Alteration of Manganese Distribution in Rats under Drug-Treatments

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SUMMARY

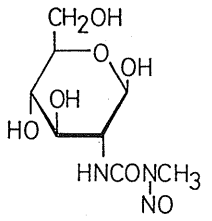
Distribution of manganese in organs and subcellular fractions of rats treated with an experimental diabetogenic drug, streptozocin (STZ), or a platinum-containing anticancer drug, cisplatin was investigated.

Male Wistar rats were given a single intravenous injection of 60 mg/kg STZ, and a subcutaneous injection of 5 mg/kg cisplatin. Manganese content was analyzed by neutron activation analysis. Treatment of STZ resulted in unchanged Mn levels in most organs. Manganese content, however, was decreased in the liver mitochondrial fraction and increased in a supernatant fraction. Manganese levels in both the liver and kidney of rats treated with cisplatin were increased after 7 days of drug administration.

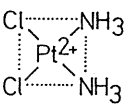
These results indicate that the distribution of trace elements such as manganese in organs and subcellular fractions depends on the pathological conditions caused by drug administration.

INTRODUCTION

Manganese is an essential element in living matter and is known to accumulate in organs[1]. Various enzymes require manganese for their activities[2]. These enzymes include pyruvate carboxylase, superoxide dismutase, RNA and DNA polymerases, manganese-catalase and others. Therefore, a deficiency or excess of manganese may alter some biological functions. Exogeneous substances such as drugs and foods may change the intrinsic distributions of manganese. We are interested in the molecular functions of manganese in pathological conditions as well as in normal conditions. The present study reports the distributions of manganese in various organs and its subcellular localization in the livers of streptozocin (STZ)-induced diabetic, and cisplatin (anticancer drug)-treated rats to examine alterations in the essential metal in pathological conditions.



Streptozocin (STZ)



Cisplatin

EXPERIMENTAL

Male Wistar rats weighing 170—200 g were allowed free access to a commercial pellet (Oriental MF, Oriental Yeast Co. Tokyo, Japan) and given tap water *ad libitum*. The manganese contents in the pellet and water were 64.5 μg/g and 24.0 ng/g, respectively. Rats (n=2–5 in each group) were given a single *ip* injection of STZ (60 mg/kg) to induce diabetes, and another series of rats (n=2 in each group) received a single *sc* injection of cisplatin (5mg/kg). Diabetic condition thus induced was monitored by a measurement of blood glucose level by the method previously described[3]. Manganese treatment was carried out by *ip* injection of 15 mg/kg body weight/day of $MnCl_2.4H_2O$ for 2 weeks. Organs were pooled from each group. Subcellular fractionation was followed by the method of Hogeboom. Protein was measured by Lowry's method. Details of neutron activation analysis have been presented elsewhere[4].

RESULTS

Manganese distribution in rat organs; Table 1 (columns 1 and 3) shows the manganese content in various organs of rats before and after the manganese treatment. Naturally occurring manganese levels were low in many organs, but relatively high contents of manganese were found in the pancreas, kidney and liver. The metal contents in these organs were greatly enhanced by the administration of manganese.

Table 1. Distribution of manganese in organs of rats treated with STZ and/or $MnCl_2$

Organ	Mn level (nmol/g protein) after the treatment					
	(1) Control	(2) STZ	(3) Saline-Mn	(4) STZ-Mn	(5) Mn-STZ-Saline	(6) Mn-STZ-Mn
Brain	8.8	10.0	40.2	41.7	38.6	81.6
Thymus	ND	0.4	21.6	29.2	96.5	78.1
Heart	ND	ND	–	–	–	–
Lung	ND	ND	–	–	–	–
Pancreas	35.8	30.7	499.7	192.7	101.4	659.8
Spleen	ND	ND	52.2	23.5	64.0	29.2
Kidney	25.8	22.7	181.7	77.2	55.7	189.5
Liver	67.7	73.1	115.7	114.3	142.8	166.1
Blood glucose level (mg/100ml)	75±9	415±49	72±9	340±53	253±20	240±13

ND = not detected. (–) = not determined.

Effect of STZ on manganese distribution; As shown in Table 1 (columns 2–4), STZ administration

did not affect the natural levels of manganese. When animals with STZ-induced diabetes were given manganese, less manganese accumulated in the spleen, pancreas and kidney than in the organs of manganese-treated normal rats. However, the manganese content in the brain, thymus and liver of the diabetic rats was not increased by manganese administration.

Effect of STZ pre- and post-administered manganese; Since the added manganese was not retained in many organs of the diabetic rats (Table 1, column 4), whether or not the pre-administered manganese is affected by the later-administered STZ is examined. Columns 3 and 5 indicate that the STZ-induced diabetes reduced the pre-administered manganese in organs such as the pancreas and kidney, but increased it in the thymus, spleen and liver. The result of column 6 shows that manganese after the manganese-STZ administration accumulated in most organs except thymus and spleen.

Manganese distribution in the liver subcellular fractions; Although the liver is a primary site of drug metabolism, the manganese content in the whole liver was not greatly affected by STZ-induced diabetes. Therefore, the subcellular distribution of manganese in the liver was examined. In Table 2, most manganese was localized in nuclear and mitochondrial fractions (column 1). With STZ (column 2), the content in the nuclear fraction was unchanged, but that in the mitochondrial and microsomal fractions reduced. Manganese administration after STZ, however, increased the content in mitochondria (columns 3 and 4). The change of order in STZ and manganese administrations did not remarkably alter the distribution of manganese (columns 4 and 5). Manganese administration after the manganese-STZ treatment enhanced the manganese accumulation in all subcellular fractions (columns 5 and 6).

Table 2. Distribution of manganese in liver subcellular fractions of rats treated with STZ and/or $MnCl_2$

Subcellular Fraction of Liver	Mn level (nmol/g protein) after the treatment					
	(1) Control	(2) STZ	(3) Saline-Mn	(4) STZ-Mn	(5) Mn-STZ-Saline	(6) Mn-STZ-Mn
Nuclear	110.0	110.9	144.7	140.9	158.7	261.0
Mitochondrial	93.9	62.6	126.0	175.8	161.6	257.0
Microsomal	12.9	8.7	30.0	31.6	32.1	70.7
Supernatant	14.6	23.8	56.7	69.4	83.3	176.7

Effect of cisplatin on manganese distribution in rat liver and kidney; Cisplatin-administration produced no alterations of manganese levels after 24 hr. but increased after 7 days for the whole homogenates of both liver and kidney, compared with the levels of control organs (Table 3). However, the manganese levels of subcellular fractions showed complicated features; the manganese content in the nuclear and mitochondrial fractions decreased after 24 hr. but increased after 7 days of drug administration, while the contents of microsomal and supernatant fractions gradually decreased by day after the treatment of drug.

DISCUSSION

It is possible that pathological conditions may alter the distribution of manganese in organs and cellular compartments, and unbalanced manganese levels result in biological disorders. In this study, we examined the STZ effects on naturally occurring manganese levels, the pre-STZ supplemented manganese levels and the post-STZ supplemented manganese levels in various organs. The results obtained are summarized in Tables 1 and 2. The natural manganese levels were low in most organs and the levels were not altered by the diabetic conditions. However, once diabetes occurred, the manganese levels in the spleen, pancreas and kidney were not elevated to the levels in control rats by the addition of manganese. The study also examined whether the effect of STZ in reducing manganese levels is overcome by the second addition of manganese. Administration after STZ elevated the manganese contents in the brain, pancreas, kidney and liver, but the contents in the thymus and spleen remained low. The manganese level in the liver was relatively insensitive to STZ.

The subcellular distribution of manganese was altered by the STZ-induced diabetes, suggesting that such an essential trace element as manganese can be transferred from one compartment to another according to the pathological conditions.

A single injection of cisplatin produced the alteration of manganese levels depending on days after the drug-treatment (Table 3). It seems likely that for the first 24 hr. the effect of cisplatin does not occur but after 7 days both the elevation of total manganese level and the alteration of manganese distribution in subcellular fractions in both the kidney and liver are produced.

Table 3. Distribution of endogenous manganese in liver and kidney of rats treated with cisplatin

Organ	Fraction	Mn level (nmol/g protein)		
		Control	Cisplatin-treated	
			24hr.	7 days
Liver	Whole homogenate	71.8	71.2	81.6
	Nuclear	129.4	103.2	141.9
	Mitochondrial	117.0	102.7	175.9
	Microsomal	64.4	49.1	35.1
	Supernatant	18.4	20.8	5.2
Kidney	Whole homogenate	35.2	35.8	45.6
	Nuclear	56.2	36.8	84.9
	Mitochondrial	54.8	37.8	92.4
	Microsomal	22.2	22.8	22.8
	Supernatant	7.0	0.6	0.0

On the basis of these results, it may tentatively be indicated that by STZ-treatment, endogenous

manganese shifts to soluble fraction from mitochondrial and microsomal fractions, while by cisplatin-treatment, naturally occurring manganese re-distributes to nuclear and mitochondrial fractions from microsomal and soluble fractions. Therefore, it may be very possible that the distribution of trace elements such as manganese in organs and their subcellular fractions depends on the pathological conditions caused by drug administration.

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