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• BASIC RESEARCH •

Effect of manganese on heat stress protein synthesis of new-born rats

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Abstract

AIM: **To study the effect of manganese (Mn) on heat stress protein 70 (HSP70) synthesis in the brain and liver of new-born rats whose mother-rats were exposed to Mn.**

METHODS: **32 female rats were randomly divided into four groups. One group was administrated with physiological saline only as control group, the other three groups were administrated with 7.5, 15 and 30 mg·kg^{-1} manganese chloride ($MnCl_2$) by intraperitioneal injection every two days for two weeks. After delivery, the mother-rats received $MnCl_2$ unceasingly for a week with the same method. Then the contents of Mn, Zn, Cu and Fe in the livers of the new-born rats were determined by atomic absorption spectroscopy; The level of HSP70 in the brains and the livers of the new-born rats as detected by Western-dot-blotting, and the SOD activities were measured simultaneously.**

RESULTS: **The contents of Mn in the livers of new-born rats of the experimental groups (respective 1.38±0.18, 2.73±0.65, 3.44±0.89μg·g^{-1}) were significantly increased compared with the control group (0.88±0.18μg·g^{-1}; P<0.01); The contents of Fe in the livers of new-born rats of 15 and 30 mg·kg^{-1} experimental groups (426±125, 572±175μg·g^{-1}, respectively) were significantly increased compared with the control group(286±42μg·g^{-1};P<0.05); the levels of Zn in the livers of the new-born rats of three experimental groups(254±49, 263±47, 213±28μg·g^{-1}, respectively) were lower than those of the control group (335±50μg·g^{-1}; respective$P$<0.05,P<0.01); and the levels of Cu showed no significant difference among the four groups (three experimental groups: 75±21, 68±241 and 78±18μg·g^{-1}; control group: 83±9μg·g^{-1}; P>0.05). There was a significant increase in the levels of HSP70 in the brains of new-born rats of the 30 mg·kg^{-1} group (19.5×10^3±1.3×10^3 A; control group: 14.3×10^3±1.4×10^{3}A; P<0.01),and the levels of HSP70 in the livers of new-born rats of three experimental groups(respective 19.6×10^3±3.9×10^{3}A, 18.5×10^3±3.8×10^{3}A, 22.4×10^3±1.9×10^{3}A) also increased than control group(13.3×10^3±1.0×10^{3}A;P<0.01), but the SOD activities showed no significant difference among brains of the four groups (experimental groups: 5.04±0.43, 4.83±0.48, 4.60±0.84 ku·g^{-1}; control group: 4.91±0.37ku·g^{-1};P>0.05). The SOD activities in the livers of 15mg·kg^{-1} group (5.41±0.44 ku·g^{-1}) was lower than the control group(5.95±0.36 ku·g^{-1}; P<0.05).**

CONCLUSION: **While mother-rats were exposed to manganese, the metabolisms of Mn, Zn and Fe of new-born rats in the livers were influenced and were situated in a stress status, thus HSP70 syntheses is induced in the brains and livers of new-born rats, but the mechanism of this effect in the developmental toxicity of Mn remains to be further studied.**

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INTRODUCTION

Manganese(Mn) has bi-directional effect on the mammal[1-3], both deficient and excess intake of Mn result in altered enzymatic reactions and brain function because of its essential element nutrition[4] and neurotoxicant effect. With exposure to higher concentrations is harmful to health[5,6]. Excessive manganese has been associated with neurobehavioral deficits and neurological and/or neuropsychiatric illness[7]. As a nervous toxicant, Mn damages central nervous system, and has procreant toxicity. Mn also could transplacenta to embryo and affects growth of offspring[8,9]. Thus, excessive manganese also is an embryotoxicant and fetotoxicant in mammals[10]. but the mechanism of this effect has not been elucidated yet. It is well known that heat stress / shock proteins (Hsps) are induced by a series of occupational factors and their main biological action is to participate in thermotolerance and toxicant tolerance. Hsps participate in protein synthesis, folding, assemblage and intracellular protein transport as the molecular chaperones[11,12]. It has not been reported whether these functions are relative with toxicity of Mn. The HSP70 levels and the SOD activities of the brains and livers and the trace-element contents of the livers were measured in the new-born rats after their mother-rats were exposed to the manganese.

MATERIALS AND METHODS

Animal Treatment

Thirty-two healthy Wistar female rats that weigh 200~240g (obtained from center of experimental animal of Tongji Medical University) were randomly divided into four groups. Except one group as control, the other three groups were treated with Manganese chloride tetrahydrate ($MnCl_2 \cdot H_2O_2$). By mean of single intraperitioneal injection administration every two days for two weeks, the doses were 7.5, 15 and 30 mg·kg^1 respectively. The control group was similarly injected physiological saline. Then the rats (1 ♂ : 1 ♀) were housed together. The next morning gestation was defined as d 0 when sperms were founded in the vaginal smear. After

pregnancy, the mother-rats were treated with $MnCl_2$ at the same concentration of before, then harvested at time points up to a week with the same method as before. These female rats received $MnCl_2$ 11 times. The newborn rats were killed by cervical dislocation in 24 h. The brain and liver of the newborn rats was reserved in -20℃ immediately.

Determination of trace elements

The reserved livers were put into roaster for 2 h at 105℃ and reserved into desicator. Drying sample accurately Weighed up 0.0500~0.1000g was put into conical flask and added 2 mL nitric acid (G.R), 0.5 mL perchloric acid (G.R) and digested to dry approximately. After cooling, the digestive sample was dissolved with redistilled water that was distilled for 4 times, the sample was put into 10 mL test tube and fixed volume to 5 mL. Trace elements were measured with atomic absorption spectroscopy (Spectr AA-40 atomic absorption apparatus, VARIAN corp., USA). Mn was measured with graphite stove atomic absorption spectroscopy analysis, and adopted flame atomic absorption spectroscopy to mensurate Cu, Zn and Fe.

Brains and livers homogenate

Prepared homogenates of the brains and livers were done as described by routine technique. Mensurated contents of the protein of the brains and livers as Lowry.

Detection of SOD activities

Taking equal quality proteins of the brains and livers to determine SOD activities in the brains and livers with SOD kits supplied by Nanjing Jiancheng Bioengineering Graduate Institute.

Determination of HSP70

Equal amount proteins of the brains and livers was taken to determine the HSP70 levels in the brains and livers with Western-dot-blotting[13]. After Western blotting, HSP70 was detected using anti-HSP70 rabbit polyclonal antibodies (provided by Lab of Cell & Developmental Genetics, University Laval, Canada) and anti-rabbit horseradish peroxidase-conjugated secondary antibodies (Sigma), and visualized using 3-3'-diaminobenzidine (DAB). The results were quantified by a densitometry (CS-930, Japan).

Statistic analysis

The results were analyzed with SAS programs in IBM-PC.

RESULTS

Effect of Mn on female rat Reproduction

After female rats exposed to Mn, their gestation periods had no significant difference from the control group (P>0.05). The parturition indexes seem to reduce along with manganic dosage increment, but the difference is not significant compared with control group(P>0.05) yet. Average of fetiferous quality of puerperal female rats seem to reduce in 7.5 and 15mg·kg^{-1} groups, but the differences are not significant compared with control group (P>0.05) yet. However, 30 mg·kg^{-1} group significantly reduced compared with control group(P<0.01), Average of fetiferous quality of pregnant female rats in 15 and 30 mg·kg^{-1} groups significantly reduced compared with the control group(P<0.05,P<0.01). The viability index of 1-day-old filial rats in 30mg·kg^{-1} group significantly reduced compared with the control group (P<0.01). The newborn rats' weight and height showed not significant difference among four groups (P>0.05, Table 1).

Effect of Mn on traceelements in newborn rats

The manganese contents of the new-born rats' livers in three experimental groups significantly increased compared with control group (P<0.01), and there was a dose-dependent increase in these groups. The levels of zinc in the new-born rats' livers in three experimental groups were lower than those of control group too (P<0.05,P<0.01,respectively). The iron contents of the new-born rats' livers in 15 and 30mg·kg^{-1} experimental groups were higher than control group(P<0.05, P<0.01, respectively), but the copper levels of the newborn rats' livers showed no significant difference among four roups (P>0.05, Table 2).

Table 1 Effect of Mn on reproduction in female rats ($\bar{x}\pm s$, n=8)

Mn (mg·kg^{-1})	Gestation (d)	Puerpera female rats (n)	Paturition index (%)	Tatol Filial rats (n)	puerperal female rats Fetiferous quality (n)	pregnant female rats fetiferous quality (n)	Livabilit of d 1 filial rats (%)	Newborn rats' mass (g)	Newborn rats' height (cm)
control	21.3±0.8	7	87.5	68	9.7±2.1	8.5±3.9*	98.5	5.89±0.49	5.2±0.25
7.5	21.8±1.2	6	75.0	41	6.8±3.1	5.1±4.1	95.1	5.47±0.54	5.1±0.36
15	22.2±0.8	5	62.5	33	6.6±2.7	4.1±3.9^a	87.9	5.38±0.53	4.98±0.35
30	22.7±1.5	3	37.5	15	5.0±2.6^a	1.8±2.9^b	73.3^b	5.13±0.51	4.87±0.38

^aP<0.05,^bP<0.01, *vs* control.

Effect of Mn on SOD activities in newborn rats

Although the SOD activities of the newborn rat' brains seem to reduce in 15, 30mg·kg^{-1} groups and increase in 7.5mg·kg^{-1} group, but no significant difference showed among all the four groups (P>0.05). The SOD activities of the newborn rat' livers were lower in 15mg·kg^{-1} group than control group(P<0.05), but showed no significant difference among 7.5, 30mg·kg^{-1} and control groups (P>0.05, Table 3).

Table 2 Effect of Mn on traceelements in newborn rats' livers ($\bar{x}\pm s$, μg·g^{-1})

Mn (mg·kg^{-1})	Filialrats (n)	Mn	Cu	Zn	Fe
control	5	0.88±0.18	83±9	335±50	286±42
7.5	5	1.38±0.18^b	75±21	254±50^a	271±88
15	7	2.73±0.65^b	68±24	263±47^a	426±125^a
30	5	3.44±0.89^b	78±18	213±28^b	572±175^b

^aP<0.05, ^bP<0.01, *vs* control.

Table 3 Effect of Mn on SOD and HSP70 in brains and livers ($x \pm s$)

Mn ($mg \cdot kg^{-1}$)	Filial Rats (n)	Brain		Liver	
		SOD / $ku \cdot g^{-1}$	HSP70/$\times 10^3$ A	SOD / $ku \cdot g^{-1}$	HSP70/$\times 10^3$ A
control	8	4.91±0.37	14.3±1.4	5.95±0.36	13.4± 1.0
7.5	9	5.04±0.43	16.0±1.8	5.96±0.41	19.6±3.9[b]
15	7	4.83±0.48	14.4±1.4	5.41±0.44[a]	18.5±3.8[b]
30	6	4.60±0.84	19.5±1.3[b]	5.87±0.68	22.4±1.9[b]

[a]$P<0.05$,[b]$P<0.01$,*vs* control

Effect of Mn on HSP70 synthesis in newborn rats

The level of HSP70 in the brain showed no significant difference among 7.5, 15$mg \cdot kg^{-1}$ and control groups ($P>0.05$), but showed significantly increased between 30$mg \cdot kg^{-1}$ and control groups ($P< 0.01$). The level of HSP70 in the livers significantly increased in three experimental groups to be compared with control group ($P<0.01$, Table 3).

DISSCUSION

The fetiferous quality reduces after female rats are imbued with Mn; parturition index also tended to reduce along with the manganese dosage increase, and livability of d 1 old filial rats significantly reduced along with the manganese dosage increase. The fact illustrates that manganese influences female rats' reproductive function. Manganese could transplacenta to enter filial central nervous system[15]. Yang BN reported that the new-born rats' manganese contents of the brain were significantly higher than those of control group with inductance coupling plasmic emanant spectroscopy after mother-rats exposed to by manganese[16]. This study determined that the new-born rats' manganese contents of the livers are significantly higher than control group with atomic absorption spectroscopy after mother-rats exposed to manganese, and have a dose-effect relationship. The results show that the manganese could be transferred to the embryo by placental barrier and accumulate in filial rat's brains and livers after mother-rats to be exposed by manganese in gestation. It has not been reported that the effect of Mn on other microelements in the newborn rats' liver of intoxicanted mother-rats. This study also detected that the levels of Zn in the new-born rats' livers of three experimental groups were lower than those of control group, and the iron contents in the new-born rats' livers of and 30$mg \cdot kg^{-1}$ treated groups were higher than the control group, but the copper levels showed no significant difference among four groups. It was obvious that the zinc and iron metabolism were disturbed in the livers of new-born rats after mother-rats exposed to manganese. Zn is an essential microelement and has much to do with many enzymatic bioactivities. Zn is very important to maintain normal body growth and reproductive function. While the body zinc dificiency, the hormone syntheses reduce and their activities decrease, which, therefore, result in serious obstruction of the growth, children' testicles agenesis, reducing spermic quantity, weakening spermic activity, agenesis of the immune apparatus in the thymus gland etc and immune estate. While serous zinc contents descend, it is degressive that the levels of neuropeptide somatostatin and arginine-vasopressin have much to do with capability of the learning and memory in rats' hippocampi, and make the rats' capability of learning and memory clearly decline[17]. When filial rats lack the zinc during the lactation and weaning stage, the brain weight, the hippocampal weight, the serum and hippocampal zinc concentrations are significantly lowered, and proportion of induced long-term potentiation (LTP) is zero and proportion of active avoidance response decreased profoundly[18], Cognition is a field of thought processes by which an individual processes information through skills of perception, thinking, memory, learning and attention. Zinc deficiency may affect cognitive development by alterations in attention, activity, neuropsychological behavior and motor development. The exact mechanisms are not clear but it appears that zinc is essential for neurogenesis, neuronal migration, synaptogenesis[19]. Excessive manganese arising zinc deficiency could interfere with neurotransmission and subsequent neuropsychological behavior.

This study determined zinc decrease in newborn rats' livers after mother-rats were administrated by manganese, it tallies with the phenomenon that as we observed, filial rats' neurobehavior functions deferred and the capability of learning and memory weakened[20]. The result enlightens that the effect of Mn on filial rats' developmental toxicity might be responsible for the manganese which leads to the zinc deficiency in the brain and liver. A new pathway is suggested in the manganese mechanism research on filial rats' neurotoxicity. Fe is also an essential microelement and has important physiologic function. The study also discovered that newborn rats' irons to heighten in the livers after mother-rats were imbued by manganese. The most actions are antagonistic between Fe and Zn. It is uncertain that whether the manganese cumulated in the liver to arise the zinc decreases and results in heightening Fe antagonistically. So, the reason needs further study.

Mn has an especial affinity with the mitochndrial and can largely cumulate in the cells that contain plentiful mitochndrial, so the manganese content is highest in the liver and the content is higher in the spleen, the kidney and brain[21]. The manganese mainly damages extrapyramidal system on central nervous system,, which is related to specifically organization and the neurons distribution in the substantia nigra and corpus striatum. Many melanin neurons and aminergic neurons are distributed in the dense area of substantia nigra. They have a speciality that collect and cumulate the amine and metal elements, such as manganese etc. Superfluous manganeses on one hand activate the cytochrome oxidase P-450 to engender the free radicals to deplete the sulthydyls and impair cellular resistivity, Mn on the other hand can also produce plentiful free radicals to damage mitochondrial, and to disturb energy metabolism, then mediate the toxicity of excitability amino acids and result in increasing intracellular calcium, activating the calcium-dependent protease, nuclease, phosphatase and promoting the cellular recessive denaturalization. The increase of calcium ions again, promotes the production of free radicals as result, forms a vicious circle. Therefore, cumulative manganese may destroy the protective barrier of the melanin neurons and constantly releases to beget continual toxicity in the extrapyramidal system). The free radicals play very important role in manganese neurotoxicity. SOD is a important enzyme among enzymes system that eliminates free radicals. The manganese is able to inhibit SOD by forming free radicals. In our study only the SOD activities of livers in 15$mg \cdot kg^{-1}$ group were lower than the control group. Although it inclined depression that the SOD activities of the livers in 7.5 and 30$mg \cdot kg^{-1}$ groups and of the brains in the three experimental groups, the differences were not significant to be compared with control group. This causation may be that SOD have a insensitivity on free radicals by the manganese toxicity, also may have other cause to be elucidated. The body was situated in a toxicant stress status and oxidative stress after the manganese was accumulated in the filial rat's brain and liver[22]. When body was situated in a toxicant stress / oxidative stress status, heat stress protein is markedly elevated *in vivo* on response of toxicant stress / oxidative stress.[23-26]. The stress response is a common physiological response from the prokaryotes to the human. It is the character of various stress responses that Hsps syntheses appear as increase or grow out of nothing in the cells. Hsps are a kind proteins that have protective function as quite conservation in the evolution. Hsps could respond stress responses by some injurious environmental factors, such as Pb[27], Cd[28], Hg[29], As[30,31], benzene[32,33], CO[34], ischemia[35], psychological tension[36]etc. Hsps have been proposed as

general markers of cellular aggression and their use for environmental monitoring is often suggested[37]. Hsps endow with resumptive capability of the cells or biology from various stress responses and protect them to keep from the damages of these factors[38-46]. The primary signification of Hsps is to keep protect the cells from suffering from ill conditions of high temperature, low oxygen, heavy metal and illness etc[47-54]. Therefore it is obvious that thermotolerance was formed, in other words, after the cells or organisms were exposed to subthermal death point, the livability significantly increases on the thermal death point. HSP70 exerts main action in the form of stress tolerance. HSP70 has important protective function in intracellular hereditary substance DNA, the process of biologic growth, development, differentiation and regulation[55-57]. HSP70 connects with many internal bioactive substances and take part in many biochemical processes. It is principal that HSP70 promote protein synthesis, folding, assemblage, transportation and take part in the elimination of metamorphic proteins as molecular chaperones under non-stress conditions[58-62]. These important functions may have to do with the function of the thermotolerance and poison tolerance. When cells experience thermal or toxicant stress, Hsps take on a new role, conserved from poison to humans, of protecting cells from the detrimental effects of stress. This role takes on added significance for the embryo in which the developmental program must be read linearly, with little opportunity to cycle backward to complete a missed segment of the program. Thereby Hsps will afford protection to the human embryo/fetus exposed to thermal/toxicant tress[63-65].

This study showed that some HSP70 were synthesized in normal newborn rats' brains and livers. The HSP70 contents of the newborn rats' brains in Low and medium dosage groups had a few increase, but the differences were not significant compared with control group. The HSP70 contents in the newborn rats' brains of high manganese dosage group significantly increased. The HSP70 contents all significantly increased in the newborn rats' livers of three experimental groups to be compared with control group. These results indicated that HSP70 had a certain degree role in normal growth and the manganese had effect on HSP70 syntheses in the newborn rats' brains and livers. However the HSP70 syntheses were low in very few newborn rats' brains and livers of experimental groups too. It remains to be elucidated that HSP70 synthesis is related to manganese toxicity in the pathology, clinic, susceptibility of manganese poisoning and other biochemical variety.

Through contrast affected degree of HSP70 syntheses in newborn rats' brain and livers,. induction of HSP70 is sensitive in the liver than in the brain. The reason may have to do with manganese accumulation that is higher in the liver than in the brain in the newborn rats. Recently some researcher explore HSP70 to relate to the fetation from the point of the development and had acquired many intersting results. They considered stress response to have diploid effects. On the one hand protect embryonic growth, on the other hand disturb as well as embryonic growth. Final procreant consequence mainly depend on the intensity and duration in the stress response whether to exceed self regulative level in intracellular HSP70 synthesis and also relate to injured period in fetation. Many results all had proved that HSP70 could influence embryonic growth *in vivo* and *in vitro*. HSP70 maybe is a biomaker as screening developmental toxicant[66], and excessive manganese may be considered a developmental toxicant.. Moreover, the stress status also leads to disorder of digestive system, for instance, gastrointestinal motility disorders[67,68], vasoactive intestinal peptide (VIP), neuropeptide Y(NPY) of colonic mucosa was increased[69], platelet derived growth factor (PDGF)[70], and tissue inhibitor of metalloproteinase 1 (TIMP-1) are increased[71], and heme oxygenase-1 (HO-1) mRNA and protein were highly induced and HO enzyme activity was higher after hemorrhagic shock and resuscitation (HR)[72]. Besides accelerates HSP70 synthesis *in vivo*,The manganese could induce the nitric oxide synthase(NOS) yet. The NOS begets increasing the synthesis of nitric oxide(NO). Superfluous NO could mediate neurotoxicity of excitative amino acid as a cytotoxicity molecule and could damage central nervous system in budding filial generation[73] [28], whereas, HSP70 could diminish the liver damage by NO. Re-induction of HSP70 expression by stress effect re-established resistance to NO toxicity[74].

Our study demonstrates that the excessive manganese cumulated largely filial rat's brain and liver by way of placenta after mother-rats exposed to manganese, and disturbed the microelement metabolisms of the l manganese, zinc and iron *in vivo* and injured the growth on the offspring. On the other hand the body was situated in a stress status and the embryo and filial generation growth were damaged by the inducement of HSP70 synthesis in the brain and liver. The mechanism of this effect in the developmental toxicity of Mn remains to be further reseached.

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