Korean J Biol Sci 1: 337-340, 1997

Reducing Fetal Contamination of Radiostrontium by Water Soluble Chitosan

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Key Words:
Pregnancy
Mouse
Chitosan
Radiostrontium
Chelator

The purpose of this study is to evaluate whether water soluble chitosan, a natural nontoxic chelator, can reduce fetal contamination of radiostrontium in pregnant mice. Various forms of water soluble chitosans (10% or 1% powder, or 1% solution) were given to pregnant mice before or after contamination of 0.005 μCi/B.W(g) Sr-85. Transplacental transfer of Sr-85 to fetus was 6.8±2.7% of injected dose, when Sr-85 was administered at the 20th day of pregnancy. Fetal radioactivity was significantly reduced when mother mice were treated with water soluble chitosan before contamination of Sr-85. Water soluble chitosans of 10% or 1% powder, or 1% solution significantly reduced fetal retention of Sr-85 to 2.3±0.7%, 2.7±0.8%, and 2.0±0.9%, respectively. However, fetal contamination was not reduced, when water soluble chitosans of 10% or 1% powder, or 1% solution were administered after maternal contamination of Sr-85. From these data we can conclude that water soluble chitosan could reduce fetal contamination of radiostrontium in pregnant mice, when given before the pregnant mice were exposed to radiostrontium.

Radiostrontium is one of potential environmental radiotoxins which can be produced by nuclear accidents (Ilyin et al., 1975). It has been reported that when radiostrontium contaminates a pregnant mother, the contamination of embryo or fetus is unavoidable (Natsusaka and Nishimura, 1962; Ujeno, 1983; Auvinen et al., 1994). Although many chemical chelators such as ethylenediaminetetraacetate (EDTA), diethylenetriamine pentaacetic acid (DTPA), sodium tetra-methylene diamine-tetraacetate and sodium thicotate were proven effective in eliminating radiostrontium from animal body (Ortega and Gomez, 1989), their own toxicities limit clinical use to human, especially to pregnant woman. Therefore, a nontoxic chelator is needed to eliminate or block contamination of radiotoxins.

Chitosan has been introduced as a chelating agent of heavy metal owing to its chelating property of a divalent positive ion (Laurance et al., 1980). Water soluble chitosan was very effective in eliminating radiostrontium contaminated in the body of mouse (Bom et al., 1994). It was proven nontoxic (Arai et al., 1968; Kim et al., 1996). The purpose of the present study was to investigate how effectively water soluble chitosan can reduce the fetal contamination of radiostrontium in pregnant mice.

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Materials and Methods

Experimental animals

Experimental animals were pregnant mice (40 - 50 g) of ICR strain which were purchased from Sam Yuk Laboratory Animal and mated in the animal laboratory of Chonnam University Medical School. The mice were bred in the polycarbonate cage (40×25×17 cm) in room temperature (23±2℃) with food and water supplied *ad libitum*.

Chitosan and radiostrontium (Sr-85)

Water soluble chitosan of molecular weight less than 10,000 and 75 to 90 percent of deacetylation was purchased from Yosu Chungmu Industrial. It was dissolved in saline to be 1% and 10% solutions.

Radiostrontium ($^{85}SrCl_2$, hereafter, simply referred to as Sr-85) was purchased from DuPont, and diluted in saline to be 0.25 μCi/ml. Sr-85 of 0.005 μCi/B.W. (g) was administered orally using orogastric tubes. Although Sr- 90 is a main nuclear product which can be produced from nuclear accidents, Sr-85 was used in this experiment for the experimental safety. Sr-85 has a similar radiochemical property with a short half life (64.5 d) compared to Sr-90 with a long physical half life (28.8 yr).

Experimental groups

Experimental groups consisted of 7 groups (8 mice in each group). In three groups, water soluble chitosan was administered to female mice as 10% or 1% powders, and 1% solution once a day for 60 d (groups 1, 2, and 3, respectively). They were mated with male mice at 40th day, and were given 0.005 μCi/B.W. (g) Sr-85 at 60th day (20th day of pregnancy). In another three groups (24 pregnant mice), Sr-85 was administered at the 20th day of pregnancy, and then water soluble chitosan was administered through orogastric tubes as 10% or 1% powders, and 1% solution (groups 4, 5, and 6, respectively, 8 mice in each group). Control group (n=8) received Sr-85 at the 20th day of pregnancy, and was fed with usual food without chitosan.

Measurement of radioactivity

To measure the remaining radioactivity in organs of the mother, they were sacrificed after birth. Gamma radiation from organs of them were separately counted using a gamma counter (Hewlett-Packard) with energy window 450-600 KeV. They were corrected for their decay. Fetal whole body radioactivity was also measured at the first day after birth. Retention of Sr-85 in each organ and fetus was expressed as percent of injected dose.

Changes of birth rate after chitosan feeding

To examine whether chitosans used in this experiment cause any change in birth rate, 68 female mice were fed with water soluble chitosan and their birth rates were counted. Four experimental groups were set as follows; Group 1, 15 female mice fed with 10% of chitosan powder at the same time as mating; Group 2, 18 female mice fed with 1% of chitosan (powder) at the same time as mating; Group 3, 15 female mice fed with 1% of chitosan (solution) at the same time as mating; and Control group, 20 female mice with general food. Then a change in mean birth rate was observed. Among the F_1 group of the experimental mice, 33 female mice were divided into the three groups which were fed the same chitosan foods. Their birth rates were also counted.

Statistical analysis

Differences of Sr-85 retention in fetus and mother were tested by analysis of variance (ANOVA) test using PC version of SAS. P value less than 0.05 was considered as significant.

Results

Transplacental transfer of Sr-85 to fetus was 6.8 ± 2.7% of injected dose, when Sr-85 was administered 20th day of pregnancy. Fetal radioactivity was significantly reduced when mother mice were treated with water soluble chitosan before and during pregnancy ($p<0.001$, Table 1). However, fetal retention was indifferent, when water soluble chitosan was treated after oral contamination of Sr-85 ($p=0.27$, Table 1).

Table 1. Fetal uptake of radiostrontium (Sr-85) following chitosan administration

Control[a]	Group 1[b]	Group 2[c]	Group 3[c]	Group 4[d]	Group 5[e]	Group 6[e]
6.8±2.7[f]	2.3±0.7	2.7±0.8	2.0±0.9	6.7±2.4	6.1±2.7	6.3±2.7

a. General food feeding group.
b. Chitosan powder (10%) was given for 60 d before radiostrontium administration.
c. Chitosan powder (1%) was given for 60 d before radiostrontium administration.
d. Chitosan powder (10%) was given after radiostrontium administration.
e. Chitosan powder (1%) was given after radiostrontium administration.
f. Mean±SD, $P<0.001$ vs control.

Water soluble chitosans used in this study failed to affect birth rates of both F1 and F2 generations ($p>0.05$ vs control, Table 2).

Discussion

Blocking transplacental transfer of radiostrontium from mother mice to feus was proven possible by water soluble chitosan. It was possible when the mother mice were treated with water soluble chitosan for a long period before and during pregnancy. When radiostrontium was contaminated during pregnancy, however, water soluble chitosan could not eliminate contaminated radiostrontium from the fetus.

The route of radiostrontium contamination is absorption through intestine or entering the blood through wounds. When absorbed, it is then deposited into bones (Engstrom et al., 1968; Ilyin et al., 1975). As the fetal skeletal system develops at the end of pregnancy, calcium ions of the mother are transferred to the fetus. Radiostrontium comes into fetus through placenta under the influence of calcium ion stream. It was reported that when pregnant rats were contaminated by radiostrontium, its transfer rate increased as the period of pregnancy became longer: 0.1% of transfer rate on the 15th day of pregnancy, 0.51% on the 17th day, 2.6% on the 18th day, and 4.6% on the 19th day

Table 2. Mean birth number after the treatment of chitosan in mice

	Control[a]	Group 1[b]	Group 2[c]	Group 3[d]
No of mother	20	15	18	15
Birth No (F1)	156	119	146	125
Average birth No (F1)	7.8±1.7[e]	7.9±1.9	8.1±1.7	8.3±3.0
No of mother	12	10	11	12
Birth No (F2)	89	77	89	94
Average birth No (F2)	7.4±1.4	7.7±2.5	8.1±1.3	7.8±1.6

a. General food feeding group.
b. Chitosan powder (10%) was given at mating.
c. Chitosan powder (1%) was given at mating.
d. Chitosan solution (1%) was given at mating.
e. Mean±SD.

(Ruhmann et al., 1963; Natsusaka and Nishimura, 1982). Stather et al. (1992) reported that the transfer rate of radiostrontium was 5.3%. When radiostrontium was injected into tails of pregnant mice and rats, the fetal mice on the 20th day of pregnancy has much higher concentration of radiostrontium than the fetal rats on the 21st day of pregnancy (Onyskowova and Josifko, 1985). In the case of the mouse contaminated by radiostrontium on the 19th day of pregnancy through intraperitoneal injection, 5.4% of injected dose were transferred to fetus after 4 h, while extremely small amount of radiostrontium was transferred to fetus when radiostrontium was injected into the mouse on the early period of pregnancy or before mating (Holmberg et al., 1960). The transfer rate of 6.8% in this study was slightly higher than the previous reports.

Exoskeleton of crustaceans is composed of chitin, protein, and inorganic salts. Chitin is a linear β-(1→4) homoglycan composed of 2-acetamido-2-deoxy-D-glucopyranosyl (GlcNAc) residue and an available biopolymer obtained commercially from shrimp and crab shells. Chitosan is a chemical variation formed by deacethylation of chitin from exoskeleton of crustanceans, and it is nontoxic and natural polymer (Kurita et al., 1977). Having a regular distribution of aliphatic primary amino groups, chitosan exhibits remarkable ability to form complexes with transition metals and salts with some acids in aqueous solution. Chitin and chitosan have a number of applications (Muzarelli, 1983). In the biomedical division, its derivatives are served as detoxicants after being poisoned by radiostrontium in the animal body (Nishimura et al., 1994). Recently, the use of chitosan as a natural polymer of drug carriers has received much attention in the pharmaceutical field because of their good biocompatibility (Miyazaki et al., 1994). Kim et al. (1996) reported that chitosan caused no chromosomal aberration, while EDTA caused significant chromosomal damage. In this study, we found that the birth rate was not changed for two generations after the treatment of chitosan to pregnant mice. However, the duration of administration of chitosans was different from that in the contamination experiment. We did not test for any mutagenicity or teratogenicity in this study. Therefore, we could not conclude that chitosan is safe to use during pregnancy inspite of no change in birth rate.

For the mechanism of reducing fetal contamination of Sr-85, we propose the diminished availability of Sr-85 in fetus when water soluble chitosan was treated to female mice before and during pregnancy. Water soluble chitosans circulating in blood can chelate Sr-85 in the blood. And chitosan-strontium complex could be excreted through kidney (Kim et al., 1996). However, when Sr-85 is contaminated first, water soluble chitosan cannot reduce the contamination of fetus.

In summary, water soluble chitosan did not alter birth rate and could reduce the fetal contamination of radiostrontium, when it was administered before pregnant mice were contaminated by Sr-85.

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[Received January 31, 1997; accepted March 27, 1997]