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Enhanced gastrointestinal absorption of drugs in rats pretreated with the synthetic immunomodulator, levamisole

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The effect of levamisole on drug absorption from the rat small intestine has been investigated by means of an in-situ recirculation technique. The absorption of salicylic acid, sulphanilamide and aminopyrine was significantly increased by the intraperitoneal administration of levamisole (2 mg) 1 day before the absorption studies, but there was no significant effect on absorption from the small intestine of indomethacin, bromthymol blue, sulphafurazole (sulfisoxazole), quinine, sulphanilic acid, phenol red (phenolsulfonphthalein), L-tryptophan and fluorescein isothiocyanate-dextrans. The effect of levamisole on the absorption from the small intestine of salicylic acid was marginally dose- and time-dependent, the maximal effect being observed after pretreatment with 2 mg of levamisole 1 day before the absorption studies. Sulphanilamide, similarly, was better sorbed from the small intestine and also from the stomach in the presence of levamisole. The intraperitoneal administration of levamisole may influence the absorption of some low molecular weight drugs from the gastrointestinal tract.

It has been suggested that substances modifying the biological response might offer an effective and less toxic alternative or adjunct to conventional forms of treatment such as chemotherapy, radiotherapy and surgery. In cancer chemotherapy, such agents have been coadministered with other drugs. Recently, Machkova et al (1986) reported that pretreatment of mice with the immunomodulator muramyl dipeptide 1 h before methotrexate injection resulted in delayed absorption of methotrexate from subcutaneous tissues.

Levamisole, a biological response modifier, possesses immunopotentiating activities without direct anticancer effects, and is used in association with anticancer drugs, non-steroidal anti-inflammatory drugs, or analgesic-antipyretic drugs in the treatment of cancer (Symoens & Rosenthal 1977). However, few studies have been made to investigate the pharmacokinetic interaction between levamisole and other drugs. Our purpose has been to investigate the effect of levamisole on the absorption of some drugs using an in-situ recirculation of intestine or an in-situ loop of stomach.

Materials and methods

Levamisole was purchased from Aldrich Chemical Company, Inc. Indomethacin and fluorescein isothiocyanate-dextrans (average molecular weight, 65 600) were obtained from Sigma Chemical Co., St Louis, MO. Aminopyrine was obtained from Wako Pure Chemical Industries, Ltd. Salicylic acid, bromthymol blue, sulphanilamide, sulphafurazole (sulfisoxazole), quinine, sulphanilic acid, phenol red (phenolsulfonphthalein), L-tryptophan, tetramisole and all other reagents were of reagent grade obtained from Nakarai Chemical Co., Ltd.

Intestinal absorption studies. The isotonic buffer solution (pH 6·5) was prepared from 0·123 M Na_2HPO_4 and 0·163 M NaH_2PO_4. The concentration of drugs dissolved in this solution was 1 mM for salicylic acid, aminopyrine, and L-tryptophan, 0·1 mM for indomethacin, bromthymol blue, sulphanilamide, sulphafurazole, quinine, sulphanilic acid and phenol red, and 100 μg mL^{-1} for fluorescein isothiocyanate-dextrans.

Gastric absorption studies. The isotonic buffer solution (pH 1·1) was prepared from 0·05 M NaCl and 0·1 M HCl. Sulphanilamide was dissolved in this solution at a concentration of 0·5 mM.

Animals and pretreatment of levamisole. Male Wistar rats, 180–230 g, were housed in a stainless cage placed in a room maintained at 20–25 °C and on 12 h light-dark cycles. They had free access to water and diet for at least 2 days before use after which they were weighed and pretreated with levamisole, tetramisole or the solvent (saline) given intraperitoneally (0·5 mL) or intravenously (0·25 mL). Levamisole (2 mg), was also given intraluminally dissolved in the drug solution which was recirculated.

In-situ recirculation technique of the intestine. The procedure was as described by Kimura et al (1981). Rats were anaesthetized with i.p. pentobarbitone and the small or large intestine cannulated. Drug solution (40 mL for the small intestine, 20 mL for the large intestine), kept at 37 °C, was recirculated through the intestine for 1 h at 5 mL min^{-1} using a peristaltic pump. Then the perfusate in the intestine was withdrawn and the lumen washed with pH 6·5 buffer solution. The washings were combined with the perfused solution and made up to 100 mL with pH 6·5 buffer solution. The amount of drug disappearing from the lumen was calculated as the difference between the amounts of the drug in the initial and the final solutions.

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In-situ loop method of stomach. The method of Kimura et al (1981) was used. Rats were fasted overnight one day before the experiments, but allowed free access to water. Drug solution (4 mL) was held in the stomach for 30 min and the amount of drug taken up was calculated by the difference in the amounts of the drug between the initial and the final solutions.

The drugs were assayed by conventional spectrophotometric or spectrofluorometric techniques.

The results were analysed statistically with the Student's *t*-test. Differences with a P value of less than 0·05 were considered significant.

Results and discussion

To assess the effect of levamisole on the intestinal absorption of salicylic acid, sulphanilamide and sulphafurazole were chosen as rapidly or moderately absorbable drugs, whereas sulphanilic acid and phenol red were the poorly absorbable drugs and aminopyrine and quinine, which are rapidly or moderately absorbable, were the basic compounds. As shown in Table 1, the small intestine absorption of salicylic acid, aminopyrine and sulphanilamide was significantly increased by the pretreatment with levamisole compared with saline (0·9% NaCl) treatment as control. No significant change was observed with indomethacin, bromthymol blue, sulphafurazole, quinine, sulphanilic acid, phenol red, L-tryptophan, or fluorescein isothiocyanate-dextrans. Levamisole did not modify the absorption of salicylic acid or sulphanilamide from the large intestine. The other drugs were not studied. Intestinal absorption of salicylic acid is influenced by the mesenteric blood flow (Beubler & Lembeck 1976) and in addition our previous reports showed the intestinal absorption of salicylic acid to be significantly decreased by systemic anaphylaxis due to the reduced blood flow from the rat small intestine (Nakamura et al 1982b; Yamamoto et al 1984a, b, c). From these observations, it seems likely that changes in the mesenteric blood flow may be responsible for the enhanced absorption of drugs by the pretreatment with levamisole. In addition, the enhanced absorption of both salicylic acid, an anionic drug, and aminopyrine, a cationic drug, was observed by the levamisole, suggesting that this effect was not associated with the charge of the ionized drugs. On the other hand, intestinal absorption of indomethacin and bromthymol blue, which were chosen as drugs binding to the intestinal tissue (Hucker et al 1966; Nakamura et al 1976; Yamamoto et al 1985, 1986), was not affected by levamisole pretreatment. The absorption of poorly absorbable drugs such as sulphanilic acid and phenol red, whose absorption was reported to be enhanced by surfactants, bile salts (Kakemi et al 1970a; Kimura et al 1972), and disease states (ulcer) (Nakamura et al 1982a, 1983, 1984), was also not influenced by levamisole (i.p.), nor was the intestinal absorption of the macromolecular compound fluorescein isothiocyanate-dextrans. These findings suggest that progressive loss of structural integrity of the epithelium does not occur on pretreatment with levamisole. L-Tryptophan is absorbed mainly by an active transport system at a low concentration (Kakemi et al 1970b). That the intestinal absorption of 1 mM L-tryptophan was not affected by levamisole, suggested that levamisole may not have an effect on the drug absorption by the active transport system.

Table 1. The effect of levamisole on drug absorption from the rat small intestine. The absorption was examined by means of an in-situ recirculation technique for 1 h. Levamisole (2 mg) dissolved in 0·5 mL of saline was injected intraperitoneally 1 day before the absorption studies. Results are expressed as the mean ± s.d. with the number of experiments in parentheses.

Drugs	Concn (mM)	% Absorption in 1 h		Increase, fold
		Control	Levamisole	
Indomethacin	0·1	72·4 ± 4·2 (4)	70·1 ± 5·2 (4)[a]	0·97
Salicylic acid	1·0	64·0 ± 1·4 (4)	81·0 ± 2·1 (5)[b]	1·27
Aminopyrine	1·0	58·0 ± 3·1 (4)	73·4 ± 1·3 (4)[b]	1·27
Bromthymol blue	0·1	49·0 ± 6·5 (6)	53·1 ± 5·3 (6)[a]	1·08
Sulphanilamide	0·1	44·8 ± 2·9 (4)	64·1 ± 2·3 (5)[b]	1·43
Sulphafurazole	0·1	43·0 ± 7·5 (4)	42·7 ± 7·1 (4)[a]	0·99
Quinine	0·1	31·5 ± 5·3 (4)	31·8 ± 4·0 (4)[a]	1·01
Sulphanilic acid	0·1	7·8 ± 0·5 (5)	8·0 ± 0·4 (5)[a]	1·03
Phenol red	0·1	6·4 ± 1·0 (5)	6·1 ± 0·9 (5)[a]	0·95
L-Tryptophan	1·0	74·4 ± 3·2 (4)	76·9 ± 3·3 (4)[a]	1·03
Fluorescein isothiocyanate-dextrans	100 μg mL^{-1}	2·8 ± 0·7 (4)	3·3 ± 1·1 (4)[a]	1·18

[a] Not significantly different.
[b] $P < 0·001$, compared with each control.

The effect of levamisole on the absorption of salicylic acid was marginally dependent on the dose of levamisole (0·01–8 mg i.p.) and on the time of its administration (4 h–6 days). The maximal effect was observed after pretreatment with 2 mg of levamisole 1 day before the absorption studies. We also found sulphanilamide absorption to be enhanced in the stomach ($P < 0·001$) as well as the small intestine, but not in the large intestine. Absorption of salicylic acid was not significantly changed in the stomach or large intestine. Nor was any significant effect noted in the intestinal absorption of salicylic acid and sulphanilamide when levamisole was given in advance intravenously or simultaneously by intraluminal administration. A significant increase in salicylic acid and sulphanilamide absorption was observed after the pretreatment with tetramisole, a racemic mixture of levamisole and dexamisole, the enhancement of the absorption 2 mg of tetramisole being similar to that of 1 mg of levamisole.

It would appear from the findings that intraperitoneal pretreatment with levamisole influences the absorption of some drugs from the gastrointestinal tract but the mechanism involved remains to be resolved.

REFERENCES

Beubler, E., Lembeck, F. (1976) Naunyn-Schmiedeberg's Arch. Pharmacol. 292: 73–77

Hucker, H. B., Zacchei, A. G., Cox, S. V., Brodie, D. A., Cantwell, N. H. R. (1966) J. Pharmacol. Exp. Ther. 153: 237–249

Kakemi, K., Sezaki, H., Konishi, R., Kimura, T., Murakami, M. (1970a) Chem. Pharm. Bull. 18: 275–280

Kakemi, K., Sezaki, H., Nakano, M., Suzuki, E. (1970b) Ibid. 18: 2176–2182

Kimura, T., Sezaki, H., Kakemi, K. (1972) Ibid. 20: 1656–1662

Kimura, T., Kim, K. S., Sezaki, H. (1981) J. Pharmacobiodyn. 4: 35–41

Machkova, Z., Farghali, H., Janku, I., Masek, K. (1986) IRCS Med. Sci. 14: 71–72

Nakamura, J., Yoshizaki, Y., Yasuhara, M., Kimura, T., Muranishi, S., Sezaki, H. (1976) Chem. Pharm. Bull. 24: 683–690

Nakamura, J., Takada, S., Ohtsuka, N., Heya, T., Yamamoto, A., Kimura, T., Sezaki, H. (1982a) Ibid. 30: 2291–2293

Nakamura, J., Yamamoto, A., Takada, S., Kimura, T., Sezaki, H. (1982b) J. Pharmacobiodyn. 5: 278–284

Nakamura, J., Takada, S., Ohtsuka, N., Heya, T., Yamamoto, A., Kimura, T., Sezaki, H. (1983) J. Pharm. Pharmacol. 35: 369–372

Nakamura, J., Takada, S., Ohtsuka, N., Heya, T., Ueda, S., Hamaura, T., Yamamoto, A., Kimura, T., Sezaki, H. (1984) J. Pharmacobiodyn. 7: 485–491

Symoens, J., Rosenthal, M. (1977) J. Reticuloendothel. Soc. 21: 175–221

Yamamoto, A., Nakamura, J., Takada, S., Kimura, T., Sezaki, H. (1984a) J. Pharm. Sci. 73: 48–52

Yamamoto, A., Nakamura, J., Takada, S., Takeda, M., Hashida, M., Kimura, T., Sezaki, H. (1984b) J. Pharmacobiodyn. 7: 728–736

Yamamoto, A., Nakamura, J., Takada, S., Takeda, M., Hashida, M., Kimura, T., Sezaki, H. (1984c) Ibid. 7: 737–746

Yamamoto, A., Utsumi, E., Hamaura, T., Nakamura, J., Hashida, M., Sezaki, H. (1985) Ibid. 8: 830–840

Yamamoto, A., Utsumi, E., Sakane, T., Hamaura, T., Nakamura, J., Hashida, M., Sezaki, H. (1986) J. Pharm. Pharmacol. 38: 357–362

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Effects of a 5-lipoxygenase inhibitor, REV-5901, on leukotriene and histamine release from human lung tissue in-vitro

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REV-5901, α-pentyl-3-(2-quinolinylmethoxy)benzene-methanol, is an arylmethylphenyl ether derivative which inhibits 5-lipoxygenase activity of leukocytes. Its effects on the release of leukotrienes, induced by antigen and calcium ionophore, from human lung tissue in-vitro have been examined. At 1 and 10 μM it significantly inhibited the release of leukotrienes induced by both stimuli. At 10 μM it also inhibited antigen-induced histamine release. These results suggest that REV-5901 may be useful in clinical disorders such as asthma in which leukotriene release may be involved.

Metabolites of arachidonic acid, produced via the 5-lipoxygenase pathway possess a wide range of biological actions which suggest that they may be involved in immediate hypersensitivity and inflammatory reponses (Lewis & Austen 1981). Considerable evidence has accumulated to implicate leukotrienes in the pathophysiology of various lung diseases including asthma (O'Driscoll & Kay 1982). In particular, the sulphidopeptide leukotrienes (LTs), leukotriene C_4 (LTC_4) and leukotriene D_4 (LTD_4) are potent bronchoconstrictors in man (Barnes et al 1984). Furthermore, these LTs induce airway hyper-responsiveness, which is a fundamental abnormality in asthma (Boushey et al 1980), in normal humans (Barnes et al 1984).

One approach to developing a better treatment for asthma has been to synthesize new compounds which inhibit the 5-lipoxygenase pathway. REV-5901, α-pentyl-3-(2-quinolinylmethoxy)benzene-methanol is an arylmethylphenyl ether that inhibits 5-lipoxygenases in rat and human leukocytes in-vitro with respective EC50 values of 0·16 and 5 μM (Coutts et al 1985). Furthermore, it demonstrates selectivity for 5-lipoxygenases, being inactive against 12-lipoxygenase from rat platelets and cyclooxygenase from sheep seminal vesicles in concentrations of 100 and 200 μM, respectively (Coutts et al 1985).

The present experiments were designed to determine the effects of REV-5901 on the release in-vitro of leukotriene and histamine from human lung tissue, induced by immunological and non-immunological stimuli.

Materials and methods

Human lung tissue was obtained from operative specimens resected from patients with carcinoma of the lung after approval had been obtained from the Human Ethics Review Committee of the University of Sydney. Lung fragments were prepared, sensitized and challenged as described previously (Armour et al 1981). Briefly, the tissue was washed free of blood, cut into small fragments (approximately 3 mm^3) then incubated overnight in serum with a high titre of specific immunoglobulin E (IgE) to *Dermatophagoides pteronyssinus*. The following day, the fragments were repeatedly washed in warm Tyrode's solution, divided into repli-

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