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Enhanced absorption of salicylic acid and sulfanilamide by the synthetic immunomodulator levamisole in rats

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Summary

The effect of levamisole on drug absorption from the gastrointestinal tract was investigated by means of an in situ recirculation technique for the small or large intestine and an in situ loop method for the stomach. Intestinal absorption of salicylic acid and sulfanilamide was significantly increased by the i.p. administration of levamisole 1 day before the absorption studies, whereas no significant effect was noted in the intestinal absorption of these drugs by the intravenous pretreatment or simultaneous intraluminal administration of levamisole. The effect of levamisole on the absorption of salicylic acid was dose- and time-dependent and the maximal effect was observed by the pretreatment with 2 mg of levamisole 1 day before the absorption studies. On the other hand, levamisole also increased the absorption of sulfanilamide in the stomach but not in the large intestine. A significant increase in salicylic acid and sulfanilamide absorption was observed by the pretreatment of tretramisole. These results suggested that i.p. administration of levamisole may influence the absorption of some low-molecular-weight drugs from the gastrointestinal tract.

Introduction

Immunochemotherapy is nowadays one of the most promising fields in cancer therapy. Although chemotherapeutic agents have a dramatic effect on tumor cells, their side effects such as suppressed immune responses may make it difficult to obtain effective clinical results in many cases. Therefore, immunomodulators have been widely used for various kinds of cancer with anticancer chemotherapeutic agents to improve the chemotherapeutic activity of these agents (Oh-hashi et al., 1978; Pearson et al., 1972). In general, the effective results of this combination therapy may be due to the prevention of immunosuppression caused by the anticancer agents. However, little attention has been paid to the pharmacokinetic interaction of immunomodulators and other drugs, such as anticancer agents. Recently, Machkova et al. (1986) reported that pretreatment of mice by the synthetic immunomodulator muramyl dipeptide 1 h before methotrexate injection resulted in delayed absorption of methotrexate from subcutaneous tissues. In addition, we have previously demonstrated that intestinal absorption of sali-

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cylic acid, sulfanilamide, and aminopyrine was significantly enhanced by i.p. administration of levamisole (Utsumi et al., 1987). These results indicated that immunomodulators influenced the absorption and disposition of other drugs. Hence, the present study concerns the effect of levamisole on drug absorption from the gastrointestinal tract in more detail.

Materials and Methods

Materials

Levamisole was purchased from Aldrich Chemical Company, Inc. Salicylic acid, sulfanilamide, tetramisole and all other reagents were of reagent grade obtained from Nakarai Chemical Co., Ltd.

Preparation of drug solution

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, and 0.1 mM for sulfanilamide.

Gastric absorption studies. The isotonic buffer solution (pH 1.1) was prepared from 0.05 M NaCl and 0.1 M HCl. Salicylic acid and sulfanilamide were dissolved in this solution at a concentration of 3 mM and 0.5 mM, respectively.

Animals and pretreatment of levamisole

Male Wistar rats weighing 180–230 g, were used in these studies. The rats were housed in the stainless cage placed in a room maintained at 20–25 °C on 12 h light–dark cycles. Rats were weighed and pretreated with levamisole. For i.p. injection, levamisole dissolved in 0.5 ml of physiological saline was injected into the peritoneal cavity. Tetramisole was administered in the same manner as the i.p. administration of levamisole. For i.v. injection, levamisole dissolved in 0.25 ml of physiological saline was injected into the femoral vein under light ether anesthesia. Injections were given 1 day before starting the recirculation of drug solution, unless otherwise indicated. In the control experiments, the same volume of physiological saline was administered i.p. or i.v. For intraluminal administration, levamisole (2 mg) was dissolved in the drug solution and was recirculated simultaneously with the drug solution.

Absorption studies

Absorption studies were performed using an in situ recirculation technique and an in situ loop method.

The in situ recirculation of the intestine procedure was as described previously (Yamamoto et al., 1986). Rats were anesthetized with pentobarbital (19.4 mg/kg) given by i.p. injection, and the small or large intestine was cannulated for an in situ recirculation method. Drug solution (40 ml for the small intestine, 20 ml for the large intestine) kept at 37 °C was recirculated through the intestine at 5 ml/min using a peristaltic pump. At the end of the perfusion experiments, the perfusate in the intestine was withdrawn and the lumen was 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 that disappeared from the lumen was calculated as the difference between the amounts of the drug in the initial and the final solutions.

The absorption from the stomach was examined by means of an in situ loop method (Kimura et al., 1981). Rats were fasted overnight prior to the experiments, but allowed free access to water. Drug solution (4 ml) was kept in the stomach for 30 min and the amount of drug absorbed in 30 min was calculated by the difference in the amounts of the drug between the initial and the final mucosal solutions.

Analytical methods

Salicylic acid. Sample solution (3 ml) was acidified with 0.1 ml of 35% HCl and extracted with 7 ml of chloroform. The organic phase (5 ml) was then shaken with 5 ml of 0.1 M NaOH and the optical density of the aqueous phase was determined at 295 nm (Nakamura et al., 1982).

Sulfanilamide. Sulfanilamide was diazotized, coupled with 2-diethylaminoethyl-1-naphthylamine, and then was extracted with iso-amyl alcohol after the addition of sodium chloride. The

optical density of the organic layer was determined at 555 nm (Yamamoto et al., 1984a)

Statistical analyses

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

Effect of administration route of levamisole on drug absorption from the rat small intestine

Rats were treated with levamisole and intestinal absorption of salicylic acid and sulfanilamide was examined by means of an in situ recirculation technique for 1 h. Levamisole (2 mg) was administered i.p. or i.v. 1 day before the perfusion experiment. Salicylic acid and sulfanilamide were chosen as model drugs because the maximal increase in their absorption was observed by the pretreatment with levamisole in our previous report (Utsumi et al., 1987). As demonstrated in Fig. 1, intestinal absorption of salicylic acid (A) and sulfanilamide (B) was significantly increased by i.p. administration of levamisole, whereas no significant effect was noted in the intestinal absorption of these drugs by i.v. pretreatment or simultaneous intraluminal administration of levamisole. Therefore, this result indicated that the enhancement effect of drug absorption by levamisole was dependent on the administration route of this immunomodulator. The result that intraluminal administration of levamisole had no effect on drug absorption suggested that levamisole might not interact with the epithelial membrane directly. Furthermore, because levamisole is known to be extensively metabolized in the liver (Symoens and Rosenthal, 1977), the different effect between i.v. and i.p. administration of levamisole might be explained by the amount of levamisole metabolites which might be more effective to the enhanced absorption of the drugs than levamisole itself.

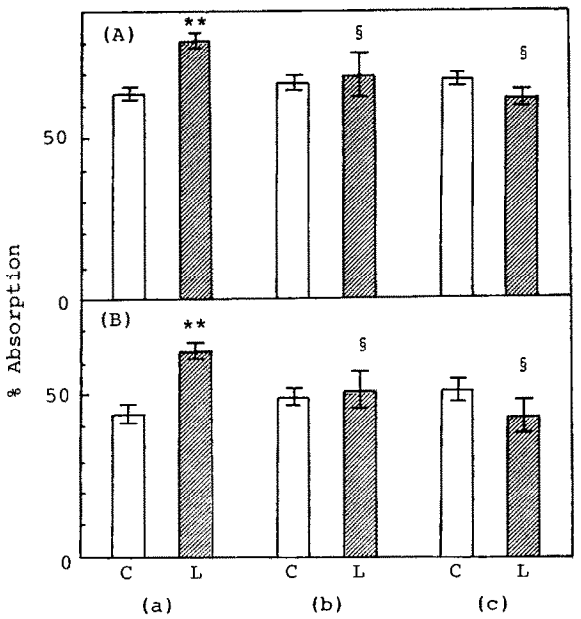

Fig. 1. Effect of administration route of levamisole on salicylic acid (A) and sulfanilamide (B) absorption from the rat small intestine. Intestinal absorption of drugs was examined by means of an in situ recirculation technique for 1 h. Levamisole (2 mg) was administered i.p. (a), intraluminally (b), i.v. (c) as described in Materials and Methods. Results are expressed as the mean ± S.D. of at least 4 rats. C, control; L, levamisole; **, $P < 0.001$, §, not significantly different, compared with the control.

Effect of levamisole on time course of drug absorption from the rat small intestine

Fig. 2 shows the effect of intraperitoneal levamisole on time course of salicylic acid and sulfanilamide absorption from the rat small intestine. The absorption process of these drugs seemed to follow the apparent first-order kinetics even in the case of levamisole pretreatment. In addition, the enhanced absorption of drugs by levamisole has been already observed 15 min after starting the recirculation of the drug solution.

Dose dependency of levamisole-induced enhancement of salicylic acid absorption from the rat small intestine

The effect of levamisole dose on the intestinal absorption of salicylic acid is shown in Table 1. Animals were pretreated i.p. with 0.01–8 mg of levamisole 1 day before the absorption studies. A significant increase in salicylic acid absorption was observed in rats pretreated with 0.1–4 mg of levamisole, whereas no significant change was noted in rats pretreated with 0.01 and 8 mg of

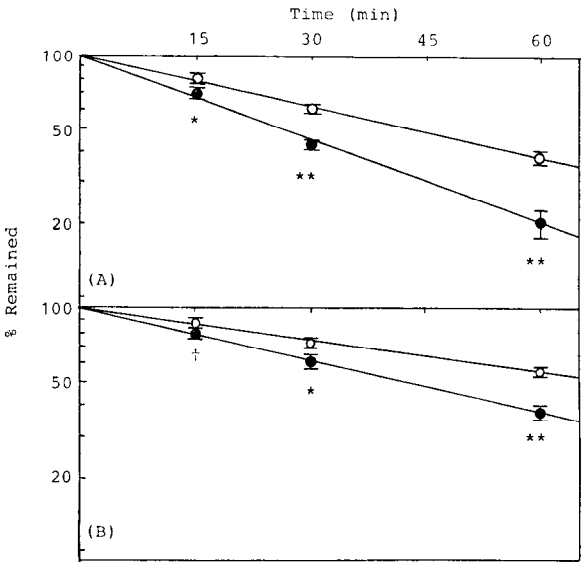

Fig. 2. Effect of levamisole on time course of salicylic acid (A), and sulfanilamide (B) absorption from the rat small intestine. Intestinal absorption of drugs was examined by means of an in situ recirculation technique for 15, 30 and 60 min. Levamisole (2 mg) dissolved in 0.5 ml of saline was injected i.p. 1 day before the absorption studies. Results are expressed as the mean ± S.D. of at least 4 rats. ○, control; ●, levamisole; **, $P < 0.001$; *, $P < 0.01$; †, $P < 0.05$, compared with the control.

levamisole. A maximum effect was obtained in rats following pretreatment with 2 mg of levamisole. In a previous paper (Sampson, 1978), it was reported that the immunostimulating effect of levamisole was dose-dependent and that at high concentrations, suppression rather than augmentation of the immune response was observed. Moreover, similar dose–response effects were also obtained in terms of tumor growth (Sampson, 1978). That is, tumor inhibition was found to be dose-dependent (0–8 mg/kg) in rats, and at high doses tumor inhibition did not occur, while at lower doses the growth of the cancer was inhibited. These findings are in good agreement with the absorption studies and suggest that some immunological factors may influence the enhanced absorption of drugs.

Effect of timing of the levamisole pretreatment on the salicylic acid absorption from the rat small intestine

The effect of timing of the levamisole pretreatment on the intestinal absorption of salicylic acid was also examined and the results are presented in Table 2. Animals were pretreated i.p. with 2 mg of levamisole 4–5 h, 1, 2, 4, and 6 days before the absorption studies. The enhanced absorption of salicylic acid was noted in rats pretreated with levamisole 1, 2 and 4 days before the absorption studies. However, no significant change was observed in the intestinal absorption of salicylic acid by the pretreatment of levamisole 4–5 h and 6

TABLE 1

Dose dependency of levamisole-induced enhancement of salicylic acid absorption from the rat small intestine

Dose	% Absorption in 1 h	Increase
Control (saline)	64.0 ± 1.4 (4)	
Levamisole 0.01 mg	68.9 ± 8.3 (4) [a]	1.08×
Levamisole 0.1 mg	75.4 ± 2.1 (4) [b]	1.18×
Levamisole 1 mg	73.0 ± 6.2 (4) [d]	1.14×
Levamisole 2 mg	81.0 ± 2.1 (5) [b]	1.27×
Levamisole 4 mg	76.0 ± 5.6 (6) [c]	1.19×
Levamisole 8 mg	69.2 ± 6.0 (4) [a]	1.08×

Intestinal absorption of salicylic acid was examined by means of an in situ recirculation technique for 1 h. Levamisole (0.01–8 mg) dissolved in 0.5 ml of saline was injected i.p. 1 day before the absorption studies. Results are expressed as the mean ± S.D. with the number of experiments in parentheses. [a] Not significantly different, [b] $P < 0.001$, [c] $P < 0.01$, [d] $P < 0.05$, compared with the control.

TABLE 2

Effect of timing of the levamisole pretreatment on salicylic acid absorption from the rat small intestine

Pretreatment time	% Absorption in 1 h	Increase
Control (saline 1 day)	64.0 ± 1.4 (4)	
Levamisole 4-5 h	68.0 ± 4.8 (6) [a]	1.06×
Levamisole 1 day	81.0 ± 2.1 (5) [b]	1.27×
Levamisole 2 days	73.1 ± 6.7 (6) [c]	1.14×
Levamisole 4 days	72.2 ± 6.5 (4) [c]	1.13×
Levamisole 6 days	67.5 ± 6.3 (6) [a]	1.05×

Intestinal absorption of salicylic acid 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 i.p. 4–5 h, 1, 2, 4 and 6 days before the absorption studies. Results are expressed as the mean ± S.D. with the number of experiments in parentheses. [a] Not significantly different, [b] $P < 0.001$, [c] $P < 0.05$, compared with the control.

days before the absorption studies. The most effective condition for enhanced salicylic acid absorption was observed by the i.p. pretreatment with 2 mg of levamisole 1 day before the absorption studies. It was known that although levamisole was rapidly excreted from the body into the urine, the pharmacological and immunological effects of the drug were prolonged (Ramot et al., 1976). The result of this report suggests that the enhanced absorption of salicylic acid by the pretreatment of levamisole may be closely related to the prolonged pharmacological effect of levamisole.

Regional differences in the effect of levamisole on drug absorption

The effect of levamisole on the gastric and the intestinal absorption of salicylic acid and sulfanilamide was examined in order to investigate whether the enhanced absorption of these drugs by levamisole would be observed in all segments of the gastrointestinal tract or not. As shown in Table 3, site specificity is evident. The absorption of salicylic acid from the small intestine was enhanced by the pretreatment of levamisole (2 mg), but not in the stomach and the large intestine. On the other hand, the absorption of sulfanilamide was significantly enhanced in the stomach and the small intestine, although it was not influenced in the large intestine. The difference in levamisole absorption promoting effect between salicylic acid and sulfanilamide in the stomach was not clearly defined. However, these results may be partly explained on the difference of changes in mesenteric blood flow because these drugs, well absorbed drugs, were affected by the mesenteric blood flow (Yamamoto et al., 1984b and c; 1985). Overall, the action of levamisole on intestinal absorption of drugs was found to be more effective than that on gastric absorption of drugs in spite of the enhancement of sulfanilamide absorption (ratio = 1.67, compared with the control).

TABLE 3

Regional differences in the effect of levamisole on drug absorption

	% Absorption		Increase
	Control	Levamisole	
Salicylic acid			
Stomach	43.9 ± 2.9 (4)	48.0 ± 7.2 (8) [a]	1.09×
Small intestine	64.0 ± 1.4 (4)	81.0 ± 2.1 (5) [b]	1.27×
Large intestine	44.4 ± 4.7 (4)	45.4 ± 4.1 (4) [a]	1.02×
Sulfanilamide			
Stomach	2.1 ± 0.5 (6)	3.5 ± 0.2 (4) [b]	1.67×
Small intestine	44.8 ± 2.9 (4)	64.1 ± 2.3 (4) [b]	1.43×
Large intestine	11.2 ± 1.3 (4)	11.0 ± 0.5 (4) [a]	0.98×

The effect of levamisole on the gastric and the intestinal absorption of drugs was examined by means of an in situ loop method for 30 min and in situ recirculation technique for 1 h, respectively. Levamisole (2 mg) dissolved in 0.5 ml of saline was injected i.p. 1 day before the absorption studies. Results are expressed as the mean ± S.D. with the number of experiments in parentheses.

[a] Not significantly different, [b] $P < 0.001$, compared with the control.

Effect of tetramisole on the drug absorption from the rat small intestine

The effect of tetramisole, which is the racemic mixture of levamisole and its D-isomer dexamisole, on drug absorption from the rat small intestine is shown in Fig. 3. Animals were pretreated i.p. with 2 mg of tetramisole 1 day before the absorption studies. A significant increase in salicylic acid (A) and sulfanilamide (B) absorption was observed and the value of the enhanced absorption percentage by 2 mg of tetramisole was almost equal to that by 1 mg of levamisole. In addition, there was a statistically significant difference between the absorption percentage for 2 mg tetramisole and 2 mg levamisole ($P < 0.01$ for salicylic acid, $P < 0.001$ for sulfanilamide). It has been reported that though both of tetramisole and levamisole have the anthelmintic and immunopotentiating effects, dexamisole, the D-isomer, is almost completely devoid of immunomodifying properties (Renoux and Renoux, 1974). These findings suggest that dexamisole is ineffective in the enhanced absorption of these drugs from the gastrointestinal tract as well as the immunomodulating effect.

These results lead us to conclude that i.p. pretreatment of levamisole may influence the absorption of some drugs from the gastrointestinal tract. The mechanism by which intestinal absorption of salicylic acid and sulfanilamide was enhanced by the pretreatment of levamisole is not defined at

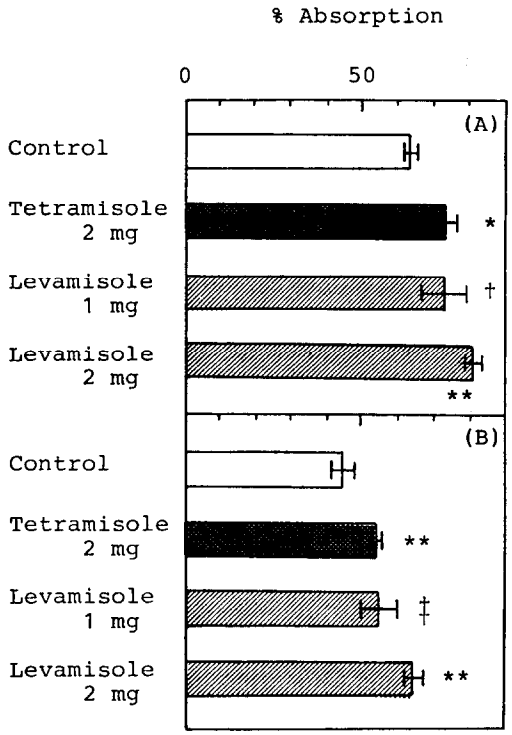

Fig. 3. Effect of tetramisole on salicylic acid (A) and sulfanilamide (B) absorption from the rat small intestine. Intestinal absorption of drugs was examined by means of an in situ recirculation technique for 1 h. Tetramisole (2 mg) dissolved in 0.5 ml saline was injected i.p. 1 day before the absorption studies. Results are expressed as the mean ± S.D. of at least 4 rats. **, $P < 0.001$; *, $P < 0.01$; ‡, $P < 0.02$; †, $P < 0.05$; compared with the control.

present. In this case, various mechanisms may be considered. One of the most important mechanisms is the alteration of the mesenteric blood flow by the pretreatment of levamisole. It is well known that the absorption rates of rapidly absorbed drugs are controlled by the mesenteric blood flow. Beubler and Lembeck (1976) demonstrated that the absorption rate of salicylic acid was affected by the intestinal blood flow. Therefore, changes in the vascular system by the pretreatment of levamisole might influence the absorption rate of salicylic acid and sulfanilamide. Another possibility is that changes in the mucosal permeability of drugs induced by levamisole may contribute to the enhanced absorption of drugs. Moreover, it was known that an increase in cyclic GMP (cGMP) and a decrease in cyclic AMP (cAMP) were observed in vitro in the presence of levamisole (Symoens and Rosenthal, 1977) and cyclic nucleotide causes significant increases in absorption of drugs (Nakamura et al., 1979). The changes in the cyclic nucleotide level induced by levamisole may also play an important role in the enhancement effect on drug absorption. More extensive investigation will be needed to elucidate the mechanism of the observation in this paper.

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