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Effect of Several Adsorbents on the Gastrointestinal Absorption of Paraquat

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The effect of several adsorbents on paraquat poisoning was investigated (1) by measuring the saturated amount of the poison adhered on the adsorbents *in vitro* and (2) by assaying the blood level of paraquat in the rat in *in situ* intestinal absorption experiments. Activated charcoal powder, natural aluminum silicate, and cationic exchange resins (calcium polystyrene sulfonate and sodium polystyrene sulfonate) were used as adsorbents. The steady-state blood level of paraquat in its absorption experiment with the cationic exchange resins was markedly lower than those without the resins or with other adsorbents. A good relationship was achieved between the calculated AUC or absorption rate (*in situ*) and the saturated adsorption amount (*in vitro*). The rank order of the effect was sodium polystyrene sulfonate>calcium polystyrene sulfonate>natural aluminum silicate>activated charcoal powder. The effect of sodium polystyrene sulfonate after intestinal washing with physiological saline was also measured, and a synergistic effect (marked decrease in blood paraquat level) was found as compared with the intestinal washing alone. The simultaneous use of G.I. washing and powerful adsorbent was scientifically proven to be most beneficial.

Key words: Paraquat, Detoxification, Adsorbent, G.I. absorption, *In situ* perfusion

INTRODUCTION

Paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride) is a contact herbicide that is widely used in the agricultural industry to aid in the harvesting of a wide variety of crops (Sager, 1987). It widespread use stems from its usefulness as an effective nonselective weed eliminator. It is a permanently charged cation and is dissociated at all pH values (Clark *et al.*, 1966). Despite its usefulness, paraquat is a well-known lung, nail, and ocular toxicant (Smith *et al.*, 1990; Sammann *et al.*, 1969; Cant *et al.*, 1968). The major cause of systemic toxicity leading to death is accidental oral ingestion (Haley, 1979). Washing of the gastrointestinal (G.I.) tract and/or the administration of a purgative are generally used in first-aid treatment to eliminate paraquat from the body (Ukai and Kawase, 1985). Activated charcoal powder, natural aluminum silicate and dextran sulfate have been used as adsorbents in paraquat poisonings (Neuvonen, 1982; Ameno and Fuke, 1987; Ukai *et al.*, 1987), and now cationic exchange resins, calcium polystyrene sulfonate and sodium polystyrene sulfonate are also used (Nokata *et al.*, 1984), because paraquat exists in the cationic form also in the G.I. tract (Takagi, 1983).

The present study sought to determine the amount of paraquat adsorbed by the adsorbents by *in vitro* and rat *in situ* experiments to examine the effect of such adsorbents in more details.

MATERIALS AND METHODS

Materials

Paraquat dichloride was obtained from ICI Japan Co., Ltd. (Tokyo, Japan). Four adsorbents; activated charcoal powder (Wako Pure Chemical Industries, Ltd., Osaka, Japan), natural aluminum silicate (Adsorbin® Sankyo Co., Ltd., Tokyo), calcium polystyrene sulfonate (Kalimate® Nikken Chemicals Co., Tokyo) and sodium polystyrene sulfonate (Kayexalate® Torii Pharmaceutical Co., Tokyo) were selected.

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Measurement of Amount of Paraquat Adsorbed

After preincubating 50 ml of phosphate buffered saline (pH 7.4) containing 50 mg of paraquat dichloride at 37℃ for 20 min, 50 mg of a particular adsorbent was added to the solution. One ml of the solution was withdrawn at 0, 5, 10, 15, 30, 60 and 120 min and filtered through a membrane filter (CO20A013A, Advantec Toyo, Tokyo). The filtrate (0.2 ml) was diluted with distilled water and assayed the paraquat concentration in the filtrate and the amount of paraquat adhered on the adsorbent. The experimental period (120 min) was enough to determine both the poison concentration in the solution and the amount of the poison adsorbed at equilibrium.

After preincubating 2 ml of distilled water or phosphate buffered saline (pH 7.4) containing 2 mg of paraquat dichloride at 37℃ for 20 min in the same method, 0.2, 0.5, 1, 2, 4, 6 or 8 mg of a particular adsorbent was added to the solution. The sample was filtrated at 120 min through the membrane filter and the saturated amount of paraquat adsorbed was determined.

Saturated amount of paraquat adsorbed was also measured using 2 ml of Japan Pharmacoepia (JP) 12-disintegration fluids (first fluid, pH 1.2 or second fluid, pH 6.8) containing 2 mg each adsorbent and enough amount of paraquat dichloride (1 mg for activated charcoal and natural aluminum silicate, 8 mg for cationic exchange resins, which was selected by referencing the data in distilled water and phosphate buffered saline).

Intravenous Infusion Experiment

Wistar rats weighing about 250 g (Saitama Laboratory Animals, Saitama, Japan) were used in all animal experiments. The time course of plasma concentration of paraquat after intravenous (*i.v.*) infusion at a rate of 250 μg/h was followed, and the total body clearance of the poison was calculated from the infusion rate and steady-state plasma concentration. Blood samples (0.3 ml each) were periodically withdrawn from the jugular vein and centrifuged to obtain plasma. The same volume of physiological saline was then injected intravenously. Samples were kept in a freezer until analysis.

In situ Perfusion Experiment

The *in situ* intestinal perfusion experiments were done in the ordinary manner (Karino *et al.*, 1982) using overnight-fasted rats. A rat anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg) was fixed on an operating board and its abdomen was opened. After ligature of the bile duct, silicone tubing (3 mm i.d., 4 mm o.d.) was cannulated into both ends of the upper duodenum to the lower ileum, and the contents of the lumen were washed out through the cannulas with physiological saline solution warmed to 37℃ (approximately 100 ml) until the emerging fluid was clear. Then, the proximal of the two cannulas was connected to the outlet of a perfusion pump (Microtube pump MP-3, Eyela Co., Tokyo) and the saline solution remaining in the lumen was drained with air at a speed of 5 ml/min. Thereafter, each drug solution prewarmed to 37℃ in the reservoir was recirculated through the cannulas at 5 ml/min. The positions of the inlet and outlet tube in the animal were fixed at the same height as the pump to avoid any hydrostatic pressure effect. The drug solution in the reservoir was stirred during the experiment, and the decrease in body temperature was prevented by a heat lamp. A blood sample (0.3 ml) was withdrawn from the jugular vein immediately before the perfusion experiment for a control and the same volume of physiological saline was injected. After the perfusion, the length of the perfused intestine was measured by placing a thread along the curved axis of the intestine to check variation among the experiment (The length was 90-110% of the mean). The perfusate contained paraquat dichloride and each adsorbent (1 mg/ml each) in phosphate buffered saline (pH 7.4). Decrease of free paraquat concentration in the perfusate was negligible. Blood (0.3 ml) was periodically sampled over 4 h and the same volume of saline was injected as above. Area under the blood concentration of paraquat-time curve (AUC) from 0 to 4 h was calculated by the trapezoidal method and zero-order absorption rate of the poison was obtained by its blood level at pseudo steady state and total body clearance.

The effect of sodium polystyrene sulfonate after intestinal washing with physiological saline was also measured by the *in situ* perfusion experiment. There were three groups in this experiment. The perfusion experiment of paraquat dichloride was carried out in the same method for every group described above except over 2 h. The perfusate contained sodium polystyrene sulfonate only for the third group. Each perfusate was exhausted at 2 h. Then the experiment was continued to another 4 h (totally 6 h) for the first group (control). Physiological saline without and with sodium polystyrene sulfonate was perfused from 2 to 6 h for the second and third groups, respectively. Blood (0.3 ml) was taken as the same as above.

Determination of Paraquat

Paraquat was determined by an HPLC method. Each sample (20 μl) adequately diluted and contained diquat as an internal standard was injected into the HPLC. The HPLC system consisted of a pump system

(LC-6A, Shimadzu Seisakusho, Kyoto, Japan), a UV detector (SPD-6A, Shimadzu Seisakusho), a Chromatopac (CR-3-A, Shimadzu Seisakuhso) and a stainless-steel column (4.0φ×150 mm) packed with ion-exchange resin (ES-502C, Asahipak, Tokyo, Japan). Conditions were: elution phase, adequate mixture of 0.1 M Na_2HCO_3 and 0.1 M H_3BO_3 containing 0.3 M KCl (adjusted pH at 9.5) : methanol (1 : 1); flow rate 1.0 ml/min; room temperature; detection, UV 257 nm. Sensitivity of this method was about 1 μg/ml.

Data Analysis

Statistical analysis was performed with a Student's t-test. Linear regression analysis was adopted to evaluate the *in vitro-in situ* relationship. Most experiments were done quintuplicatedly and the values are presented as the mean with S.E. When no average could be obtained, the data were shown one by one.

RESUCTS

Figure 1a and b show the adsorption isotherms of paraquat (amount of paraquat adsorbed vs. its free concentration in a solution at equilibrium) on the various adsorbents in distilled water and pH 7.4 phosphate buffered saline, respectively. The adsorption rate was equilibrated so rapidly (data not shown) that each amount of paraquat at the final sampling time (120 min) was represented. The amount of the poison adsorbed increased with increase in the concentration of the adsorbent used. Each profile may show Type I isotherm, which can be represented by the following Langmuir isotherm equation (Martin, 1993).

$$M = M_{max} a C_f / (1 + a C_f) \quad (1)$$

in which M and M_{max} are the mass and saturated mass of paraquat adsorbed per gram of adsorbent at free paraquat concentration, C_f, and a is a coefficient relating to adsorption and desorption rates. Equation 1 can be expressed by,

$$C_f/M = 1/M_{max} \cdot C_f + 1/(M_{max} a) \quad (2)$$

The saturated amount of paraquat adsorbed can be determined by a plateau level in Fig. 1. But the value can be obtained more exactly by the Eq. 2.) Figure 2 shows C_f/M vs. C_f relationship. Since all points were on a line for each adsorbent and each solution, it was confirmed that paraquat adsorption on these adsorbents obeys Type I isotherm. Slope of the lines in Fig. 2 is reciprocal of saturated amount of paraquat adsorbed ($1/M_{max}$). Calculated M_{max} was very close to that obtained from the amount of paraquat adsorbed at the plateau region in Fig. 1. Table 1 summarizes the M_{max} value for distilled water and phosphate buffered saline (from Fig. 2 and Eq. 2) together with that

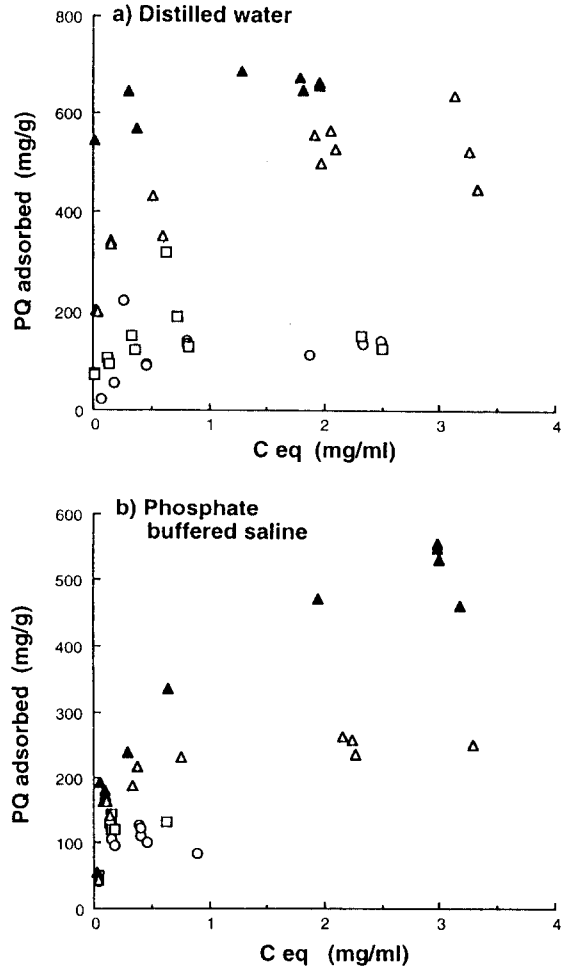

Fig. 1. Adsorption isotherms for paraquat on the four adsorbents in distilled water (a) and phosphate buffered saline (b)
Symbols: activated charcoal powder (○), natural aluminum silicate (□), calcium polystyrene sulfonate (△), and sodium polystyrene sulfonate (▲).
PQ: paraquat

for JP 12 first and second disintegration fluids (pH 1.2, 6.8)(obtained from one point-experiment, see the experimental part in detail). Cation exchange resins (calcium polystyrene sulfonate and sodium polystyrene sulfonate) adsorbed paraquat much more than activated charcoal powder or natural aluminum silicate. These resins were effective in all solutions tested in this experiment. In the case of activated charcoal, the amount of paraquat adsorbed was highest in distilled water. Low pH and addition of salts decreased the adsorption. In the case of natural aluminum silicate, however, only pH affected the adsorption amount. In case of both the cation exchange resins, the order of the saturated amount adsorbed was the second fluid for dissolution>distilled waster>the first fluid> phosphate buffered saline. Cations for the buffer cons-

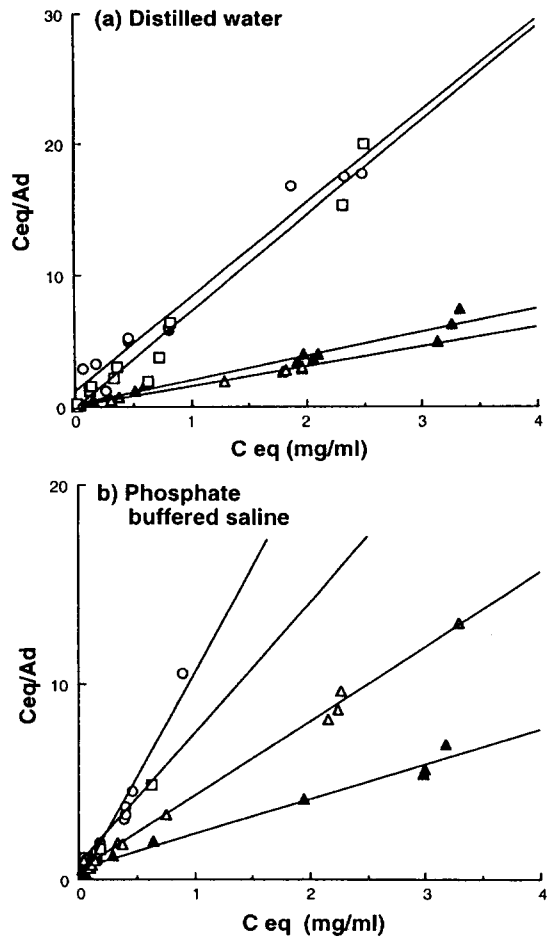

Fig. 2. Double reciprocal plot of the paraquat adsorption.
Symbols: the same as in Fig. 1.
Each regression equation was obtained by the least square method.

Table I. Saturated Amount of Paraquat Adsorbed on the Several Adsorbents[a]

	Distilled water	PBS[b]	1st fluid	2nd fluid
Activated charcoal powder	140.6	89.22	6.89	99.76
Natural aluminum silicate	136.69	138.89	37.55	173.74
Calcium polystyrene sulfonate	543.07	261.54	481.91	579.59
Sodium polystyrene sulfonate	668.72	561.29	433.65	769.60

[a] mg paraquat/1 g of adsorbent
[b] phosphate buffered saline

tituents were supposed to affect the adsorption on the cation exchange reins, but no clear tendency was found on the effect of the constituents.

Prior to the *in situ* intestinal perfusion experiment, a continuous *i.v.* infusion experiment was done to measure the total body clearance of paraquat. Figure 3 shows the time course of paraquat concentration in plasma after *i.v.* infusion. Elimination half-life was so short that the steady-state concentration was easily

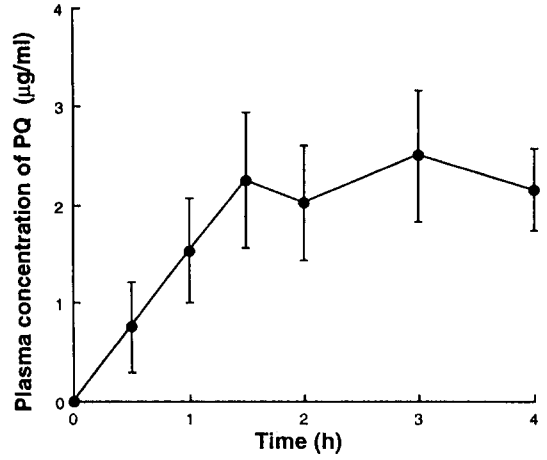

Fig. 3. Plasma concentration of paraquat during continuous *i.v.* infusion in rats.
Each point represents the mean ± S.E. of 5 rats.

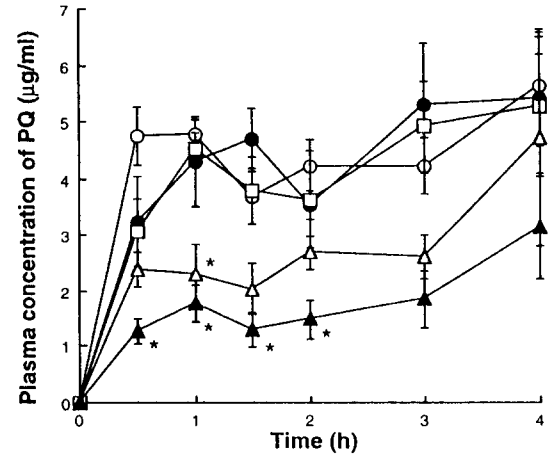

Fig. 4. Effect of adsorbents on the intestinal absorption of paraquat in rats.
Symbols: control (●), activated charcoal powder (○), natural aluminum silicate (□), calcium polystyrene sulfonate (△), and sodium polystyrene sulfonate (▲).
*: $p<0.05$ for comparison with control.
Each point represents the mean ± S.E. of 5 rats.
PQ: paraquat

calculated to be 2.16± 0.53 μg/ml as an average plasma concentration from 1.5 to 4 h. Total body clearance was then calculated from the average plasma level and the infusion rate (250 μg/h), and the value was 158± 36 ml/h.

Figure 4 shows the time course of paraquat concentration in plasma in the *in situ* intestinal perfusion experiment; these were similar to those for the *i.v.* infusion, which suggests that the intestinal absorption of paraquat obeyed zero-order kinetics in rats. When paraquat alone in buffer solution was perfused, the steady-state plasma level was about 5 μg/ml. Simulta-

Table II. Effect of several adsorbents on the pharmacokinetic parameters of paraquat in rats

	AUC (μg/ml)	K (μg/h)
Control	18.9± 3.4	831± 140
Activated charcoal powder	18.2± 0.2	771± 47
Natural aluminum silicate	16.3± 0.5	727± 195
Calcium polystyrene sulfonate	9.99± 2.18	404± 81
Sodium polystyrene sulfonate	4.55± 1.26[a)]	196± 68[a)]

Each value represents the mean ± S.E. of 5 experiments.
[a)] Significant in comparison with control ($p<0.05$).

neous use of paraquat with activated charcoal powder and natural aluminum silicate reduced the plasma level of paraquat, although no significant difference was found. In contrast, cationic exchange resins significantly decreased the plasma level as compared with no adsorbent (control), with the effect of sodium polystyrene sulfonate being especially notable. The steady-state plasma level when paraquat was used simultaneously with sodium polystyrene sulfonate was about 1.5 μg /ml, which was about one fourth that for paraquat alone.

Table 2 shows $AUC_{0\text{-}4h}$ and zero-order absorption rate constant, K, for each case. The K-value was obtained by the steady-state plasma level and total body clearance as measured in the *i.v.* infusion experiment. This table showing the effect of cation exchange resins, especially sodium polystyrene sulfonate, is more quantitatively understandable than Fig. 4.

Next, the *in vitro-in situ* correlation was investigated. Figure 5a shows the relationship between the saturated amount of paraquat adsorbed (*in vitro*, at 1 mg/ml for the concentration of adsorbent, from Figs. 1 and 2, Table I) and $AUC_{0\text{-}4h}$ (*in situ*, from Table II), and Fig. 5b shows the saturated amount adsorbed (*in vitro*, Table I) and absorption rate (*in situ*, from Table II) of paraquat. Both lines are linear, indicating good *in vitro-in situ* relationships. The higher the *in vitro* adsorption of paraquat was, the lower was the *in situ* intestinal absorption of paraquat. It is thus clear that paraquat absorption is dependent on the presence and ability of an adsorbent. Hence, it is of great importance to identify a powerful adsorbent for use in treating paraquat poisoning.

Faster first aid is also a key factor in the treatment of paraquat poisoning (Chiyo *et al.*, 1984). As shown in Fig. 4, adsorbent was simultaneously used with paraquat; in the next experiment, paraquat was intestinally pre-perfused *in situ* 2 h before all the paraquat was replaced by physiological saline, and then the *in situ* intestinal perfusion experiment was carried out with adsorbent alone. The pre-perfusion period was set at 2 h because the steady-state plasma level was obtained earlier than this following the intestinal perfusion of paraquat (Fig. 4). Figure 6 shows the synergy

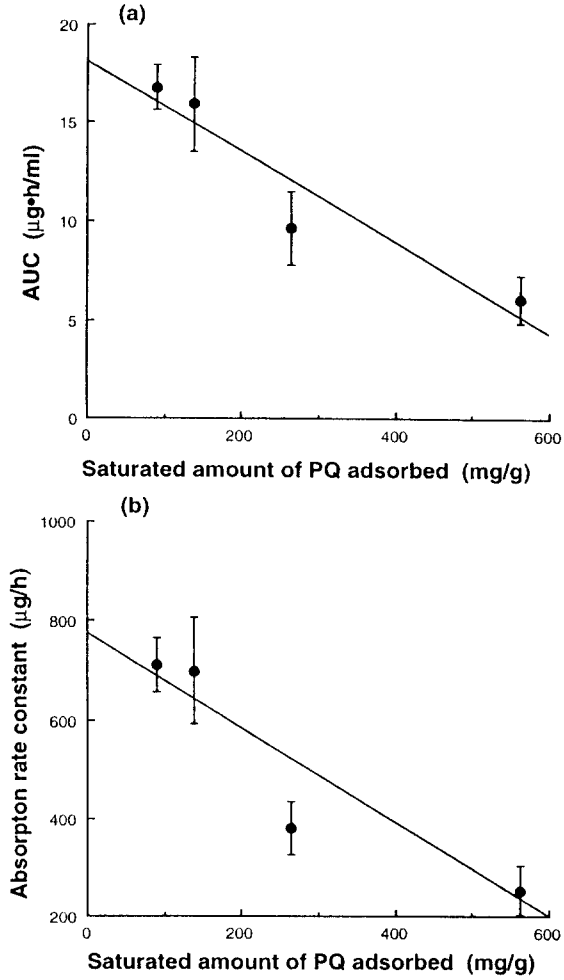

Fig. 5. Relationship between saturated amount of adsorbed (*in vitro*) and AUC (a) or absorption rate (b) (*in situ*) of paraquat
Each point represents the mean ± S.E.
PQ: paraquat

effect of adsorbent and intestinal washing on the intestinal absorption of paraquat. Without intestinal washing at 2 h, the plasma level of the poison increased slightly even when there was no further perfusion of it. This gradual increase may be due to impairment of the renal function (Ameno and Fuke, 1987) and remnants of the paraquat on the intestinal mucosa. In contrast, continuous intestinal washing reduced the plasma level by about one third, and addition of the adsorbent sodium polystyrene sulfonate further significantly decreased the plasma level. It is clear that adsorbent does function to eliminate paraquat from the intestine.

DISCUSSION

The *i.v.* continuous infusion experiment conducted was intended to measure total body clearance of pa-

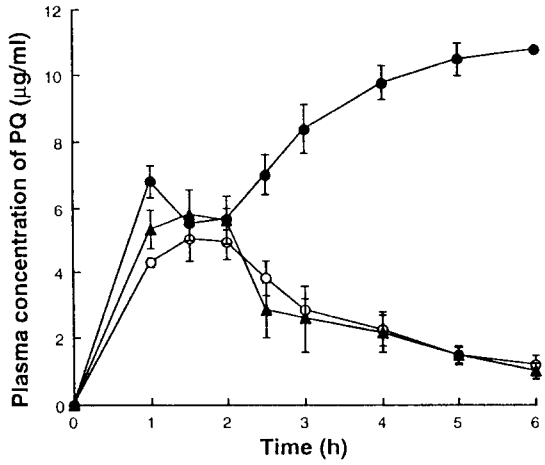

Fig. 6. Synergy effect of adsorbent and intestinal washing on the intestinal absorption of paraquat.
Symbols: without washing (●), washing alone (○), washing with sodium polystyrene sulfonate (▲)
Each point represents the mean ± S.E. of 5 rats.
PQ: paraquat

raquat and was used in place of an *i.v.* bolus injection because initial plasma concentration after the latter is generally so high that some of the toxic effects of paraquat may remain, *i.e.* interruption of elimination (renal or hepatic functions) or, in the worst case, death. In addition, a low *i.v.* dose of paraquat is not really useful because of its low sensitivity for assay. In *i.v.* continuous infusion, however, the concentration level in plasma can be easily set so as to prevent toxicity and measure the plasma level.

The *i.v.* infusion rate used, 250 μg/h, was based on a preliminary experiment involving adjusting the steady-state plasma concentration of paraquat for the *i.v.* infusion, 2.16± 0.55 μg/ml (Fig. 3), to that for *in situ* intestinal perfusion, 1-7 μg/ml (Fig. 4). Although the pharmacokinetics of paraquat may be non-linear, linear kinetics was assumed within a certain plasma level range in this experiment.

The adsorption isotherm for paraquat on four adsorbents was shown in the Langmuir isotherm. The saturated amount of paraquat adsorbed was different among the adsorbents. Sodium polystyrene sulfonate was the most effective of the four adsorbents in paraquat adsorption. Table 1 shows a comparison of the saturated adsorption in four different solvents at 1 mg/ml of each adsorbent. Since cation exchange resins were effective not only at pH 1.2 but also at pHs 6.8 and 7.4, they would have a detoxification effect in the stomach as well as in the small intestine.

Results of the *in vitro* experiments (Figs. 1 and 2, Table I) and the *in situ* experiments (Fig. 4, Table II) clearly showed the benefit of cationic exchange resins, which were more powerful than natural aluminum silicate and activated charcoal powder in counteracting paraquat poisoning. Adsorbents may not be homogenous in the G.I. tract. A gradual increase in the steady-state blood level of paraquat for every adsorbent as shown in Fig. 4 may be due to sediment or stagnation of the adsorbent at the upper intestine. Intensity and rank order of the effect of the adsorbent, however, were shown clearly. A good relationship was found between the *in vitro* and *in situ* data (Fig. 5). The *in vitro* adsorption data may be a good index for clinical use.

In addition to the adsorption by adsorbents in the G.I. tract, gastric washing is also important for first aid. Since simultaneous use of the gastric washing and the application of adsorbent was more effective than use of an adsorbent alone (Fig. 6), the combination is shown to be a useful tool for the treatment of paraquat poisoning.

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