In Vitro Study of the Adsorption Characteristics of Drugs

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The adsorption characteristics of eight adsorbents, cholestyramine, colestimide, aluminum silicate, sucralfate, aluminum hydroxide, calcium polystyrene sulfonate, carbon sphere and medicinal carbon, on the drugs such as methotrexate, antidepressants, mizoribine and ciprofloxacin hydrochloride were investigated *in vitro*. Medicinal carbon showed an excellent adsorption of all the tested drugs while the carbon spheres showed a high but slow adsorption characteristic. Cholestyramine and colestimide showed a higher adsorption in methotrexate than the other adsorbents. Aluminum silicate and calcium polystyrene sulfonate showed higher adsorption in four antidepressants, clomipramine hydrochloride, imipramine hydrochloride, mianserin hydrochloride and trazodone hydrochloride. In mizoribine, there were no adsorbents that showed higher adsorption except for the medicinal carbon. In ciprofloxacin hydrochloride, aluminum preparations and calcium polystyrene sulfonate showed higher adsorption characteristics. It is suggested that several adsorbents are potentially useful treatments for drug overdoses, but that these adsorbents have the possibility of decreasing the effects of the co-administered medicines.

Key words adsorption; adsorbent; drug interaction; *in vitro*

The role of the enterohepatic circulation, in which a drug excreted within bile is reabsorbed, is an important problem at the time of drug intoxication. Although high-dose methotrexate has showed the superior effectiveness in osteosarcoma *etc.*, there were many patients who showed a slow elimination in our hospital. The slow second phase of methotrexate elimination after intravenous high-dose therapy appears to be due to non-renal mechanisms (biliary excretion, gut secretion, enteral reabsorption).[1)] Patients attempting suicide often take antidepressants and enterohepatic circulation was reported for some antidepressants.[2)] It is useful to administer the adsorbents to reduce the adverse effects of such drugs. On the other hand, the adsorbents may decrease the absorption of the co-administered drugs and attenuate the efficacy. There were no reports that the adsorption characteristics of adsorbents were simultaneously investigated. The present study was carried out to evaluate and to compare the influence of eight adsorbents on the adsorption rate of seven co-administrated drugs *in vitro*.

MATERIALS AND METHODS

Materials Drugs Tested: Four antidepressants and methotrexate which are easy to appear intoxication, mizoribine, the immunosuppressant and ciprofloxacin, the antimicrobial agent which are clinically important were investigated.

Clomipramine hydrochloride and imipramine hydrochloride (Novartis Pharma, Tokyo, Japan), mianserin hydrochloride and trazodone hydrochloride (Nippon Organon, Osaka, Japan), mizoribine (Asahi Kasei, Osaka, Japan), ciprofloxacin hydrochloride (Bayer, Osaka, Japan), and methotrexate (Methotrexate® parenteral 50 mg, 50 mg/V, Nihon Wyeth Lederle, Tokyo, Japan, Lot. No. 215-2) were used.

Adsorbents: Medicinal carbon (Medicinal carbon®, Kenei, Osaka, Japan, Lot. No. 1J17), carbon sphere (Kremezin®, Kureha Chemical, Tokyo, Japan, 21S4), cholestyramine (Questran®, 4 g/9 g, Bristol, Tokyo, Japan, QSBA661), colestimide (Cholebine®, 500 mg/T, powdered and sieved with a 48 mesh screen, Mitsubishi-Tokyo, Tokyo, Japan, DF01), calcium polystyrene sulfonate (Kalimate®, Nikken Chemicals, Tokyo, Japan, 35129A), aluminum silicate (Adsorbin®, Sankyo, Tokyo, Japan, NH008), sucralfate (Ulcermin®, 900 mg/g, Chugai, Tokyo, Japan, C2B06), aluminum hydroxide gel (Alumigel®, 990 mg/g, Chugai, Tokyo, Japan, J0I01) were used.

***In Vitro* Adsorption Studies** Adsorbents (10 mg as the component drug) were added to the tested drug solutions with different concentrations in 5 ml of the second fluid of Japan Pharmacopoeia XIV Disintegration Test (0.05 M phosphate buffer pH 6.8). Because the oral daily doses of the tested drugs are about 100—200 mg/d and those of the adsorbents are about 3—10 g/d, the ratio of the tested drugs to the adsorbents were 1 : 10, 1 : 20 and 1 : 100 in the solutions. The resulting suspension was incubated for 30 min at 37 °C (Taiyo Circle Shaker, Taiyo, Tokyo, Japan, and Thermo Elecon Model KTH-2, Kayagaki, Tokyo, Japan). The suspension was filtered through a membrane filter (Durapore® membrane filter, 0.45 μm, Millipore, Tokyo, Japan, R2BN45081). The concentrations of each drug were measured by spectrophotometric analysis at the maximum absorption wavelength using a Hitachi model U-2000 double beam spectrophotometer (Hitachi, Tokyo, Japan). The maximum wavelengths were 252 nm with clomipramine hydrochloride, 250 nm with imipramine hydrochloride, 278 nm with mianserin hydrochloride, 246 nm with trazodone hydrochloride, 277 nm with mizoribine, 271 nm with ciprofloxacin hydrochloride and 302 nm with methotrexate. The correlation coefficients of the calibration line of all drugs were 1.000, the coefficients of variation were less than 1.1% and the limits of detection were 0.1—0.5 μg/ml in all tested drugs. The percentage adsorption of drugs was calculated using the equation (1−absorption after incubation/absorption before incubation)×100 (%). All data are expressed as the mean±S.D.

***In Vitro* Adsorption Study of Carbon Sphere** Sixty milligrams of carbon spheres was added to four drug solutions (clomipramine hydrochloride, imipramine hydrochloride, trazodone hydrochloride and methotrexate) of different concentrations in 30 ml of the second fluid of Japan Pharma-

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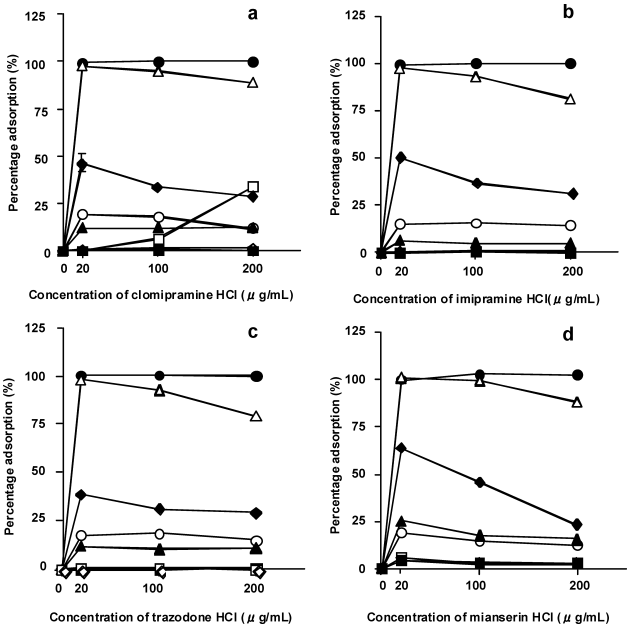

Fig. 1. Percentage Adsorption of Four Antidepressants by the Absorbents

Adsorbates: clomipramine HCl (a), imipramine HCl (b), trazodone HCl (c) and mianserin HCl (d). Adsorbents: medicinal carbon (●), aluminum silicate (△), calcium polystyrene sulfonate (◆), carbon sphere (○), cholestyramine (▲), sucralfate (□), colestimide (■) and aluminum hydroxide gel (◇). Each point represents the mean±S.D. (n=5).

copoeia XIV buffer (0.05 M phosphate buffer pH 6.8). The resulting suspension was stirred with a stirrer at room temperature. The supernatant was then collected at 1, 3, 5 and 7 h (at 24 h in methotrexate). The concentrations of each drug were measured by spectrophotometric analysis at the maximum absorption wavelength. All data are expressed as the mean±S.D. The adsorption parameters were calculated by Langmuir equation as follows.

$$1/S=(1/S_\infty)+(1/S_\infty K)\times(1/C)$$

S; adsorpted amount, S_∞; maximum adsorptive capacity, K; adsorption equilibrium constant, C; concentration of drug

RESULTS

Adsorption Studies of Eight Adsorbents Medicinal carbon showed the highest adsorption characteristic in all the tested drugs. In four antidepressants, clomipramine hydrochloride, imipramine hydrochloride, mianserin hydrochloride and trazodone hydrochloride, aluminum silicate and calcium polystyrene sulfonate showed a higher adsorption (Fig. 1). In methotrexate (MTX), cholestyramine and colestimide showed a higher adsorption than the other adsorbents (Fig. 2). In mizoribine, there were no adsorbents except for medicinal carbon, which showed a high adsorption (Fig. 2). In ciprofloxacin hydrochloride, aluminum silicate, sucralfate, aluminum hydroxide gel powder, which was prepared from powdering Alumigel® and sieving with a 48 mesh screen, and calcium polystyrene sulfonate showed higher adsorption characteristics as shown in Fig. 2.

Adsorption Studies of Carbon Sphere The percentage adsorption of all four tested drugs increased in a time-dependent manner (Fig. 3). The maximum adsorptive capacities of the antidepressants calculated by Langmuir equation were higher than that of MTX (Table 1).

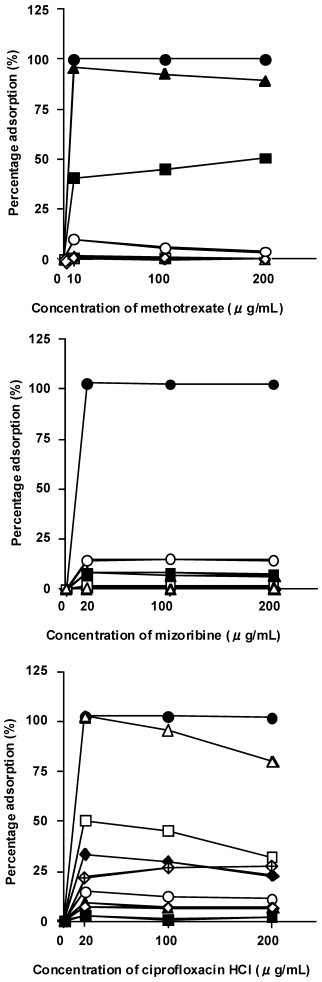

Fig. 2. Percentage Adsorption of Methotrexate, Mizoribine and Ciprofloxacin by the Absorbents

Adsorbents: medicinal carbon (●), cholestyramine (▲), colestimide (■), carbon sphere (○), calcium polystyrene sulfonate (◆), aluminum silicate (△), sucralfate (□), aluminum hydroxide gel (◇) and aluminum hydroxide gel powder (⊕). Each point represents the mean±S.D. (n=5).

DISCUSSION

Medicinal carbon can bind with many drugs such as acetaminophen, phenobarbital, theophylline *etc.*,[3] and it is not absorbed in the gut. Medicinal carbon has numerous cavities, and many drugs bind with the cavity wall. Activated charcoal given orally showed significantly lowered MTX serum levels.[4] In our study, medicinal carbon showed excellent adsorption characteristics for all tested drugs. Therefore, it is suggested that medicinal carbon is useful to reduce the toxicity in the patients with acute drug intoxication. However, medicinal carbon is an impalpable powder, therefore, it is difficult to easily ingest. Carbon spheres, which have a petroleum carbohydrate origin, are used for uremic improvement and are easier to intake than medicinal carbon. Therefore, carbon sphere was expected to administer to the drug intoxication patients. It was reported that carbon spheres adsorbed phenobarbital and theophylline *in vitro*.[5] Although the percentage adsorption of the carbon spheres was higher in our study, the adsorption manner was slower than medicinal car-

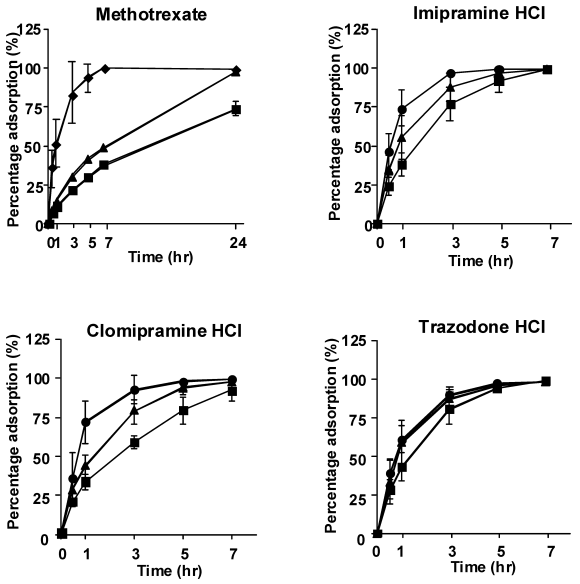

Fig. 3. Time Course of Percentage Adsorption of Methotrexate and Antidepressants by Carbon Sphere

Drug concentrations; 200 μg/ml (■), 100 μg/ml (▲), 20 μg/ml (●), 10 μg/ml (◆). Each point represents the mean±S.D. (n=5). The percentage adsorption was calculated using the equation (1−absorption after incubation/absorption before incubation)×100 (%).

Table 1. Adsorption Parameters Calculated by Langmuir Equation

	Methotrexate	Clomipramine	Iimipramine	Trazodone
Maximum adsorptive capacity (mg/g)	63	81	115	294
Adsorption equilibrium constant (l/g)	4000	1240	967	170

bon. It is suggested that carbon spheres might not be valid for acute drug intoxication, but that it might be effective when slow adsorption is useful.

The slow elimination of MTX may be accompanied by adverse effects after high-dose therapy. It is known that the enterohepatic circulation plays an important role in the pharmacokinetics of MTX. Because MTX is an anionic drug with two carboxyl groups in its structure, cholestyramine and colestimide, anion-exchange resins, can accelerate the excretion of the drug in patients suffering from methotrexatre toxicity. An 11-year-old girl with an osteosarcoma was administered a high-dose of MTX and then given cholestyramine. The serum MTX concentration 24 h after the MTX infusion was about 50% of the concentration measured after the first course of MTX given without cholestyramine.[6] Additionally, it was also reported that cholestyramine and colestimide had high adsorption capacities for MTX in an *in vitro* study.[7,8] In our study, medicinal carbon, cholestyramine and colestimide showed high adsorptions for MTX, in addition, the carbon spheres showed a slow but high adsorption manner. It is suggested that such drugs might reduce the possible toxicity by MTX.

Suicide attempt patients often take antidepressants. It is necessary to administer adsorbents at the time of acute drug intoxication. Some of the antidepressants were also reported the enterohepatic circulation.[2] It was reported that the bioavailability of amitriptyline was impaired by the concomitant administration of sucralfate.[9] The adsorption of the tricyclic antidepressants onto cholestyramine was also demonstrated in an *in vitro* study.[10] In our study, medicinal carbon and aluminum silicate showed higher adsorption characteristics than sucralfate and cholestyramine. It was reported that the blood levels of chlorpromazine were lowered following concomitant antacid addition, and that this effect was most likely due to the adsorption of chlorpromazine into the gel structure of the antacid.[11] It is suggested that not only medicinal carbon but also aluminum silicate are useful to eliminate these antidepressants. On the other hand, when these drugs are taken at the same time, the efficacy of the antidepressants may be reduced.

In our study, calcium polystyrene sulfonate, a cation-exchange resin, also showed the adsorption of imipramine hydrochloride, clomipramine hydrochloride, mianserin hydrochloride, trazodone hydrochloride and ciprofloxacin hydrochloride based on their cationic properties. When calcium polystyrene sulfonate and these drugs are taken at the same time, calcium polystyrene sulfonate may reduce the efficacy of these co-administered drugs.

There are many reports about the interaction between quinolones and polyvalent cations. It was reported that the prostatitis treatment was probably failed according to the interaction between ciprofloxacin and sucralfate,[12] and that the bioavailability of ciprofloxacin decreased an average of 30% with sucralfate pretreatment.[13] From an *in vitro* study, significant modifications of the dissolution profiles of ciprofloxacin were reported to decrease 17.89% of the maximum amount dissolved due to the presence of aluminum.[14] It is suggested that the formation of the complex between the quinolone and the corresponding cations occurs. In our study, aluminum, such as aluminum silicate, sucralfate and aluminum hydroxide gel powder, and calcium polystyrene sulfonate showed a high adsorption rate, and the efficacy of ciprofloxacin might be reduced against bacteria. In mizoribine, the adsorbents used did not show a high adsorption rate except for medicinal carbon.

Thus, these adsorbents showed the adsorption characteristics by physical adsorption (carbon and antacids), complex formation of ion-exchange resins (cholestyramine, colestimide and polystyrene sulfonate) and chelate formation (aluminum). It is suggested that several adsorbents are potentially useful treatments for drug overdoses, but that these adsorbents have possibility of decreasing the efficacy of the co-administered drugs. Because the adsorption ratios of the adsorbents are different in each co-administered drugs, medication managements such as the selection of drugs, medication history and administration time are necessary for the pharmacotherapy of patients.

CONCLUSION

Medicinal carbon showed an excellent adsorption of all tested drugs and carbon sphere had a slow adsorption manner. Anion-exchange resins adsorbed methotrexate, cation-exchange resin and aluminum silicate adsorbed four antidepressants. It is suggested that these adsorbents are useful for drug overdose, but the effects of the co-administered drugs may be attenuated.

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