animals treated with MeHg used in the present study. Signs of Minamata disease, such as spasm, leg crossing, or deafness, were also not demonstrated. The effects of gestational exposure to methylmercury with/without therapeutic agents(thiamin, BAL or its combination) on reproductive and fetal growth parameters are shown in Table 1. The number of dead fetuses and reasorptions per litter was increased at the mercury only treatment(30% fetal death rate) compared to at the control(no treatment) group. The number of implantations per litter was not affected by the regimen of therapeutic agents including even mercury only treatment. All of therapeutic agents were

Table 1. Effects of gestational treatment with thiamin 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)	Placental 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	0.120±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+$VB_1$100	10.7±0.3(96)	9.4±1.5(84)[b]	2.3±0.7(12)[b]	1.39±0.05[c,e]	23.1±0.8[e]	0.101±0.008[c,e]
Hg+$VB_1$200	10.3±0.4(103)	9.9±1.9(99)[f]	0.4±0.1(4)[f]	1.47±0.04[e]	23.9±0.8[e]	0.108±0.008[e]
Hg+$VB_1$300	9.8±0.7(88)	9.3±0.9(83)[c]	0.6±0.1(5)[f]	1.30±0.09[c,e]	22.1±1.3[c,e]	0.114±0.008[f]
Hg+BAL	10.5±0.3(105)	9.9±1.0(99)[f]	0.6±0.3(6)[f]	1.41±0.05[c,e]	23.2±1.2[e]	0.102±0.909[c,e]
Hg+BAL+$VB_1$200	10.4±0.5(104)	10.1±0.3(101)[f]	0.3±0.1(3)[f]	1.48±0.06[e]	24.8±0.7[e]	0.112±0.006[e]
Hg+BAL+$VB_1$300	10.7±0.3(96)	9.5±0.7(86)[e]	1.2±0.4(10)[e]	1.53±0.10[e]	25.1±1.2[f]	0.133±0.007[e,e]

$VB_1$100=thiamin 100mg/kg b.w., $VB_1$200=thiamin 200mg/kg b.w.,
$VB_1$300=thiamin 300mg/kg b.w., BAL5.0=BAL 5.0mg/kg b.w.
[a]Values expressed as mean±SEM(# of fetuses); n=9-10 litters
[b]Significantly different from controls ($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 2. Effects of gestatioal treatment with thiamin on the incidence of malformed fetuses in CD-1 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(15)[d]	8	7
Hg+$VB_1$100	7.3±1.2(7)[d,f]	5	3
Hg+$VB_1$200	3.8±1.3(4)[e,e]	1	3
Hg+$VB_1$300	5.0±1.8(5)[d,f]	2	3
Hg+BAL5.0	6.8±2.0(7)[c,f]	2	7
Hg+BAL+$VB_1$200	4.8±1.8(5)[c,e]	2	5
Hg+BAL+$VB_1$300	4.2±1.5(4)[c,e]	1	4

$VB_1$100=thiamin 100mg/kg b.w., $VB_1$200=thiamin 200mg/kg b.w.,
$VB_1$300=thiamin 300mg/kg b.w., BAL5.0=BAL 5.0mg/kg b.w.
[a]Values expressed as mean±SEM(# of fetuses); n=9-10 litters
[b]Significantly different from controls ($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$).

reduced the number of dead fetuses and reabsorption and were increased the number of live fetuses compared with those in the mercury only treatment. Especially, combined treatment(BAL + thiamin 200mg/kg b.w.) showed same result of the number of live fetuses with the control.

Fetal growth parameters(i.e., fetal body weight and crown-rump length), as indicated in Table 1, were reduced in mice treated with mercury only and in some treament group regardless with dose-dependent manner. However, a dose-dependent return in placental weight to normal(no treatment) group was

Table 3. Effects of gestatioal treatment with thiamin on maternal organ weights in CD-1 mice exposed to 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[d]	0.45±0.04[d]
Hg+$VB_1$100	8.01±0.24(9)[e]	1.46±0.06[e]	1.98±0.12[e]	0.40±0.02[e]
Hg+$VB_1$200	8.14±0.44(10)[e]	1.55±0.09[e]	2.25±0.33[e]	0.47±0.03[c,e]
Hg+$VB_1$300	8.87±0.57(9)[e]	1.49±0.02[e]	2.13±0.20[e]	0.45±0.09[d]
Hg+BAL5.0	8.22±0.33(10)[e]	1.44±0.04[e]	2.12±0.15[e]	0.41±0.02
Hg+BAL+$VB_1$200	8.09±0.41(10)[e]	1.35±0.03[f]	2.08±0.18[e]	0.36±0.04[e]
Hg+BAL+$VB_1$300	8.77±0.49(9)[f]	1.42±0.05[f]	1.86±0.20[d]	0.40±0.03

$VB_1$100=thiamin 100mg/kg b.w., $VB_1$200=thiamin 200mg/kg b.w.,
$VB_1$300=thiamin 300mg/kg b.w., BAL5.0=BAL 5.0mg/kg b.w.
[a]Values expressed as mean±SEM(# of fetuses); n=9-10 litters
[b]Significantly different from controls ($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 thiamin on maternal weight gains, water and feed consumption in CD-1 mice exposed with mercury

Treatment	Maternal weight gains(g)	Water consumption(ml/day)	Feed consumption(g/day)
Control	7.4±0.4(10)	8.2±0.3	7.0±0.2
Hg	6.0±0.6(9)[d]	4.5±0.2[d]	5.6±0.2[d]
Hg+$VB_1$100	6.5±0.5(9)[d]	5.0±0.3[d]	6.0±0.1[d]
Hg+$VB_1$200	6.9±0.4(10)[d]	5.3±0.2[d]	6.2±0.1[c]
Hg+$VB_1$300	6.1±0.4(9)[d]	5.1±0.3[d,e]	6.5±0.5[c]
Hg+BAL 5.0	7.0±0.4(9)[c]	7.1±0.6[f]	6.7±0.5[c]
Hg+BAL+$VB_1$200	7.0±0.3(9)[c]	6.9±0.4[e]	6.9±0.4[e]
Hg+BAL+$VB_1$300	6.3±0.4(9)	7.0±0.4[e]	6.4±0.4[4]

$VB_1$100=thiamin 100mg/kg b.w., $VB_1$200=thiamin 200mg/kg b.w.,
$VB_1$300=thiamin 300mg/kg b.w., BAL5.0=BAL 5.0mg/kg b.w.
Values expressed as mean±SEM(# of mice).
[a]Maternal weight gain calculated minus contribution of gravid uterus.
[b]Significantly different from controls ($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$).

observed. These effects on growth parameters were particularly apparent in the mercury only treatment without any therapeutic application. Generally, conventional antidote(BAL) treatment with thiamin (200, 300mg/kg b.w.) resulted in improvement of the deleterious effects possibly produced by mercury exposure compared to a single treatment.

The effects of gestational exposure to mercury in the incidence of malformed fetuses are presented in Table 2. The percentage of malformed fetuses was increased at the mercury only treatment and was statistically different from both control and other treatment groups. Even though there was no difference depended on regimen of treatment, therapeutic application with mercury exposure was reduced approximately 50% of malformation incidence

affected by mercury. Malformations observed in this experiment included cleft palate, microcephaly and kinky tail.

The effects of gestational exposure of mercury on relative maternal organ weights are summarized in Table 3. Relative maternal liver, kidney and spleen weights were increased in the group treated with mercury only. On the other hand, relative maternal brain weights in mice exposured to mercury only was decreased compared to the no treatment group. All group treated with therapeutic agents(thiamin, BAL and combined) showed beneficial effects against toxicological influences of mercury to maternal organ weight but difference was found when compared to no treated mice.

The effects of gestational exposure to mercury on maternal weight gains, water and feed consumption are indicated in Table 4. Maternal weight gain, water and feed consumption were affected by mercury exposure when terminal weight was determined without the contribution of the gravid uterus. When the weight of the gravid uterus was included in the calculation of gestational weight gain, dams in the highest dosage group gained significantly less weight (data not shown). Water consumption was reduced at the other treatment group but less than the mercury only treatment. The reduction in water consumption in the mercury treatment group was statistically different from both control and other treatment groups. In spite of the decreased water comsumption, maternal mortality was not observed in any of the treatment groups.

Discussion

The present study demonstrates that thiamin alone or in combination with BAL reduces the reproductive toxicity of mercury. It appears that thiamin prevents incidence of dead fetuses from mercury exposure. The present study indicates that 20ppm of mercury is teratogenic. The most striking effect was an increased incidence of cleft palate. It has been suggested that methylmercury inhibited the elevation of the palate[15].

A previous study demonstrated the teratogenicity of a single oral dose of 25 mg/kg of methymercury (MeHg) on day 10 of gestation in ICR mice, in particular, its complete inhibitory effect on palatal closure[3]. This study demonstrates similar results.

This inhibitory effect of 25 mg/kg MeHg on palatal closure can be almost completely prevented by the intraperitoneal injection of 370 mg/kg/day of Tiopronin, an antidote against heavy-metal poisonings. It has been reported that maternally administered MeHg immediately penetrates fetuses and stays in fetal tissues for a long period[16,17]. The occurrence of cleft palate in the present study seems to be a result of a delay in fetal development necessary for the palatal shelves to acquire a potential to elevate as reported previously by Yasuda et al[18]. The critical stage prior to elevation must require multiple factors besides the growth of the palatal shelves[19]. A subtle but reproducible morphological change in palatal shelves was observed in the fetuses treated with even the lower dose of MeHg(10-15mg/kg)[18].

Although the mechanism of the thiamin-mercury interaction in the body remains unclear, it has been suggested that thiamin facilitates the removal of heavy metals from body fluids and other tissues by the formation of readily excretable complexes[5-8,20]. Increased urinary excretion of heavy metal(lead) with thiamin supplementation has been also demonstrated[21]. Thiamin is thought to be nontoxic in humans unless it is administered in doses which are many thousands of time higher than the recommended dietary intake[9].

Thiamin, when given simultaneouly with lead, may inhibit or interfere with the absorption of lead in tissues, possibly via the formation of a lead-thiamin or lead-thiamin metabolite complex[5,6,22]. Thiamin has been shown to complex in vitro with other heavy metals, such as copper and cadmium[23-27]. Therefore similar complex formation may be anticipated with mercury and thiamin, resulting in decreased body accumulation of mercury. Regardless of the mechanism involved, the improved efficacy of the combined treatment suggests a potential role for thiamin in combination with BAL or other chelating agents in the treatment of mercury intoxication, especially, during gestation period, although further studies are required to evaluate the degree of clinical

improvement associated with thiamin treatment.

Summary

Pregnant CD-1 mice were exposed to methylmercury in the drinking water at concentration of 20ppm with subcutaneous treatment of thiaminHCl(vitamin B_1) (100mg, 200mg or 300mg/kg b.w.) or BAL(5.0 mg/kg b.w.) under the alone or combined base at the therapeutic agents from day 6 to 15 of gestation. Fetal growth parameters, including body weight and crown-rump length in the mice exposed to mercury, were reduced as placental weight compared to those in the control group(no treatment). The incidence of dead fetuses/resorption and malformed fetuses(especially cleft palate) was also increased even in the group treated with thrapeutic agents as well as in the mercury only treated group. However, all kinds of alteration indicated above, possibly induced by mercury, reduced/or decreased significantly compared to those of control. A subtle indication of maternal toxicity was noted in most experimental animals as evidenced by decreased water consumption and increased relative liver weight. The present study confirmed that methylmercuric chloride is embrytoxic and teratogenic in CD-1 mice when administered during organogenesis and that thiamin administration may have therapeutic application for the treatment or prevention against of deleterious effects induced by mercury during gestation period.

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