demonstrated for other 5-nitroimidazole drugs (Cosar et al 1966).

These results suggest that the lactam nucleus is an important molecular moeity for antiprotozoal activity in the 2-substituted 5-nitroimidazoles.

This work was generously funded by the Centre National de la Recherche Scientifique and the Groupe Rhône-Poulenc. O.J. is a recipient of a CNRS-RP Research Grant (1985–1988).

### References

Audibert, P., Placidi, M., Giovannangeli, G., Cristau, B., Gasquet, M., Delmas, F., Andrac, A., Timon-David, P. (1979) Dérivés de l'acide p-arsanilique. Influence d'une N-substitution sur les propriétés antiparasitaires. Ann. Pharm. Fr. 37: 483–490

Breccia, A., Cavalerri, B., Adams, G. E. (1982) Nitroimidazoles: Chemistry, Pharmacology and Clinical Application. Plenum Press, New York

Cosar, C., Crisan, C., Horclois, R., Jacob, R. M., Robert, J., Tchelitcheff, S., Vaupré, R. (1966) Nitroimidazoles. Préparation et activité chimiothérapeutique. Arzneim. Forsch./Drug. Res. 16: 23–29

Docampo, R., Moreno, S. N. J. (1984) Free radical intermediate in the antiparasitic action of drugs and phagocytic cells. In: Pryor, W. A. (ed.) Free Radicals in Biology. vol VI, Academic Press, New York, pp 243–288

Halliwell, B., Gutteridge, J. M. C. (1986) Free Radicals in Biology and Medicine. Clarendon Press, Oxford

Hubert-Habart, M., Kokel, B., Menichi, G. (1987) Réflexions sur la chimiothérapie des cancers. Actualité Chimique: 105–114

Jentzer, O., Vanelle, P., Crozet, M., Maldonado, J., Barreau, M. (1991) Nouveaux 5-nitroimidazoles à noyau lactame hautement actifs: préparation et propriétés antibactériennes. Eur. J. Med. Chem. In press

Josephy, P. D., Mason, R. P. (1985) Nitroimidazoles. In: Anders, M. W. (ed.) Bioactivation of Foreign Compounds. Academic Press, Orlando, pp 451–483

Kernbaum, S. (1988) 5-Nitroimidazoles et infections à anaérobies. In: Giroud, J. P., Mathé, G., Meyniel, G. (eds) Pharmacologie Clinique. Bases de la Thérapeutique. 2nd, Expansion Scientifique, Paris, pp 1512–1519

Nair, M. D., Nagarajan, K. (1983) Nitroimidazoles as chemotherapeutic agents. In: Jucker, E. (ed.) Progress in Drug Research. vol 27, Birkhauser Verlag, Basel, pp 163–252

Taylor, E. R. A., Baker, J. R. (1968) The Cultivation of Parasites In Vitro. Blackwell Scientific Publications, Oxford

Vanelle, P., De Meo, M. P., Maldonado, J., Nouguier, R., Crozet, M. P., Laget, M., Dumenil, G. (1990) Genotoxicity in oxazolidine derivatives: influence of the nitro group. Eur. J. Med. Chem. 25: 241–250

Vanelle, P., Crozet, M. P., Maldonado, J., Barreau, M. (1991) Synthèse par réaction $S_{RN}1$ de nouveaux 5-nitroimidazoles antiparasitaires et antibactériens. Ibid. 26: 167–178

Walsh, J. S., Wang, R., Bagan, E., Wang, C. C., Wislocki, P., Miwa, G. T. (1987) Structural alterations that differentially affect the mutagenic and antitrichomonal activities of 5-nitroimidazoles. J. Med. Chem. 30: 150–156

---

J. Pharm. Pharmacol. 1991, 43: 736–738
Communicated March 11, 1991

© 1991 J. Pharm. Pharmacol.

# Gastrointestinal absorption of quaternary ammonium compounds correlated to their binding to small intestinal brush border membrane in rat

HIROSHI SAITOH, NOBUTAKA SAITOH, KEN ISEKI, KATSUMI MIYAZAKI, *Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan*

**Abstract**—The relationship between absorption of quaternary ammonium compounds (QACs) from rat intestine and their in-vitro binding to isolated brush-border membrane has been examined, using a series of n-alkyltrimethylammoniums. The binding of these QACs gradually increased with each extension of unbranched hydrocarbon chain from octyltrimethylammonium to tetradecyltrimethylammonium. However, hexyltrimethylammonium and heptyltrimethylammonium failed to bind to the membrane. On the other hand, the disappearance of these QACs from rat jejunal loop also increased with the length of hydrocarbon chain over the range of 8·9 to 71·3%. A good correlation was found between binding to the brush-border membrane and disappearance from jejunal loop. From these results, it was suggested that the size of the hydrophobic part of a QAC molecule was a principal determinant of both absorption and membrane binding, and that the absorption of QACs, with an appropriate sized hydrophobic part, was closely associated with the degree of binding to the membrane.

To elucidate the intestinal absorption mechanisms of quaternary ammonium compounds (QACs), we have studied the transport characteristics of these drugs by the intestinal brush-border membrane vesicles, and found that the binding of QACs to the membrane is a first step in the specialized transport mechanism driven by physiological membrane potentials (Saitoh et al 1987, 1988a, b, 1989). We have also demonstrated that the structure of the hydrophobic part of various organic cations is a determinant factor of the binding activities (Saitoh et al 1990). It remains unclear, however, whether the differences in the binding among QACs would be reflected in their absorption behaviour. In the current study, we have developed a simple and sensitive assay for n-alkyltrimethylammoniums and examined in-vitro binding to the brush-border membrane, in-situ absorption, and the correlation between the two.

Correspondence: K. Miyazaki, Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan.

### Materials and methods

We expressed each n-alkyltrimethylammonium as the number of carbons of the n-alkyl group; hexyltrimethylammonium (C6), heptyl- (C7), octyl- (C8), nonyl- (C9), decyl- (C10), undecyl- (C11), dodecyl- (C12), and tetradecyl- (C14). C12 and C14 were purchased from Nakalai Tesque, Inc. (Kyoto, Japan) and Tokyo Kasei Kogyo, Co. Ltd (Tokyo, Japan), respectively. Other QACs were synthesized in our laboratory as reported previously (Saitoh et al 1990).

The procedure for isolating the brush-border membrane from rat small intestine and the binding study to the membrane were described previously (Iseki et al 1989; Saitoh et al 1990). The absorption experiment was carried out at pH 6·5 using the in-situ loop technique of Levine & Pelikan (1961). A jejunal loop (10 cm) was prepared in a male Wistar rat, 200–250 g, and the proximal ligature of the loop placed about 10 cm from the pylorus. After washing the loop gently with 10 mL of a modified Ringer solution (Schultz et al 1966), 50 $\mu$M QAC (1 mL) dissolved in the Ringer solution was injected into the loop. After 30 min, the contents of the loop were withdrawn as completely as

possible, and the lumen was washed with the Ringer solution to give a volume of 5 mL. For the determination of tissue concentration of methylchlorpromazine, scraped mucosa was homogenized with 1 mL of 20 mM sodium dodecyl sulphate (SDS) and diluted to a volume of 5 mL with the same SDS solution.

The assay method of n-alkyltrimethylammonium was newly developed as follows; 0·5 mL of 2 mM sodium 2-naphthalenesulphonate as an ion-pair reagent and 3 mL of dichloromethane were added to a sample solution (0·5 mL). The mixture was then shaken for 15 min and centrifuged at 1000 *g* for 5 min. After evaporation of 2 mL of the organic layer, the residue was reconstituted with 0·2 mL of distilled $H_2O$ containing resorcinol as a standard. 2-Naphthalenesulphonate in the solution was determined by HPLC. The conditions for HPLC (apparatus and column) were the same as described previously (Saitoh et al 1990). Other conditions were as follows: mobile phase, 0·025 M $KH_2PO_4$ adjusted to pH 2·5 with phosphoric acid–methanol (55:45); flow rate, 0·5 mL $min^{-1}$; column temperature, 50°C; detector wavelength, 274 nm; and detector sensitivity, 0·01–0·04 aufs.

Polypropylene tubes were used in all steps to minimize the adsorption of QACs to test tubes. If necessary to correct the adsorption, a known amount (0·5 nmol) of corresponding QAC was added to a sample solution. Protein concentration of membrane suspension was determined according to Lowry et al (1951) with bovine serum albumin as a standard. Methylchlorpromazine was determined as reported previously (Saitoh et al 1990).

## Results

The present assay method of n-alkyltrimethylammoniums lacking UV absorbance, is based on an ion-pair extraction method with an appropriate counter-anion. The anion used here is required to satisfy the following conditions; i) not to transfer into organic layer by itself, ii) to form a lipophilic ion-pair with n-alkyltrimethylammonium quantitatively, and iii) to be highly UV-absorbing. Among various organic anions tested, 2-naphthalenesulphonate was the most suitable and could be determined by HPLC with the conditions presented in Materials and methods. The coefficients of variation at the initial concentration of 10 μM were 4·39% for C8 and 3·12% for C12. Minimum detectable amounts were 15 pmol (C8) and 8 pmol (C14) per injection. It was demonstrated that the assay method developed here was more sensitive and reproducible compared with the method described elsewhere (Denkert et al 1981; Larson & Pfeiffer 1983).

Fig. 1 shows the binding behaviour of eight n-alkyltrimethylammoniums to the brush border membrane. The binding gradually increased with each extension of the unbranched hydrocarbon chain from C8 to C14. On the other hand, C6 and C7 failed to bind under the present experimental conditions. The binding behaviour of these QACs did not correlate with their lipid solubility (data not shown). These findings directly indicated that the size of the hydrophobic part of molecule was an important factor in QAC binding to the brush border membrane. The curve shown in Fig. 1 was very similar to that of inhibitory effects of these QACs on methylchlorpromazine binding (Saitoh et al 1990), and there was a good correlation (r = 0·988) between binding behaviour and inhibitory effect, suggesting the presence of common binding sites on the membrane.

The absorption of n-alkyltrimethylammoniums following injection into the jejunal loop is shown in Fig. 2. The percentage of disappearance after 30 min was gradually increased with the

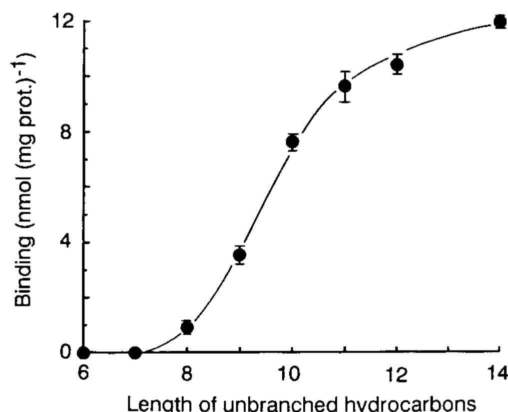


FIG. 1. Binding of eight n-alkyltrimethylammoniums to rat small intestinal brush-border membrane. The concentration of n-alkyltrimethylammonium was 16 μM. Each point represents the mean ± s.e.m. of 5–7 measurements with three membrane preparations, some s.e.m. lie within the symbol area.

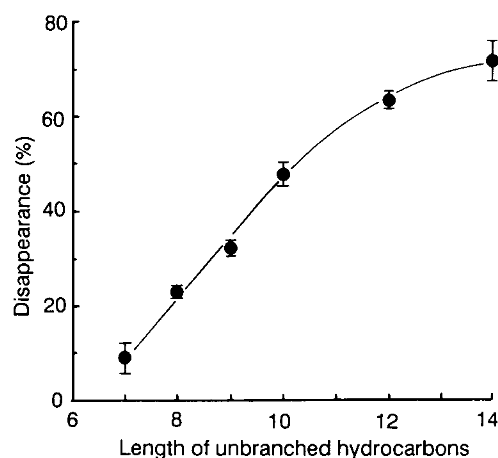


FIG. 2. Absorption of six n-alkyltrimethylammoniums from rat intestinal loops. The dose was 1 mL of 50 μM n-alkyltrimethylammonium in modified Ringer solution (pH 6·5) per 10 cm loop. Each point represents the mean ± s.d. of 3–4 experiments.

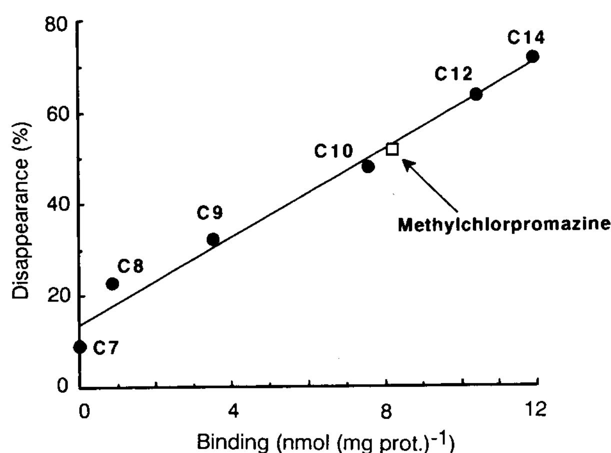


FIG. 3. The relationship between binding of n-alkyltrimethylammoniums to rat small intestinal brush-border membrane and their disappearance from rat intestinal loops. The line was determined by linear regression analysis (r = 0·991).

length of the hydrocarbon chain over a wide range of 8·9 to 71·3%. The relationship between binding to the brush border membrane and the disappearance are shown in Fig. 3. A good correlation was found between the two ($r = 0.991$), demonstrating that the absorption of n-alkyltrimethylammonium, which lacks binding activity, was extremely low. Moreover, the binding and absorption data of methylchlorpromazine, a QAC having a large tricyclic structure, were well fitted to the regression line shown in Fig. 3. The amount of methylchlorpromazine accumulated in mucosa was found to be 20% of the initial dose. The net absorption calculated from the difference between disappearance and mucosa accumulation amounted to ca. 30% of initial dose. It was, therefore, obvious that the disappearance shown in Fig. 3 did not imply the binding to the brush-border membrane alone, although mucosa concentration of n-alkyltrimethylammoniums could not be determined due to the interference of endogeneous substances.

## Discussion

In the present study, it has been shown that the absorption of QACs is closely associated with the degree of binding to the brush border membrane and that the size of the hydrophobic part of a QAC molecule is a principal determinant of both absorption and membrane binding. We have reported previously that physiological membrane potentials across the brush border membrane (inside-negative) is a driving-force to move a QAC, which is bound to the membrane, into the epithelium (Saitoh et al 1988a). It is possible that the movement of a QAC into epithelium is more sensitive to the membrane potentials when there is a large concentration gradient between the membrane and cell interior as a result of higher membrane binding. Therefore, it seems reasonable that QAC absorption should be correlated to the degree of membrane binding.

It should be noted that both binding and absorption exhibited slight saturability with extension of hydrocarbon chain length (Figs 1, 2). Although the reason is not clear, the introduction of an excessively large hydrophobic part into the QAC molecule seems not always to be effective in enhancing QAC absorption. As shown in Fig. 3, the hydrophobic part of methylchlorpromazine containing a phenothiazine ring was equivalent to that of C10. Since orally-active QACs have various structures of the hydrophobic part, it may be useful to compare their binding as a convenient estimate of their absorption.

It may be assumed that QACs without a hydrophobic part would be absorbed from the intestine with difficulty. However, some investigators have pointed out that small-molecular QACs such as choline and tetramethylammonium (TMA) are well absorbed, and that carrier-mediated transport systems participate in their membrane transport (Sanford & Smyth 1971; Kuczler et al 1977; Kessler et al 1978; Hegazy & Schwenk 1984; Tsubaki & Komai 1986). Recently, we have found that the transport mechanism of paraquat, a harmful bis-QAC, is different from that of a large QAC, with a hydrophobic part; initial uptake of paraquat by the brush border membrane vesicles is significantly inhibited by choline and TMA (unpublished data). It is, therefore, probable that small and large QACs are absorbed by different transport mechanisms.

## References

Denkert, M., Hackzell, L., Schill, G., Sjogren, E. (1981) Reversed-phase ion-pair chromatography with UV-absorbing ions in the mobile phase. J. Chromatogr. 218: 31–43

Hegazy, E., Schwenk, M. (1984) Choline uptake by isolated enterocytes of guinea pig. J. Nutr. 114: 2217–2220

Iseki, K., Sugawara, M., Saitoh, H., Miyazaki, K., Arita, T. (1989) Comparison of transport characteristics of amino β-lactam antibiotics and dipeptides across rat intestinal brush border membrane. J. Pharm. Pharmacol. 41: 628–632

Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M., Semenza, G. (1978) A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport system. Biochim. Biophys. Acta 506: 136–154

Kuczler, F. J., Nahrword, D. L., Rose, R. C. (1977) Choline influx across the brush border of guinea pig jejunum. Ibid. 465: 131–137

Larson, J. R., Pfeiffer, C. D. (1983) Determination of alkyl quaternary ammonium compounds by liquid chromatography with indirect photometric detection. Anal. Chem. 55: 393–396

Levine, R. R., Pelikan, E. W. (1961) The influence of experimental procedures and dose on the intestinal absorption of an onium compound, benzomethamine. J. Pharmacol. Exp. Ther. 131: 319–327

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265–275

Saitoh, H., Kobayashi, Y., Miyazaki, K., Arita, T. (1987) A highly sensitive HPLC method for the assay of propantheline used to measure its uptake by rat intestinal brush border membrane vesicles. J. Pharm. Pharmacol. 39: 9–12

Saitoh, H., Kawai, S., Miyazaki, K., Arita, T. (1988a) Transport characteristics of propantheline across rat intestinal brush border membrane. Ibid. 40: 176–180

Saitoh, H., Kawai, S., Iseki, K., Miyazaki, K., Arita, T. (1988b) Binding of organic cations to brush border membrane from rat small intestine. Ibid. 40: 776–780

Saitoh, H., Kawai, S., Iseki, K., Miyazaki, K., Arita, T. (1989) Transport characteristics of quaternary ammonium compounds across rat small intestinal brush border membrane. J. Pharmacobio-Dyn. 12: S-127

Saitoh, H., Noujoh, A., Chiba, Y., Iseki, K., Miyazaki, K., Arita, T. (1990) Correlation between structures of organic cations and their binding behaviours to brush border membrane isolated from rat small intestine. J. Pharm. Pharmacol. 42: 308–313

Sanford, P. A., Smyth, D. H. (1971) Intestinal transfer of choline in rat and hamster. J. Physiol. 215: 769–788

Schultz, S. G., Fuisz, R. E., Curran, P. F. (1966) Amino acid and sugar transport in rabbit ileum. J. Gen. Physiol. 49: 849–866

Tsubaki, H., Komai, T. (1986) Intestinal absorption of tetramethylammonium and its derivatives in rats. J. Pharmacobio-Dyn. 9: 747–754