Originals

STUDY ON THE ABSORPTION AND PROTEIN BINDING OF CARBARYL, DIELDRIN AND PARAQUAT IN RATS FED ON PROTEIN DIET

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Abstract.....Carbaryl was well absorbed from the small intestine of rats. In the cytosol fraction of intestinal mucosa, it was bound to a smallmolecular component (M. W. 2,200 daltons). In serum, carbaryl was bound mainly to albumin and partly to globulins. Dieldrin was slowly absorbed from small intestine. The peak of dieldrin concentration in blood was observed 2.5 hr after the administration. Binding of dieldrin to mucosal protein in cytosol was not detectable. In serum, dieldrin was bound to lipoproteins, globulins and partly to albumin. Absorption of paraquat from the small intestine was more rapid than that of dieldrin in situ study. However, in vivo study, the absorption of paraquat was the lowest among these three chemicals. In the cytosol fraction of intestinal mucosa, paraquat was bound to a small-molecular component (M. W. 3,100 daltons). In serum, it was present in unbound fraction. In the comparative studies with protein diets, absorption of paraquat from small intestine in situ was higher in low protein diet rats than that in high protein diet rats. However, difference in absorption in vitro and in histopathological study was not noted between low protein diet rats and high protein diet rats.

Key words: absorption, protein binding, carbaryl, dieldrin, paraquat

INTRODUCTION

In the previous paper (Tanaka, 1980), distribution and biliary excretion of agricultural chemicals, carbaryl, dieldrin and paraquat, were studied in rats fed on different protein diet. In that study, lower retention in organs and higher biliary excretion of chemicals were found in the group of high protein diet rats compared with those of low

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protein diet rats.

However, organ distribution of the chemicals would be influenced by absorption from gastrointestinal tract, metabolic function of liver, affinity to tissues and others.

In the present paper, we studied the effect of protein concentration in diet on gastrointestinal absorption of chemicals and the protein binding of chemicals to detect carrier components of chemicals.

Carbaryl, dieldrin and paraquat were mainly absorbed from small intestine of rats in our experiments by measuring radioactivity in portal venous blood and bile of rats administered with ^{14}C -labeled chemicals. Therefore, we injected the chemicals into small intestine of rats under the collection of bile for the purpose of avoiding the inflow of bile to small intestine, in situ study.

MATERIALS AND METHODS

Four-weeks old male Sprague - Dawley rats were divided into three groups. Group 1 was fed on a normal diet (casein 24.5%), group 2 was fed on a low protein diet (casein 5%) and group 3 was fed on a high protein diet (casein 45%) for 8-10 weeks before experiment, respectively. Diets were prepared by Japan Clear, Ltd (Japan). The composition of diets is showed in Table 1.

^{14}C -carbaryl (59.5 mCi/mmole), ^{14}C -dieldrin (85 mCi/mmole) and ^{14}C -paraquat chloride (111 mCi/mmole) were obtained from The Radiochemical Centre, Amersham (USA).

Unlabeled carbaryl, dieldrin and paraquat chloride were purchased from Wako Pure Chemicals Industries, Ltd. (Japan).

In vivo study : After 16 hr of fasting, rats were administered with each chemical via a stomach catheter (one ml of test chemical solution), under a light anesthesia with ether. Concentrations of chemicals in test chemical solution were 5 mg/ml of corn oil (10 $\mu\text{Ci/ml}$) for carbaryl, 0.46 mg/ml of corn oil (10 $\mu\text{Ci/ml}$) for dieldrin or 0.46 mg/ml of water (10 $\mu\text{Ci/ml}$) for paraquat chloride, respectively.

Tabel 1 Diet composition

	Group 1	Group 2	Group 3
Casein	24.5%(g/100g)	5.0%	45.0%
Corn starch	47.8	67.3	27.3
Vegetable oil	6.0	6.0	6.0
Cellulose	5.0	5.0	5.0
Vitamin mixture ^a	1.0	1.0	1.0
Mineral mixture ^b	6.2	6.2	6.2
Sucrose	9.5	9.5	9.5

a: Vitamin A 1000 I. U., Vitamin D₃ 200 I. U., Vitamin E 2.3, B₁ 1.3, B₂ 1.4, B₆ 1.1 and B₁₂ 0.004 mg/100g, respectively, Ca-Pantothenate 3.8 mg/100 g, Niacine 11.6 mg /100 g, folic acid 0.1 mg/100 g, Choline 228 mg/100 g.

b: Ca 1.0, P 1.0, Mg 0.27, Na 0.31; K 0.85, Mn 6.0, Fe 10.0 g/100g

One or five hr after the administration of chemicals to rats, blood samples were collected exhaustively by heart puncture under ether anesthesia, and small intestine was excised. The excised small intestines were rinsed with saline and separated into mucosa and serosa using a slide glass as a scraper. Radioactivities in mucosa, serosa, rinsed saline and blood samples were determined in a liquid scintillation spectrometer as described previously (Tanaka, 1980).

To determine the distribution of the chemicals in subcellular organelles, small intestinal mucosa was fractionated into cell debris (CD), mitochondria (Mt), microsome (Ms) and cytosol (Cs) by differential centrifugation at 4°C. One gram of mucosa (wet) was homogenized in 9 ml of cold 0.25 M sucrose solution containing 3 mM Tris-HCl and 0.1 mM EDTA by Teflon homogenizer (10 strokes, 5 min), and centrifuged at 1,000 g for 15 min. After the centrifugation, the precipitate was designated as CD fraction and the supernatant was recentrifuged at 10,000 g for 60 min, resulting precipitate (Mt) and supernatant which is separated to Ms (precipitate) and Cs (supernatant) by a centrifugation of 104,000 g for 60 min.

Protein concentration of CD, Mt, Ms and Cs was determined according to Lowry et al (1951) using bovine serum albumin as a standard, and radioactivity in those fractions was determined in a liquid scintillation spectrometer (Mark II, Nuclear Chicago) after solubilizing in NCS as described in previous paper (Tanaka, 1980).

In situ study : Small intestine of rat fasted for 16 hr was partitioned to three closed loops of 10 cm long segment tied with silk thread, and bile was collected from polyethylene tube cannulated into the bile duct under pentobarbital (30 mg/kg, i. p.) anesthesia (Bagnall, 1979).

One or five hours after the administration of 100 μ g chemicals (0.2 μ Ci) into each loops, loops were excised and rinsed with saline.

Radioactivity in mucosa, serosa or rinsed saline of each loops was determined as described above. Absorption of chemicals across small intestine was calculated by subtracting the radioactivity in mucosa, serosa and rinsed saline from total radioactivity administered.

To determine the absorption via lymph duct and biliary excretion of the chemicals, lymph liquid from truncus intestinalis and bile from bile duct were collected from the rats administered with chemicals (0.2 ml of chemicals solutions) into the small intestinal loops.

For the time course study of radioactivity in blood following oral administration of chemicals in rats, 200 μ l of blood samples were collected from Nelaton's catheter connected to femoral artery.

Protein binding of chemicals : Cytosol fraction of small intestinal mucosa of rats administered with 14 C-labeled chemicals were prepared as described in vivo study, and chemical binding proteins in cytosol was separated by a Sephadex G-25 gel filtration technique with 0.15 M NaCl.

Serum samples of in vivo experiments were separated from blood obtained by heart

puncture.

In vitro protein binding study was carried out by incubation of rat serum with ^{14}C -labeled chemicals for 60 min at 37°C .

Both of serum samples, in vivo and in vitro, were applied on a Sephadex G-200 gel filtration and an agarose gel electrophoresis.

After the Sephadex gel filtration, ^{14}C -radioactivity of each fraction was determined in a liquid scintillation spectrometer with Bray's scintillation liquid, and protein concentration of each fraction was determined by UV absorbance at 280 nm.

After the agarose gel electrophoresis, gel was cut in halves lengthwise. One half of gel was sliced in 2 mm width, and ^{14}C -radioactivity in each slice was determined in a liquid scintillation spectrometer with Bray's scintillation liquid. The remaining half of gel was stained by Ponceau 3 R or Sudan Black B to detect protein or lipoprotein.

RESULTS

In vivo study: ^{14}C -radioactivity in the whole gastrointestinal tract of rats at 1 hr and 5 hr after oral administration of chemicals is showed in Table 2. The highest radioactivity was found in paraquat-administered rats among the three chemicals used, through the dietary divided three groups. Significant difference between group 2 and group 3 was not noted in carbaryl- and dieldrin-administered rats at 1 hr and 5 hr after the administration, however only significant difference was noted in paraquat-administered rat at 5 hr ($p < 0.05$). Table 3 shows the retention of radioactivity in small intestinal mucosa or serosa of those rats. In group 1, the highest radioactivity was detected in mucosa and serosa of paraquat-administered rats at 1 hr and in those of dieldrin-administered rats at 5 hr. Radioactivity in mucosa and serosa of paraquat-administered rats at 5 hr was decreased than at 1 hr, however it was increased when dieldrin was administered. When carbaryl was administered, radioactivity existed in mucosa and serosa of group 2 was higher at 5 hr than at 1 hr, but opposite result was obtained in those of group 3. Radioactive content in mucosa and serosa of carbaryl-administered rats at 1 hr was higher in group 3 than group 2 ($p < 0.05$), and that in mucosa at 5 hr was higher in group 2 than group 3 ($p < 0.05$). Furthermore, radioactivity in mucosa and serosa of paraquat-administered rats at 5 hr was higher in group 2 than group 3 ($p < 0.05$).

Radioactive distribution in subcellular organellae of small intestinal mucosa of group 1 was showed in Fig. 1. With a carbaryl administration, the highest radioactivity was detected in Cs, and with a dieldrin administration, the lowest radioactivity was detected in Cs. In the paraquat-administered rats, the lowest radioactivity was detected in Mt. Significant difference of radioactive distribution in subcellular organellae of small intestinal mucosa was noted between group 2 and group 3.

Radioactive concentration in blood obtained from rats administered with chemicals was showed in Table 4. In group 1, radioactivity was the same level at 1 hr and at 5 hr after the carbaryl administration, however, it was higher at 5 hr than at 1 hr in dieldrin-administered rats, and it was lower at 5 hr than at 1 hr in paraquat-administered rats.

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Table 2 ^{14}C -radioactivity* in the whole gastrointestinal tract at 1 hr and 5 hr after the oral administration of chemicals in rats

	1 hr			5 hr		
	Carbaryl	Dieldrin	Paraquat	Carbaryl	Dieldrin	Paraquat
Group 1	55.09 $\pm$ 1.17	59.50 $\pm$ 0.44	95.04 $\pm$ 2.05	32.48 $\pm$ 2.35	27.39 $\pm$ 1.48	63.28 $\pm$ 7.02
Group 2	44.93 $\pm$ 3.81	61.24 $\pm$ 22.02	91.53 $\pm$ 1.00	21.73 $\pm$ 1.50	17.52 $\pm$ 3.45	51.51 $\pm$ 4.60 ^e
Group 3	54.87 $\pm$ 9.43	88.58 $\pm$ 11.41	92.43 $\pm$ 4.78	30.71 $\pm$ 3.09	34.45 $\pm$ 9.62	67.90 $\pm$ 2.50 ^e

* % of dose

Doses were described in method of text.

Values are the mean $\pm$ S. E. of three animals.

e: Significant difference between group 2 and group 3 ($p < 0.05$)

Group 1: normal diet rats, Group 2: low protein diet rats, Group 3: high protein diet rats

Table 3 Retention of radioactivity* in small intestine of rats fed on normal (group 1), low (group 2) or high (group 3) protein diet

	Carbaryl		Dieldrin		Paraquat	
	Mucosa	Serosa	Mucosa	Serosa	Mucosa	Serosa
Group 1	0.75 $\pm$ 0.09	0.45 $\pm$ 0.09	0.45 $\pm$ 0.10	0.23 $\pm$ 0.04	2.24 $\pm$ 0.26	1.47 $\pm$ 0.20
1 hr Group 2	0.42 $\pm$ 0.05 ^c	0.30 $\pm$ 0.09 ^d	0.72 $\pm$ 0.20	0.24 $\pm$ 0.08	1.64 $\pm$ 0.37	1.51 $\pm$ 0.23
Group 3	1.41 $\pm$ 0.23 ^c	0.99 $\pm$ 0.24 ^d	0.88 $\pm$ 0.24	0.24 $\pm$ 0.05	1.61 $\pm$ 0.44	1.29 $\pm$ 0.22
Group 1	0.51 $\pm$ 0.03	0.31 $\pm$ 0.04	1.39 $\pm$ 0.06	0.67 $\pm$ 0.07	0.25 $\pm$ 0.01	0.11 $\pm$ 0.02
5 hr Group 2	1.02 $\pm$ 0.02 ^e	0.55 $\pm$ 0.09	1.95 $\pm$ 0.65	1.08 $\pm$ 0.47	0.54 $\pm$ 0.06 ^f	0.38 $\pm$ 0.03 ^f
Group 3	0.71 $\pm$ 0.09 ^e	0.55 $\pm$ 0.09	1.73 $\pm$ 0.74	0.79 $\pm$ 0.43	0.21 $\pm$ 0.17 ^f	0.12 $\pm$ 0.01 ^f

* % of dose /g tissue

Rats were orally administered with chemicals.

Doses were described in method of text.

Values are the mean $\pm$ S. E. of three animals.

c,d,e: Significant difference between group 2 and group 3 ($p < 0.05$)

f: Significant difference between group 2 and group 3 ($p < 0.01$)

In a comparative study, higher radioactivity in blood of group 2 than in that of group 3 was observed at 1 hr with carbaryl administration.

In situ study: Fig. 2 shows the absorption of radioactive chemicals across small intestine and retention of those in intestinal mucosa and serosa of normal diet rats administered with 100 μg (0.2 μCi) of chemicals in each loops. The highest absorption rate was observed in carbaryl-administered rats and the lowest absorption rate was observed in dieldrin-administered rats. Retention in mucosa was the highest when paraquat was administered.

Table 5 shows the absorption and retention of radioactive chemicals for 1 hr in group 2 and group 3. Significant difference between group 2 and group 3 was observed only in absorption of paraquat. Absorption across small intestine of group 2 was higher than that of group 3.

Fig. 1 Radioactive distribution in subcellular organelles of small intestinal mucosa of normal diet rats at 1 hr ( ) and 5 hr ( ) after the oral administration of chemicals in vivo.

Doses were described in method of text.

CD: cell debris, Mt: mitochondria, Ms: microsome, Cs: cytosol

Values are the mean $\pm$ S. E. of three animals.

Table 4 Radioactive concentration (% of dose) in blood samples collected by heart puncture of rats fed on normal (group 1), low (group 2) or high (group 3) protein diet

	1 hr			5 hr		
	Carbaryl	Dieldrin	Paraquat	Carbaryl	Dieldrin	Paraquat
Group 1	0.0379 $\pm$ 0.0072	0.0051 $\pm$ 0.0019	0.0166 $\pm$ 0.0021	0.0522 $\pm$ 0.0046	0.0382 $\pm$ 0.0033	0.0026 $\pm$ 0.0022
Group 2	0.1764 $\pm$ 0.0114 ^c	0.0056 $\pm$ 0.0055	0.0492 $\pm$ 0.0126	0.0894 $\pm$ 0.0122	0.0394 $\pm$ 0.0014	0.0029 $\pm$ 0.0005
Group 3	0.0354 $\pm$ 0.0109 ^c	0.0035 $\pm$ 0.0026	0.0165 $\pm$ 0.0031	0.0596 $\pm$ 0.0082	0.0501 $\pm$ 0.0142	0.0032 $\pm$ 0.0003

Rats were orally administered with chemicals.

Doses were described in method of text.

Values were mean $\pm$ S. E. of three animals.

c: Significant difference between group 2 and group 3 ($p < 0.01$)

Radioactivity of blood in femoral artery of rats orally administered with radioactive chemicals is showed in Fig. 3. In carbaryl-administered rat, the highest radioactivity was observed immediately after administration, then the activity decreased gradually till 3.5 hr after administration. The activity increased again, kept the same level for a period of 4-7 hr after administration. In dieldrin-administered rats, the highest radioactivity was detected at 2-3 hr after the administration. In paraquat-administered rats, the highest radioactivity was detected within 1 hr after administration, and it was significantly decreased by time.

Protein binding of chemicals : The binding of chemicals with cytosol components in small intestinal mucosa of rats at 1 hr and 5 hr after the administration was studied by gel filtration with Sephadex G-25 (Fig. 4). Radioactivity was detected in small molecular fraction : 2,200 daltons for carbaryl, 3,100 daltons for paraquat. However the binding component of dieldrin was not detectable in cytosol.

Binding of chemicals to serum protein was studied by gel filtration and agarose gel electrophoresis. In vivo binding experiment, carbaryl was bound mostly to albumin and partly to globulin and lipoprotein with Sephadex G-200 gel filtration as showed in Fig. 5. Dieldrin was bound mostly to lipoprotein, globulin and partly to albumin. Binding protein of paraquat was not detectable in serum because paraquat was present only in

Fig. 2 Absorption and retention of ¹⁴C-radioactivity (% of dose) in small intestine of normal diet rats at 1 hr and 5 hr after the administration of chemicals in situ.

*100 μ g (0.2 μ Ci)/10 cm loop of small intestine

Values are the mean $\pm$ S. E. of six loops.

Fig. 3 Blood concentration of radioactivity in femoral artery of orally administered rats with chemicals under pentobarbital anesthesia
Doses are same as those described in vivo study.

Fig. 4 Sephadex G-25 separation of cytosol fraction of intestinal mucosa obtained from rats orally administered with chemicals

Fig. 5 Sephadex G-200 separation of rat serum prepared from blood obtained by heart puncture
Rats were orally administered with chemicals.

unbound fraction. In vitro binding experiment, similar results to the in vivo binding experiments were obtained by gel filtration. Binding of carbaryl to albumin, α -lipoprotein and β -lipoprotein was detected by agarose gel electrophoresis in vitro study.

Quantitative protein binding study of carbaryl and dieldrin were not performed because those chemicals were insoluble in water.

DISCUSSION

Normal diet is produced from natural materials, while low and high protein diets are produced from purified diet components. The diet effect on chemical absorption and retention were compared between group 2 (low protein diet rats) and group 3 (high protein diet rats) since Boyd (1968) reported the higher toxicity of carbaryl in rats fed on purified diets than rats fed on normal diets. In situ experiments on intestinal absorption (Fig. 2) and concentration of chemicals in blood (Fig. 3) suggested the rapid and higher absorption rate of carbaryl from rat intestine than other two chemicals examined. Moreover, higher retention and protein binding property of carbaryl in cytosol of small intestinal mucosa and in serum indicated that absorption of it would be mediated by binding component in cytosol of small intestine, and it would be carried

with serum proteins, especially albumin in blood. Higher concentration of carbaryl in blood than that of paraquat, at 5 after the administration (Fig. 3) suggested the slower excretion of carbaryl from kidney than that of paraquat because of protein binding property of carbaryl.

Dieldrin did not bind with cytosol component (Fig. 4) and its absorption from small intestine was the slowest (Fig. 3) suggesting the different absorption mechanism from other two chemicals.

Binding component of paraquat in cytosol of rat intestinal mucosa was low molecular substance different from carbaryl binding component as showed in Fig. 4. Small intestinal mucosa, contents negatively charged substances, binds to positively charged chemicals which inhibit absorption of paraquat in small intestine (Carf, 1964). Therefore, positively charged paraquat would bind to negatively charged substances in small intestine of rats. Unpresence of carrier protein of paraquat in serum may cause rapid excretion of it from kidney.

Rose et al (1974) reported the energy dependent accumulation of paraquat into lung of rat, however, absorption mechanism of paraquat in small intestine is seemed to be different from than in lung, since in vitro absorption study using small intestinal sac in Krebs solution indicated the highest passage of paraquat across small intestinal wall among three chemicals examined (unpublished data).

In the comparative studies on protein diets, chemicals (carbaryl, dieldrin, paraquat) were highly retained in organs of low protein diet rats than that of high protein diet rats (Tanaka, 1980). And when carbaryl was orally administered, high radioactivity was detected in small intestine (Fig. 3), especially in cytosol fraction of mucosa of high protein diet rats at 1 hr after administration. These results would be due to the rapid and higher biliary excretion of carbaryl as described in previous paper. In situ, absorption and retention experiments of the chemicals did not show any difference between group 2 and group 3 administered with carbaryl or dieldrin (Table 5). In the studies on paraquat absorption in situ (Table 5), it was highly absorbed from small intestine of low protein diet rats than high protein diet rats. However retention in mucosa and serosa, and passage of paraquat in vitro did not show the significant difference between low protein diet rats and high protein diet rats. Furthermore, histopathological study of intestine did not show significant difference between those two groups by light microscopy and electromicroscopy. Therefore, we cannot explain why the difference in absorption of paraquat occurred between low and high protein diet rats in situ study.

Boyd (1968, 1969) reported the higher toxicity of carbaryl and other pesticides in rats fed on protein deficient diet, but clinicopathologic syndrome or histopathological change were not found in those rats.

Further works on absorption and tissue uptake of paraquat and other chemicals are in progress in our laboratory.

Table 5 Absorption and retention of ^{14}C -radioactivity (% of dose*) for 1 hr after the administration of chemicals in small intestine of rats fed on low (group 2) or high (group 3) protein diets in situ

		Absorption (1 hr)	Retention (1 hr)	
			Mucosa	Serosa
Carbaryl	group 2	67.25 ± 0.64	2.19 ± 0.09	0.34 ± 0.03
	group 3	67.18 ± 3.18	1.97 ± 0.06	0.35 ± 0.03
Dieldrin	group 2	9.50 ± 1.11	1.50 ± 0.08	0.05 ± 0.01
	group 3	8.72 ± 0.80	1.48 ± 0.10	0.08 ± 0.01
Paraquat	group 2	16.30 ± 1.81^a	7.41 ± 0.50	1.92 ± 0.10
	group 3	6.40 ± 0.91^a	6.32 ± 0.90	3.11 ± 0.50

Values are the mean $\pm$ S. E. of 6 loops.

* $100 \mu\text{g}$ ($0.2 \mu\text{Ci}$)/10 cm loop of small intestine

a : Significant difference between group 2 and group 3 ($p < 0.01$)

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