Effect of Cadmium Administration and Aging on the Concentration of Essential Metals in Liver and Kidney

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Abstract

To investigate the changes in the concentration of essential metals in organs caused by exposure to cadmium (Cd) and by aging, Cd was administered subcutaneously in single doses of 0.3, 0.9 and 2.7 mg/kg to female rats 10 and 40 weeks of age. The animals were sacrificed on the 7th day after the administration. The concentration of Cd, zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) in the liver and the kidney was determined and the relations between the concentration of Cd and that of other metals were investigated.

- 1) In 10-week-old rats, the amount of Cd accumulated in the liver and the kidney increased linearly with increasing doses of Cd, while in 40-week-old rats, suppressed accumulation of Cd in the liver and increased accumulation of Cd in the kidney were found after administration of the high dose.
- 2) The concentration of Zn in the liver and the kidney both increased with increasing doses of Cd. There was no age-related difference.
- 3) The concentration of Cu in the liver increased in the animals which received the intermediate and the high dose, irrespective of age. The concentration of Cu in the kidney showed no changes caused by Cd administration or by aging.
- 4) The concentration of Fe in the liver increased in both 10-week-old and 40-week-old rats which received the high dose. The concentration was higher in 40-week-old rats than in 10-week-old rats. The Fe concentration in the kidney and the Mn concentration in the liver and the kidney was not affected by Cd administration or by aging.
- 5) Discriminant analysis, by age, suggested that the amount of metals in the liver and the kidney was more likely to be affected by exposure to Cd in young individuals and less likely to be affected by exposure to Cd with the increase of age.

Key words: Cadmium, Aging, Essential metals, Accumulation in body

Introduction

It is well known that high concentration of cadmium (Cd) is accumulated in the liver and kidney of some mammals, such as humans^{1, 2)}, horses^{3, 4)} and monkeys⁵⁾. Cd accumulation in humans is characterized by the fact that Cd is scarcely accumulated in the liver or kidney in fetuses or newborns but is actively accumulated in the body after birth^{2, 6-8)}, a clear sex difference is found in its concentration in organs, the concentration in females being twice as much as that in males¹⁾ and it is hardly eliminated

from the body while living⁹⁾ but is readily discharged into the blood from the liver and kidney after death.^{1, 10)}

It has been reported that Cd affects essential minerals, such as zinc (Zn) and copper (Cu), changing their concentration in the organs.^{3, 11-13)} It is possible that Cd is involved in the metabolism and accumulation of essential metals.

In the present study, we investigated the effect of Cd exposure on the concentration of such minerals in organs as Zn, Cu, iron (Fe) and manganese (Mn), which are regarded as essential metals in humans, and the effect of aging on the effect of Cd mentioned above.

Materials and Methods

Female Jcl: Sprague-Dawley rats, 8 and 26 weeks of age, were purchased from Clea Japan, Inc. Eight- and 26-week-old

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rats were maintained until 10 and 40 weeks of age, respectively, and were assigned to the experiment as the young group and old group, respectively. The animals were kept in an animal facility under controlled conditions with a temperature of 19–20 °C, relative humidity of 40–55%, and 12-hr lighting (6: 30 a.m. to 6: 30 p.m.). Tap water and Mouse/Rat Chow CE-2 (Clea Japan, Inc.) were available ad libitum. Cd was administered subcutaneously to the 10- and 40-week-old rats in a single dose of 0.3 mg/kg (low-dose group, Group II), 0.9 mg/kg (intermediate-dose group, Group III) or 2.7 mg/kg (high-dose group, Group IV). The control group (Group I) received a single subcutaneous dose of physiological saline. All the animals were sacrificed on the seventh day after dosing. The number of 10-week-old rats in each group was as follows: 11 in Groups I and II respectively and 12 in Groups III and IV respectively. As for the 40-week-old rats, there were 14 in each of the four groups. Livers and kidneys were collected and were subjected to wet ashing with sulfuric acid and nitric acid. Then the concentration of Cd, Zn, Fe and Mn was determined by flame or flameless atomic absorption spectrophotometry using a Polarized Zeeman Atomic Absorption Spectrophotometer Model 180-70 (Hitachi Co., Ltd.). The analytical data of the Cd-treated groups was compared with that of the control group using the t-test. The analytical data for both age groups was compared in the same manner. In addition, to comprehensively study the distribution of the concentration of the metals in the liver and the kidney, discriminant analysis was carried out using the SPSS/ACOS-6 at Kobe University General Information Center.

Results

The concentration of Cd in the liver and the kidney was plotted against cadmium doses (Fig. 1). The concentration of Cd in the liver increased linearly with the dose of Cd in both age groups, although it was higher in 40-week-old rats for the low and intermediate doses. In 10-week-old rats, the linear relationship was maintained even at the high dose. In 40-week-old rats, the rate of the accumulation of Cd in the liver decreased and the concentration of Cd in the liver was almost the same as that in 10-week-old rats. The concentration of Cd in the kidney increased linearly with the dose of Cd in 10-week-old rats and in 40-week-old rats. The concentration was higher in 40-week-old rats than in 10-week-old rats at the high dose.

The concentration of Zn is plotted against that of Cd in Fig. 2. The concentration of Zn in the liver increased in proportion to the concentration of Cd at Cd levels of 2 μ g/g or higher in both age groups, being about 1.2 times, 1.5 times, and 1.7 times the control level in the low-, intermediate- and high-dose groups, respectively. No age-related difference was found in the rate of increase in the concentration of Zn in the liver. The concentration of Zn in the kidney tended to increase gradually in proportion to the concentration of Cd in the kidney in both age groups, being about 1.1 times, 1.2 times, and 1.3 times the control level in the low-, intermediate- and high-dose groups, respectively. No age-related difference was found in the concentration of Zn in the kidney.

The relation between the concentration of Cu and that of Cd is depicted in Fig. 3. The concentration of Cu in the liver increased in proportion to the concentration of Cd at Cd levels of 5–6 μ g/g or higher in both age groups. In 10-week-old rats, the

concentration of Cu in the liver was about 1.2 times and 1.3 times the control level in the intermediate- and high-dose groups, respectively, showing a linear increase. In 40-week-old rats, however, the concentration of Cu in the liver was about 1.2 times the control level in both the intermediate- and high-dose groups, not showing a linear increase as it did in 10-week-old rats. The con-

Fig. 1 The relation between the dose of cadmium administered subcutaneously (mg/kg) and the concentration of cadmium in the liver and the kidney.

○: liver of 10-week-old female rat,
△: kidney of 10-week-old female rat,
●: liver of 40-week-old female rat,
▲: kidney of 40-week-old female rat
***p < 0.001 compared with 10-week-old rats.

Fig. 2 The relation between the concentration of cadmium and that of zinc in the liver and the kidney.

*p < 0.05, **p < 0.01 compared with 10-week-old rats.
++p < 0.01, +++p < 0.001 compared with the control group.
Symbols are the same as in Fig. 1.

Fig. 3 The relation between the concentration of cadmium and that of copper in the liver and the kidney.
* $p < 0.05$, ** $p < 0.01$ compared with 10-week-old rats.
++ $p < 0.01$, +++ $p < 0.001$ compared with the control group.
Symbols are the same as in Fig. 1.

Fig. 4 The relation between the concentration of cadmium and that of iron in the liver and the kidney.
* $p < 0.05$, ** $p < 0.01$ compared with 10-week-old rats.
+ $p < 0.05$ compared with the control group.
Symbols are the same as in Fig. 1.

Fig. 5 The relation between the concentration of cadmium and that of manganese in the liver and the kidney.
* $p < 0.05$ compared with 10-week-old rats.
+ $p < 0.05$ compared with the control group.
Symbols are the same as in Fig. 1.

centration of Cu in the kidney was significantly higher in 40-week-old rats in the high-dose group than it was in the control group. This was probably due to the low mean value for the control group. On the other hand, in 10-week-old rats, Cd administration caused no changes in the concentration of Cu in the kidney.

The concentration of Fe in the liver was higher and tended to vary more widely among individual animals in 40-week-old rats than in 10-week-old rats in the control group and in all of the Cd-treated groups (Fig. 4). It also tended to increase with Cd administration in both age groups. The concentration of Fe in the kidney was not affected by Cd administration or by aging.

The concentration of Mn in the liver and the kidney was not affected by Cd administration or by aging (Fig. 5).

The distributions of metals in the liver and the kidney in relation to Cd doses were subjected to discriminant analysis in all groups using the SPSS/ACOS-6. Since age-related differences were found at the 1% level, further discriminant analysis was carried out in each age group (Fig. 6). In 10-week-old rats, Function 1 is the concentration of Cd in the liver and the kidney, with a cumulative percent of 98%, and Function 2 is the concentration of Zn and Cu in the liver and the concentration of Cu in the kidney, with a cumulative percent of 1%. The hit rate determined by these functions was 100%. In 40-week-old rats, Function 1 is the concentration of Cd in the liver and the kidney and the concentration of Fe in the liver, with a cumulative percent of 97%, and Function 2 is the concentration of Zn and Cu in the liver and the concentration of Mn in the kidney, with a cumulative percent of 2%. The hit rate determined by these functions was 98%. Comparison of the territorial maps of canonical discriminant functions in different age groups (Fig. 6)

Fig. 6 Territorial map of canonical discriminant functions. Ten-week-old (upper panel) and 40-week-old (lower panel) female rats were dosed with cadmium at 0 (I), 0.3 (II), 0.9 (III) and 2.7 mg/kg (IV) and were sacrificed on the 7th day after the administration. ★ indicates a group centroid.

revealed that discrimination by Cd dose was clearer in 10-week-old rats than it was in 40-week-old rats and that particularly in Group III, to which 0.9 mg/kg Cd was given, the contribution of Function 2 was marked.

Discussion

It has been reported that in humans Cd is actively accumulated in the body after birth, shows a steady state at certain ages, and declines gradually thereafter.^{7, 8, 14} The age factor is greatly involved in such behavior of the accumulation of Cd in the organs. Therefore, in the present study, we investigated the effect of aging on the accumulation of Cd in the liver and the kidney in young and old female rats of the SD strain. The concentration of Cd in the liver and the kidney increased linearly with the Cd dose in young rats, while in old rats, the concentration of Cd in the liver showed no linear dose-response relationship at the high dose, with the accumulated amount reaching a ceiling. In contrast, an age-related increase was found in the amount of Cd accumulated in the kidney.

Shaikh et al.¹⁵ administered Cd subcutaneously in doses of 5–30 $\mu\text{mol/kg}$ to mice (4–6 months of age) of several strains and determined the concentration of Cd and metallothionein (MT) in the organs. They reported that in females, the concentration of Cd and MT in the liver reached a plateau when high doses were given, while the concentration of Cd and MT in the kidney both increased in proportion to the doses of Cd. Furthermore,

they administered Cd in doses of 25 $\mu\text{mol/kg}$ to mice 4 and 19 months of age. They found an age-related decrease in the concentration of MT in the liver but they did not find any age-related difference in the concentration of MT in the kidney. Wormser and Nir¹⁶ reported that in an experiment using rats, the Cd-induced induction and synthesis of MT in the liver and kidney decreased with age. These results suggest that acute exposure to Cd results in insufficient synthesis of MT in the liver because of aging, so that Cd which cannot bind to MT is transferred to the kidney.

Cd has been reported to be related to the changes in the concentration of essential metals in organs. In our study, in the liver in both age groups, the concentration of Zn increased proportionally to the concentration of Cd at Cd levels of 2 $\mu\text{g/g}$ or higher and the same tendency was found in the kidney. Others have also reported elevations of the concentration of Zn induced by Cd administration in rats.^{3, 11–13} Koizumi et al.³ reported that there was a positive correlation ($p < 0.001$) between Zn and Cd in the liver and kidney of horses. Torra et al.¹⁷ reported that, in humans, there was a positive correlation ($p < 0.001$) between Zn and Cd in the liver. In the study of Koizumi et al.¹⁸ using senescence-accelerated mice (SAM), the concentration of Zn in the liver and the kidney showed no age-related changes, but Cd administration increased the concentration of Zn in the liver. These results suggest that the concentration of Zn in the liver and kidney varies depending greatly on Cd in adult animals but is scarcely affected by aging.

With regard to the Cd-Cu relationship, it has been reported that Cd administration causes elevation of the concentration of Cu in the liver and kidney of rats¹³ and that Cu is positively correlated with Cd ($p < 0.001$) in the liver and kidney of horses.³ Meanwhile, it has been reported that on multiple acute exposures to toxic doses of Cd, the concentration of Cu in the liver and kidney of rats decreases at a certain concentration of Cd or higher.¹¹ In the present study, the concentration of Cu in the liver increased proportionally to the concentration of Cd at and in excess of 5–6 $\mu\text{g/g}$. Age-related differences were found in the rate of increase: a linear increase was found in young animals, while a decrease in the rate of the increase was found in old animals treated at the high dose (concentration of Cd in the liver, 36 $\mu\text{g/g}$). Thus, the concentration of Cu in the liver is considered to vary with the amount of Cd exposure and the route of the exposure, and is related to the dose when the concentration of Cd in the liver reaches or exceeds a certain level. This phenomenon persists in young rats until the concentration of Cd in the liver reaches considerably higher levels. In old rats, however, the concentration of Cd in the liver is presumed to reach a plateau at lower Cd levels than it does in young rats and to decline thereafter. It is generally considered that variations in the concentration of Cu in the liver are greatly affected by Cd administration and that such variations are modified by aging. However, it was found that the concentration of Cu in the kidney tended to increase only slightly in 40-week-old rats given a high dose of Cd and was scarcely affected by Cd administration or aging. It has been reported that the concentration of Cu in the kidney is elevated by repeated oral exposures to low doses of Cd^{13, 19} and that the concentration of Cu in the kidney of horses is positively correlated with the concentration of Cd.³ However, some investigators reported that the concentration of Cu in the kidney did not change after subcuta-

neous administration of Cd²⁰⁾ and other investigators reported that acute exposure to toxic doses of Cd caused a decrease of Cu in the kidney.¹¹⁾ On single-dose acute exposure as in the present study, no change in the concentration of Cu in the kidney was found. These findings suggest that the concentration of Cu in the kidney changes on exposure to Cd but that such changes differ according to the amount, the route and the period of exposure and are hardly modified by aging.

As regards Fe, its concentration in the liver tended to increase with Cd administration only in high-dose animals in both age groups. However, aging definitely elevated it irrespective of Cd administration. The concentration of Fe in the kidney showed no changes caused by Cd administration or by aging. Various reports^{13, 18, 20, 21)} indicate that the concentration of Fe in the organs differs according to the route of administration of Cd. Thus, Cd, when given orally, inhibits intestinal absorption of Fe, resulting in a reduction of the concentration of Fe in the organs^{13, 20, 21)} and, when given subcutaneously or intraperitoneally, it slightly increases the concentration of Fe in the organs.^{18, 20)} Our results, too, showed that Cd administered by a route other than the intestinal tract had little effect on the changes of Fe levels. On the other hand, regarding age-related changes in the concentration of Fe in the organs, a definite elevation was found in the liver. Other reports^{18, 22)} also described age-related increases in the concentration of Fe. Therefore, it can safely be said that the concentration of Fe in the organs was markedly elevated by aging.

There are some reports dealing with the relation between Cd and Mn^{13, 19)}, but none of them described any definite relation. There is hardly any report on a change of the concentration of Mn caused by aging. In the present study, the concentration of Mn in the liver and the kidney did not show any change as a result of Cd administration or aging. It is suggested, therefore, that Mn is hardly affected by these factors.

Discriminant analysis by age revealed that discrimination by Cd doses was clearer in young rats than it was in old rats. Essential metals in the liver and the kidney were possibly more sensitive to Cd in young rats so that the concentration of Zn and Cu in the liver changed at about 1 mg/kg of Cd. It was indicated, however, that in old rats, being modified by aging, the concentration of metals in the organs was less likely to be changed by Cd administration.

Among the essential metals determined in the present study,

the concentration of Zn and Cu varied depending largely on Cd. The concentration of Fe varied with age rather than with Cd. The concentration of Mn showed no Cd- or age-related change. It is suggested that the exposure to Cd is involved in the metabolism and the accumulation of essential metals. However, it is at present unknown what implications or effects these changes in concentration have in humans, in whom Cd is accumulated in high concentration. Further studies may shed more light on the relation between Cd and essential metals.

Conclusion

To investigate the changes in the concentration of essential metals in organs caused by exposure to Cd and by aging, Cd was administered subcutaneously in single doses of 0.3, 0.9 and 2.7 mg/kg to female rats 10 and 40 weeks of age. The animals were sacrificed on the 7th day after the administration. The concentration of Cd, Zn, Cu, Fe and Mn in the liver and the kidney was determined and the relations between the concentration of Cd and that of other metals were investigated.

- 1) In 10-week-old rats, the amount of Cd accumulated in the liver and kidney increased linearly with increasing doses of Cd, while in 40-week-old rats, suppressed accumulation of Cd in the liver and increased accumulation of Cd in the kidney were found after administration of the high dose.
- 2) The concentration of Zn in the liver and kidney both increased with increasing doses of Cd. There was no age-related difference.
- 3) The concentration of Cu in the liver increased in the animals which received the intermediate or the high dose, irrespective of age. The concentration of Cu in the kidney showed no changes caused by Cd administration or by aging.
- 4) The concentration of Fe in the liver increased in both 10-week-old and 40-week-old rats which received the high dose. The concentration was higher in 40-week-old rats than in 10-week-old rats. The Fe concentration in the kidney and the Mn concentration in the liver and the kidney was not affected by Cd administration or by aging.
- 5) Discriminant analysis by age suggested that the amount of metals in the liver and the kidney was more likely to be affected by exposure to Cd in young individuals and less likely to be affected by exposure to Cd with the increase of age.

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