Originals

THROMBOCYTOPENIA AND LEUKOCYTOSIS INDUCED BY SINGLE INTRAVENOUS INJECTIONS OF CADMIUM-SATURATED METALLOTHIONEINS-I AND -II IN RATS

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Abstract.....To comparably investigate hemotoxic potentials of CdCl_2 , cadmium-saturated metallothioneins-I (Cd-MT-I) and -II (Cd-MT-II), rats received single intravenous injections of one of those dissolved in saline with equivalent concentrations of Cd (0, 0.1, 0.3 and 1.0 mg Cd/kg body weight), and blood for hematological examinations was sampled at 1 and 5 days (Days 1 and 5) after the administrations. The counts of white blood cells showed dose-dependent increments in the 0.3 and 1.0 mg Cd/kg groups in Cd-MT-I and Cd-MT-II at Day 1, and returned to the normal levels at Day 5. The counts of platelets showed dose-dependent decrements in the three-doses groups of Cd-MT-I and Cd-MT-II at Day 1, and did a returning- and further increasing tendency at Day 5. The counts of red blood cells, values of hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, showed only slight and sporadic changes at Days 1 and 5. As to that thrombocytopenia and leukocytosis were dose-dependently brought by Cd-MTs and not by CdCl_2 , and as to that CdCl_2 and Cd-MTs hardly affected erythrocytes regarding their counts, sizes, hemoglobin contents etc., etiological mechanism (s) remains to be explored. However, our findings should be clinically emphasized in relation to Itai-Itai disease and Cd-intoxication.

Key words : Cadmium, metallothionein, thrombocytopenia, leukocytosis, rat.

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INTRODUCTION

Acute exposure to a high dose of cadmium (Cd) produces liver injury (Hoffman et al., 1975 ; Dudley et al., 1982), and, in contrast, chronic exposure to Cd does renal dysfunction (Friberg, 1984). This difference is surmised to be ascribable to cadmium-binding metallothioneins (MTs) which are synthesized by the liver, subsequently released into the plasma and taken up by the kidney (Dudley et al., 1985). These MTs are ion-exchange chromatographically classified to two major isoforms of metallothionein-I (MT-I) and metallothionein-II (MT-II) (Bühler and Kägi, 1974). Although the hepato- and nephrotoxic effects of CdCl₂ and cadmium-binding metallothioneins have been vigorously studied, it has been so far scarcely reported whether the CdCl₂ and cadmium-binding metallothioneins possess hemotoxic effect in the body or not.

Thus, we firstly tried to purify the MT-I and MT-II from the livers of the rats that were treated with CdCl₂, and to prepare Cd-saturated MTs (Cd-MTs). Then, administering CdCl₂, Cd-MT-I and Cd-MT-II to rats, we preliminarily studied alterations of the blood cells in general circulating system with a purpose to comparably investigate those possible hemotoxicity.

MATERIALS AND METHODS

Chemicals and Materials : Male Sprague-Dawley rats weighing nearly 200 g were obtained from Japan Charles River Co. (Atsugi, Japan), and allowed free access to water and standard diet pellet (CE-2 ; Clea Japan Inc., Tokyo, Japan) during the periods of experiments. CdCl₂ was purchased from Kanto Chemical Co. (Tokyo, Japan), and other reagents used in this study were of the highest grade available.

Purification and Cd-Saturation of MT-I and MT-II : According to the methods of Templeton and Cherian (1984) and Minkel et al. (1980), MT-I and MT-II were purified from the livers of 25 rats by gel-filtration- and anion-exchange ion-chromatographies. Cd-saturation in MT-I and MT-II was performed by the metal-exchange method that was described by Abel et al. (1987). The MT-I and MT-II saturated by Cd were freeze-dried, and kept at -80°C until use.

In the above purification steps, a FPLC system (Pharmacia LKB Biotechnology Co., Sweden) was used, and Cd contents (concentrations) were determined by the wet-digested mineralization and cation-exchange high-performance liquid chromatography with post-column reaction by 4-(2-pyridylazo)-resorcinol (Sudo et al., 1991).

Administrations of CdCl₂, Cd-MT-I and Cd-MT-II, and Sampling of Blood : CdCl₂, Cd-MT-I and Cd-MT-II were dissolved by saline, and their concentrations of Cd were adjusted to 0.01, 0.03 and 0.1 mg Cd/ml by addition of saline. Rats received single intravenous injections of the above solutions at 10 ml/kg body weight through the tail veins : accordingly, doses being 0, 0.1, 0.3, and 1.0 mg Cd/kg body

weight. The day when the administrations were done was designated as Day 0. At 1 and 5 days (Day 1 and Day 5, respectively) after the administrations, the abdominal cavity was opened under ether-anesthesia, and blood was taken from the abdominal aorta. The blood was put into tubes containing ethylenediaminetetra-acetic acid dipotassium salt, and used for the assay below.

Assay Procedures : Hematological examinations were done in the following items by a NE-6000 Hematological Analyzer (Toa Medical Electronics Co., Kobe, Japan) : counts of red blood cells, white blood cells and platelets, and, values of hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.

Statistics : Results were represented as the mean $\pm$ standard deviations (S. D.). Statistical significance was assessed by use of Student's t-test for unpaired samples, P values of less than 0.05 being considered significant.

RESULTS

One rat that had received Cd-MT-I at 1.0 mg Cd/kg body weight died at Day 4 after the administration. Excluding this case, single intravenous injections of CdCl₂, Cd-MT-I and Cd-MT-II at the three doses of 0.1, 0.3 and 1.0 mg Cd/kg body weight brought no death during the whole period of investigation.

In terms of blood cellular components (Table 1), the counts of white blood cells in the three doses of the CdCl₂-administered groups remained at the level of the saline-administered groups (control groups) at Days 1 and 5. The counts in the 0.3 and 1.0 mg Cd/kg groups in Cd-MT-I and Cd-MT-II showed dose-dependent increments at Day 1 : particularly, the values of 1.0 mg Cd/kg groups in Cd-MT-I and Cd-MT-II were approximately 2-fold of the control values. At Day 5, those returned to the control levels.

The counts of platelets at Day 1 showed decrements in the 0.1 and 1.0 mg/kg groups of CdCl₂, and in dose-dependent manner in the three-doses groups of Cd-MT-I and Cd-MT-II : the values of 1.0 mg Cd/kg groups of Cd-MT-I and Cd-MT-II remarkably fell to approximately 20 % of the control values. In contrast, the counts at Day 5 showed a returning- and further increasing tendency in the 1.0 mg Cd/kg group of CdCl₂, in the 0.3 and 1.0 mg Cd/kg groups of Cd-MT-I, and in the 0.3 mg Cd/kg group of Cd-MT-II.

The counts of red blood cells at Days 1 and 5 showed slight and sporadic changes in comparison to those of white blood cells and platelets : at Day 1, a decrement in the 1.0 mg Cd/kg group of CdCl₂, and an increment in the 1.0 mg Cd/kg group of Cd-MT-I ; at Day 5, a decrement in the 0.3 mg Cd/kg group of CdCl₂, and an increment in the 0.3 mg Cd/kg group of Cd-MT-II.

Concerned with the values of hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, only slight and sporadic changes were observed at Days 1 and 5 :

Table 1. Changes in counts of red blood cells, white blood cells and platelets, and in hematocrit values after single intravenous injections of CdCl₂, cadmium-binding metallothioneins-I and -II.

	White Blood Cells ($\times 10^4/\text{mm}^3$)		Red Blood Cells ($\times 10^4/\text{mm}^3$)		Platelets ($\times 10^4/\text{mm}^3$)		Hematocrit (%)	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
(mg Cd/kg body weight)								
Saline	1.171 $\pm$ 0.0147	1.118 $\pm$ 0.0160	658 $\pm$ 14.2	664 $\pm$ 15.4	116.7 $\pm$ 3.51	115.9 $\pm$ 3.32	41.6 $\pm$ 0.35	41.6 $\pm$ 0.30
CdCl ₂								
0.1 mg Cd/kg b. w.	1.341 $\pm$ 0.0294	0.944 $\pm$ 0.0115	641 $\pm$ 10.7	678 $\pm$ 15.0	98.2 $\pm$ 8.54**	129.1 $\pm$ 17.10	40.7 $\pm$ 1.51	42.5 $\pm$ 0.74
0.3 mg Cd/kg b. w.	1.185 $\pm$ 0.0265	1.085 $\pm$ 0.0176	647 $\pm$ 7.4	632 $\pm$ 13.7*	111.7 $\pm$ 10.66	114.2 $\pm$ 8.96	40.8 $\pm$ 0.61	40.9 $\pm$ 1.19
1.0 mg Cd/kg b. w.	1.285 $\pm$ 0.0303	1.432 $\pm$ 0.0220	616 $\pm$ 29.5**	654 $\pm$ 9.3	100.0 $\pm$ 8.86*	141.5 $\pm$ 7.29**	39.6 $\pm$ 1.31*	41.1 $\pm$ 0.82
Cd-MT-I								
0.1 mg Cd/kg b. w.	1.299 $\pm$ 0.0149	1.115 $\pm$ 0.0106	637 $\pm$ 19.1	654 $\pm$ 16.0	84.4 $\pm$ 8.46**	121.7 $\pm$ 16.75	41.4 $\pm$ 1.67	40.8 $\pm$ 1.27
0.3 mg Cd/kg b. w.	1.692 $\pm$ 0.0224**	1.126 $\pm$ 0.0217	681 $\pm$ 30.3	654 $\pm$ 28.2	66.8 $\pm$ 16.68**	130.7 $\pm$ 5.98**	42.4 $\pm$ 1.44	38.7 $\pm$ 1.29**
1.0 mg Cd/kg b. w.	2.511 $\pm$ 0.0267**	1.131 $\pm$ 0.0292	701 $\pm$ 23.6*	675 $\pm$ 125.2	28.2 $\pm$ 17.52**	139.9 $\pm$ 2.33**	43.2 $\pm$ 0.70**	41.8 $\pm$ 7.28
Cd-MT-II								
0.1 mg Cd/kg b. w.	1.506 $\pm$ 0.0430	1.044 $\pm$ 0.0051	634 $\pm$ 26.5	667 $\pm$ 13.1	87.2 $\pm$ 11.54**	124.6 $\pm$ 13.10	41.1 $\pm$ 1.02	41.5 $\pm$ 0.86
0.3 mg Cd/kg b. w.	2.062 $\pm$ 0.0331**	1.462 $\pm$ 0.0574	671 $\pm$ 14.4	838 $\pm$ 195.5*	46.5 $\pm$ 6.27**	136.2 $\pm$ 15.79*	41.6 $\pm$ 1.95	52.5 $\pm$ 12.98*
1.0 mg Cd/kg b. w.	2.132 $\pm$ 0.0710*	1.360 $\pm$ 0.0418	678 $\pm$ 26.0	655 $\pm$ 96.6	24.8 $\pm$ 10.82**	144.4 $\pm$ 28.70	42.3 $\pm$ 1.50	40.5 $\pm$ 5.64

Abbreviations : cadmium-binding metallothionein-I, Cd-MT-I ; cadmium-binding metallothionein-II, Cd-MT-II ; body weight, b. w. Values represent the mean $\pm$ S.D. Number of rats in Cd-MT-I 1.0 mg/kg body weight at Day 5 was 3, because of death in one rat at Day 4. Number in other groups was 4. Statistics, vs. Saline group in each time-point : *, $P < 0.05$; **, $P < 0.01$.

Table 2. Changes in values of hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration after single intravenous injections of CdCl₂, cadmium-binding metallothioneins-I and -II.

	Hemoglobin		Mean Corpuscular Volume		Mean Corpuscular Hemoglobin		Mean Corpuscular Hemoglobin Concentration	
	(g/dl)		(μ ³)		(pg)		(%)	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
(mg Cd/kg body weight)								
Saline	13.6±0.49	13.8±0.26	63.2±0.85	62.6±1.29	21.1±0.21	20.9±0.47	32.9±0.42	33.3±0.39
CdCl ₂								
0.1 mg Cd/kg b. w.	13.4±0.24	14.2±0.42	63.5±1.34	62.6±0.59	20.9±0.15	20.9±0.26	33.0±0.69	33.4±0.52
0.3 mg Cd/kg b. w.	13.5±0.27	13.5±0.39	63.0±0.57	64.7±2.33	20.9±0.21	21.4±0.55	33.1±0.41	33.1±0.52
1.0 mg Cd/kg b. w.	13.1±0.46	13.7±0.10	64.4±1.16	62.9±0.52	21.2±0.33	21.0±0.37	33.1±0.37	33.3±0.63
Cd-MT-I								
0.1 mg Cd/kg b. w.	13.5±0.50	13.5±0.44	65.0±1.71	62.3±1.76	21.2±0.33	20.6±0.57	32.6±0.37	33.1±0.41
0.3 mg Cd/kg b. w.	13.9±0.34	13.0±0.52*	62.3±0.79	59.2±1.45*	20.4±0.46*	19.8±0.29**	32.8±0.43	33.5±0.49
1.0 mg Cd/kg b. w.	14.2±0.22	14.0±2.83	61.7±1.37	62.0±0.71	20.3±0.37**	20.8±0.35	32.9±0.25	33.5±0.92
Cd-MT-II								
0.1 mg Cd/kg b. w.	13.4±0.26	13.8±0.21	64.8±2.07	62.2±0.39	21.1±0.64	20.6±0.17	32.6±0.22	33.2±0.19
0.3 mg Cd/kg b. w.	14.0±0.50	17.4±3.82	62.1±4.15	62.5±1.89	20.8±1.13	20.8±0.43	33.6±0.83	33.3±1.04
1.0 mg Cd/kg b. w.	13.9±0.60	13.4±2.05	62.3±0.64	61.9±1.80	20.5±0.32*	20.5±0.40	32.9±0.70	33.1±0.54

Abbreviations : cadmium-binding metallothionein-I, Cd-MT-I ; cadmium-binding metallothionein-II, Cd-MT-II ; body weight, b.w. Values represent the mean ± S.D. Number of rats in Cd-MT-I 1.0 mg/kg body weight at Day 5 was 3, because of death in one rat at Day 4. Number in other groups was 4. Statistics, vs. Saline group in each time-point : * P<0.05 ; ** P<0.01.

hematocrit, a decrease in the 1.0 mg Cd/kg group of CdCl₂ and an increase in the 1.0 mg Cd/kg group of Cd-MT-I at Day 1, and a decrease in the 0.3 mg Cd/kg group of Cd-MT-I and an increase in the 0.3 mg Cd/kg group of Cd-MT-II at Day 5 ; hemoglobin, a decrease in the 0.3 mg Cd/kg group of Cd-MT-I at Day 5 ; mean corpuscular volume, a decrease in the 0.3 mg Cd/kg group of Cd-MT-I at Day 5 ; mean corpuscular hemoglobin, decreases in the 0.3 and 1.0 mg Cd/kg groups of Cd-MT-I at Day 1 and in the 0.3 mg Cd/kg group at Day 5 ; mean corpuscular hemoglobin concentration, no changes in any groups at Days 1 and 5.

DISCUSSION

Molecular weights of MTs are 6,000-7,000 daltons, and 1-g molecule of Cd-saturated MTs contains 7-g atoms of Cd (Dunn et al., 1987). We prepared CdCl₂- and Cd-MTs solutions in order that Cd concentrations could be equivalent among each other. In this case, 1.0 mg Cd/ml solutions of Cd-MTs contained approximately 8 mg and more of Cd-MTs/ml. In order to obtain MTs, 25 rats were used in this study. However, the MTs obtained were too small to achieve this study with more than 4 in experimental number in each group. This difficulty in obtaining sufficient amounts of Cd-MTs is seemed likely to be raised as a reason why whole-body-studies of MTs have been reported quite less than those of CdCl₂.

In our study, CdCl₂ and Cd-MTs hardly affected erythrocytes regarding their counts, sizes, hemoglobin contents etc., during the whole experimental periods. Differing from our result, Cd-exposure has been reported to bring slight anemia with a decreased hemoglobin level (Bernard et al., 1979). As the etiological factors, Cd-interference with iron absorption from food in the intestines (Fox et al., 1971) and calcium-induced hemolysis (Elinder, 1985) have been raised. Nevertheless, this anemia reported is of the cases for the workers that Cd was exposed during more than several years (Bernard et al., 1979), and, of the cases for the experimental animals that Cd was consecutively administered for more than two months (Fox et al., 1971). Accordingly, the anemia manifested by Cd, which was not found in our study, is surmised to be ascribable to the length of duration in the Cd-exposure : single dosage in ours, and, consecutive and chronic exposure (dosage) in the precedents of literature.

Our major findings are that thrombocytopenia and leukocytosis were brought by Cd-MTs and not by CdCl₂ in the equivalent Cd dose-levels at 1 day after their intravenous administrations.

In terms of the thrombocytopenia observed, we surmised its etiology as follows : Cd-MTs in the blood directly affected and destroyed the platelets ; Cd-MTs in the blood were taken into the bone marrow, and selectively suppressed the platelet-production cellular system including megakaryoblasts, promegakaryocytes and megakaryocytes ; or, Cd-MTs resulted in hemorrhages in the body, and depletion of platelets was brought by resultant coagulation. Concerned with the last possibility,

several reports have revealed that the acute and chronic Cd-administrations in animals manifested hemorrhages in the liver (Tsunoo et al., 1979 ; Hata et al., 1980), in the placenta (Parizek, 1964, 1965 ; Chiquoine, 1965), and in the testes (Gunn et al., 1965 ; Berliner and Jones-Witters, 1975 ; Wada et al., 1982). This might imply that Cd-MTs possessed more severely hemorrhagic potentials than CdCl₂ did, whereas other possibilities could not be ruled out as the reasons for the manifestation of thrombocytopenia.

As to the leukocytosis observed, it has been reported that the administration of CdCl₂ in animals resulted in inflammations attending other pathohistological changes in the liver, kidneys and testes (Stowe et al., 1972 ; Dudley et al., 1985), and that the persons chronically exposed by Cd had manifested granulocytosis with a tendency toward lymphocytopenia (Shinoda et al., 1977). Accordingly, the leukocytosis in our study might be explained by the above inflammatory reaction. Nevertheless, a possibility that Cd-MTs had directly stimulated the bone marrow and/or lymphnodes and selectively accelerated the production of leukocytes including lymphocytes and others, could not be ruled out as the etiological reasons.

In conclusion, our study was preliminarily done to explore whether CdCl₂ and Cd-MTs possessed hemotoxic potentials or not, and lacked more detailed examinations including hemogram, myelogram and so on. Accordingly, our etiological consideration concerned with the Cd-MTs-induced thrombocytopenia and leukocytosis is obliged to remain within mere conjectures. In addition, in investigations of CdCl₂ and Cd-MTs in humans and animals, interests of researchers have been mainly concentrated on hepatic and renal dysfunctions (Hoffman et al., 1975 ; Dudley et al., 1982 ; Friberg, 1984) : above all, such hemotoxic effects of Cd-MTs that were found in our study have not yet been reported. Thus, our findings of Cd-MTs-induced thrombocytopenia and leukocytosis should be clinically emphasized in relation to Itai-Itai disease and Cd-intoxication. Further etiological investigations on possible hemotoxic mechanism (s) of Cd-MTs in the body remain to be done.

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