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Effect of immunomodulator on drug absorption from the gastrointestinal tract: the mechanism whereby levamisole enhanced the intestinal absorption of sulfanilamide in the rat

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Summary

Rats were pretreated intraperitoneally with levamisole and the intestinal absorption of sulfanilamide was examined by means of an in situ recirculation technique. Levamisole enhanced the in situ intestinal absorption of sulfanilamide and the effect was dependent on the dose and timing of levamisole pretreatment. The maximal effect was observed by the pretreatment with 2 mg of levamisole 1 day before the absorption studies. The increment in absorption was also confirmed by the measurement of blood concentration and the urinary excretion of sulfanilamide was examined by means of an in situ loop method. The pH value of the luminal solution, intraluminal protein and mucus, and the intestinal permeability of sulfanilamide examined by means of an in vitro everted sac experiment were not affected by the pretreatment with levamisole. However, levamisole significantly increased the intestinal blood flow measured by the hydrogen gas clearance method. Furthermore, the enhancement effect of levamisole pretreatment was almost restored to the control level when 0.2 mg of dibutyryl cyclic AMP was given intraperitoneally just before the absorption experiments. These findings suggest that the levamisole-induced increase in sulfanilamide absorption from the rat small intestine may be in part due to the increased blood flow and to the change in the cyclic nucleotide level.

Introduction

Levamisole is known to be an immunostimulant in both experimental animals and man and it has been used as an adjunct for the therapy of malignancies (Chirigos et al., 1973; Gonzalez et al., 1978). In most animal cancer models, levamisole does not suppress the growth of the primary tumors but prolongs the remission period after chemotherapy, and increases the number of long-term survivors if given after cytoreductive chemotherapy (Study Groups of Bronchogenic Carcinoma, 1975). Furthermore, some studies suggest that levamisole specifically inhibits metastasis formation (Renoux et al., 1972). In cancer patients, levamisole has been shown to restore delayed-type skin reactivity and, in Hodgkin patients, E-rossete

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formation in vitro, possibly because it restores the number of azathioprine-sensitive T cells (Amery, 1976).

In recent years, the application of non-specific immunomodulators, such as levamisole, in combination with other forms of therapy to augment host immunity, suggest that immunological control measures may prove valuable adjuncts to the control of neoplasia (Chirigos et al., 1975). Therefore, it was of utmost importance to examine the pharmacokinetic interaction of immunomodulators and other drugs. 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 (Machkova et al., 1986). In addition, Svensson demonstrated that administration of tilorone hydrochloride (50 mg/kg/day), a synthetic immunomodulator, for 4 days resulted in a 42% reduction in antipyrine clearance and 71% increase in plasma half-life (Svensson, 1986). We have also shown that intraperitoneal pretreatment with levamisole enhanced the intestinal absorption of salicylic acid and other low molecular weight drugs, although the mechanism has not been clearly demonstrated (Utsumi et al., 1987; Yamamoto et al., 1987).

The present study was undertaken to investigate the effect of the synthetic immunomodulator, levamisole on the intestinal absorption of sulfanilamide and to clarify the mechanism of its action.

Materials and Methods

Materials

Levamisole was purchased from Aldrich Chemical Co. Dibutyryl cyclic AMP was obtained from Yamasa Shoyu Co. Sulfanilamide and all other reagents were of reagent grade obtained from Nakarai Chemical Co. The isotonic buffer solution of pH 6.5 was prepared from 0.123 M Na_2HPO_4 and 0.163 M NaH_2PO_4. Sulfanilamide was dissolved in this buffer solution at a concentration of 0.1 mM for absorption studies.

Animals and pretreatment with levamisole

Male Wistar rats (180–230 g) were used in these studies. The rats were housed in a stainless steel cage placed in a room maintained at 20–25 °C on 12 h light–dark cycles. Rats were weighed and pretreated with levamisole. Levamisole (2 mg) dissolved in 0.5 ml of physiological saline was injected into the peritoneal cavity and absorption studies were carried out 4 h–6 days later.

Absorption studies

Absorption studies were carried out using an in situ recirculation technique and an in vitro everted sac technique.

In situ recirculation technique. The procedure was as described previously (Nakamura et al., 1982; Yamamoto et al., 1984a, b and 1984c, 1985, 1986). Rats were anesthetized with pentobarbital (19.4 mg/kg) given by intraperitoneal injection and the small intestine was cannulated for an in situ recirculation experiment. The entire length from the pylorus to ileo-cecal junction was used. The bile duct was ligated in all experiments. Drug solution (40 ml) kept at 37 °C was recirculated through the intestine for 1 h 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.

In vitro everted sac technique. Intestinal uptake and transport of sulfanilamide were studied by means of an in vitro everted sac experiment. The procedure was the same as that reported by Yasuhara et al. (1978). Under pentobarbital anesthesia (19.4 mg/kg), the small intestine was washed with pH 6.5 isotonic phosphate buffer solution and quickly removed from the rat. The removed intestine was everted by means of a wire inserted through the lumen. The upper 20 cm segment (jejunum) and the lower 20 cm segment (ileum) were everted and ligated at one end. As serosal fluid, 3 ml of pH 7.4 phosphate buffer solution was introduced from the opposite end. The everted sac was ligated and placed in the mucosal fluid (10 ml of pH 6.5 isotonic phosphate

buffer solution containing 0.1 mM sulfanilamide) bubbled with 5% CO_2 in O_2 at 37°C. At the end of the incubation period (30 min), the sac was cut, opened at one end, and the serosal fluid was collected. The sac was rinsed, weighed and homogenized in 4 vols. of pH 6.5 phosphate buffer solution. One ml of 30% (w/v) trichloroacetic acid was added to precipitate the protein and the supernatant of the homogenate, mucosal and serosal fluid were assayed for sulfanilamide.

Determination of sulfanilamide in blood

Rats were anesthetized with pentobarbital (19.4 mg/kg) and the entire length from the pylorus to ileo-cecal junction was used for this experiment. After the intravenous injection of 0.1 ml of heparin solution (1 unit/ml), sulfanilamide solution (1 mg/5 ml in pH 6.5 buffer solution) kept at 37°C was introduced into the entire loop of the small intestine. Blood samples (1 ml) were taken from the carotid vein at 5, 10, 20, 40 and 60 min after the administration of sulfanilamide solution. One ml of 30% (w/v) trichloroacetic acid was added to the blood samples and the supernatant of the blood samples were assayed for sulfanilamide.

Determination of urinary excretion of sulfanilamide

Sulfanilamide (1 mg) dissolved in 2 ml of distilled water was administered by gastric intubation under light ether anesthesia. Following the intubation, each animal was placed in a metabolic cage. The urine was collected at 4 h intervals for the first 12 h, and then at the following 12 h intervals (Nakamura et al., 1983).

Volume, pH, protein and mucus determination in the perfusate

The method was the same as in situ absorption studies. After recirculation of pH 6.5 buffer solution (40 ml) for 1 h, the perfusate volume and the pH of the perfusate were determined. In addition, an aliquot of the perfusate was centrifuged for 10 min at 3000 rpm and the protein and mucus concentrations in the supernatant were assayed.

Exsorption studies

The exsorption of sulfanilamide from the blood circulation to the gut lumen was studied as described previously (Kakemi et al., 1970). The operation was the same as the absorption studies. After the administration of sulfanilamide (10 mg/0.25 ml in saline) into the right femoral vein, the small intestine was perfused with pH 6.5 buffer solution at 3 ml/min. The perfusate was collected every 10 min for 1 h, and the exsorption rates of sulfanilamide and its metabolites in the perfusate were calculated.

Blood flow measurements

Intestinal blood flow in control and levamisole-treated rats was measured by a hydrogen gas clearance method (PHG 201, Unique Medical Co.) as described previously (Yamamoto et al., 1984c, 1986).

Pretreatment of dibutyryl cyclic AMP

Dibutyryl cyclic AMP (0.2 mg) was administered intraperitoneally just before starting the absorption experiments.

Analytical method

Sulfanilamide. Sulfanilamide was diazotized, coupled with 2-diethylaminoethyl-1-naphthylamine, and then was extracted with isoamyl alcohol after the addition of sodium chloride. The optical density of the organic layer was determined at 555 nm. The level of acetylated compounds was determined by the difference in total diazo reactants before and after the hydrolysis of the sample for 1 h in a boiling water bath with 1 ml of 2 N HCl (Yamamoto et al., 1984a).

Protein. Protein was determined by the method of Lowry et al. (1955) using bovine serum albumin as a standard.

Mucus. Mucus was determined by the anthrone method using D-glucose as a standard and a colorimetric reading at 620 nm (Roe et al., 1955).

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

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

Rats were pretreated intraperitoneally with 0.01–8 mg of levamisole 1 day before the absorption studies and intestinal absorption of sulfanilamide was examined by means of an in situ recirculation technique. As shown in Table 1, a significant increase in sulfanilamide 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 levamisole. A maximal effect was observed in rats following pretreatment with 2 mg of levamisole.

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

The effect of timing of levamisole pretreatment on the intestinal absorption of sulfanilamide was also examined. Rats were pretreated intraperitoneally with 2 mg of levamisole 4–5 h, 1, 2, 4 and 6 days before the absorption studies. As shown in Table 2, enhanced absorption of sulfanilamide was noted in rats pretreated with levamisole 4–5 h, 1, 2, and 4 days before the absorption studies.

TABLE 1

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

Intestinal absorption of sulfanilamide (0.1 mM) 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 intraperitoneally 1 day before the absorption studies. Results are expressed as the mean ± S.D. with the number of experiments in parentheses.

Dose	% Absorption in 1 h	Increase (fold)
Control (saline)	44.8 ± 2.9 (4)	–
Levamisole 0.01 mg	52.1 ± 7.1 (4) §	1.16
Levamisole 0.1 mg	56.3 ± 2.7 (4) *	1.26
Levamisole 1 mg	54.6 ± 4.8 (4) ‡	1.22
Levamisole 2 mg	64.1 ± 2.3 (5) **	1.43
Levamisole 4 mg	55.5 ± 5.4 (6) *	1.24
Levamisole 8 mg	49.8 ± 5.5 (4) §	1.11

** $P < 0.001$; * $P < 0.01$; ‡ $P < 0.02$; § not significantly different, compared with the control.

TABLE 2

Time dependency of levamisole-induced enhancement of sulfanilamide absorption from the rat small intestine

Intestinal absorption of sulfanilamide (0.1 mM) 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 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.

Pretreatment time	% Absorption in 1 h	Increase (fold)
Control (saline)	44.8 ± 2.9 (4)	–
Levamisole 4–5 hrs	51.7 ± 2.9 (6) *	1.15
Levamisole 1 day	64.1 ± 2.3 (5) **	1.43
Levamisole 2 days	55.3 ± 6.5 (6) ‡	1.23
Levamisole 4 days	52.8 ± 4.9 (4) †	1.18
Levamisole 6 days	51.0 ± 4.9 (6) §	1.14

** $P < 0.001$; * $P < 0.01$; ‡ $P < 0.02$; † $P < 0.05$; § not significantly different, compared with the control.

However, no significant change was observed in the intestinal absorption of sulfanilamide by the pretreatment with levamisole 6 days before the absorption studies. From these experiments (Tables 1, 2), the most effective condition for enhanced sulfanilamide absorption was observed by intraperitoneal pretreatment with 2 mg of levamisole 1 day before the absorption studies.

Blood concentration of sulfanilamide after administration to the intestinal loop in levamisole-pretreated rats

In order to confirm the enhancement effect of levamisole on sulfanilamide absorption from the rat small intestine, blood concentration–time profiles of sulfanilamide after administration to the small intestinal loop in control and levamisole-pretreated rats were examined and the results are shown in Fig. 1. The blood concentration of sulfanilamide reached a peak level at about 10 min in both groups, and significantly increased at 5, 10, and 20 min in levamisole-pretreated rats.

Urinary excretion rate of sulfanilamide and its metabolites following oral administration in levamisole-pretreated rats

The enhancement effect of levamisole on sulfanilamide absorption from the rat small in-

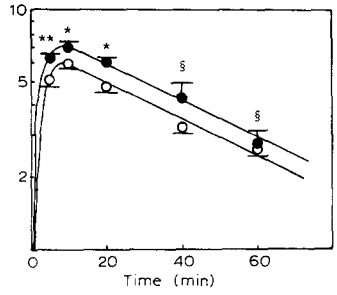

Fig. 1. Effect of pretreatment with levamisole on the blood concentration of sulfanilamide after administration to the intestine. Levamisole (2 mg) dissolved in 0.5 ml of saline was administered intraperitoneally 1 day before the experiments. Sulfanilamide (1 mg) dissolved in 5 ml of pH 6.5 buffer solution was introduced into the entire loop of the small intestine and blood samples (1 ml) were taken from the carotid vein at 5, 10, 20, 40 and 60 min after the administration of sulfanilamide solution. Results are expressed as the mean ± S.D. of at least 3 rats. Key: ○, control; ●, levamisole. ** $P < 0.001$; * $P < 0.01$; † $P < 0.05$; § not significantly different, compared with the control.

testine was also confirmed by means of urinary excretion experiments. Fig. 2 shows urinary excretion rates of sulfanilamide and its metabolites. As shown in Fig. 2, the urinary excretion rate of sulfanilamide in the urine samples of the initial 4 h was significantly increased in levamisole-pretreated rats than that in control rats. However, no significant effect was noted on the urinary excretion rate of sulfanilamide metabolites between levamisole-pretreated and normal rats.

Volume, pH, protein and mucus determination in the perfusate

In order to investigate the mechanism whereby levamisole influences the intestinal absorption of sulfanilamide, the pH value, the perfusate volume, intraluminal protein and mucus release were examined. However, these factors were not affected by the pretreatment with levamisole.

Effect of pretreatment with levamisole on the mucosal uptake, tissue accumulation, and serosal transfer of sulfanilamide

Intestinal permeability of sulfanilamide was examined by means of an in vitro everted sac experiment in levamisole-pretreated rats. As shown in Table 3, a significant decrease in ileal mucosal uptake and serosal transfer of sulfanilamide was observed in rats pretreated with levamisole, but was not observed in jejunum. Moreover, in both jejunum and ileum, no significant effect was obtained on the tissue accumulation of sulfanilamide by the pretreatment with levamisole. Overall, intestinal permeability of sulfanilamide was not affected by the pretreatment with levamisole.

Intestinal exsorption of sulfanilamide and its metabolites in levamisole-pretreated rats

Intestinal permeability of sulfanilamide was also studied by means of an in situ intestinal exsorption experiment to clarify whether it could be altered by intraperitoneal pretreatment with levamisole. However, as demonstrated in Fig. 3, no significant change was observed on the intestinal exsorption of sulfanilamide and its metabolites between levamisole-pretreated and control rats.

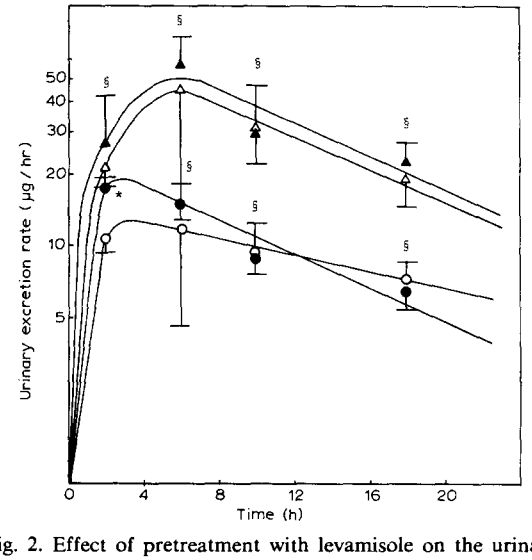

Fig. 2. Effect of pretreatment with levamisole on the urinary excretion rates of sulfanilamide. Levamisole dissolved in 0.5 ml of saline was administered intraperitoneally 1 day before the experiments. Sulfanilamide (1 mg) dissolved in 0.5 ml of saline was administered by gastric intubation under light ether anesthesia and the urine was collected at 4, 8, 12, and 24 h in a metabolic cage. Results are expressed as the mean ± S.D. of 4 rats. Key: ○, control (sulfanilamide); ●, levamisole (sulfanilamide); △, control (metabolites); ▲, levamisole (metabolites). * $P < 0.01$; § not significantly different, compared with the control.

TABLE 3

Effect of levamisole on the mucosal uptake (M), tissue accumulation (T), and serosal transfer (S) of sulfanilamide (% of dose)

Mucosal uptake, tissue accumulation and serosal transfer of sulfanilamide (0.1 mM) was examined by means of an in vitro everted sac technique for 30 min. 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.

		Control	Levamisole
M	Jejunum	19.9 ± 3.9 (3)	18.5 ± 6.2 (4) §
	Ileum	19.0 ± 1.6 (4)	17.0 ± 1.0 (6) †
T	Jejunum	10.3 ± 1.0 (3)	10.4 ± 0.5 (4) §
	Ileum	8.6 ± 0.6 (4)	9.6 ± 1.0 (6) §
S	Jejunum	5.9 ± 0.8 (3)	5.7 ± 0.2 (4) §
	Ileum	6.5 ± 0.2 (4)	6.0 ± 0.3 (6) †

† $P < 0.05$; § not significantly different, compared with the control.

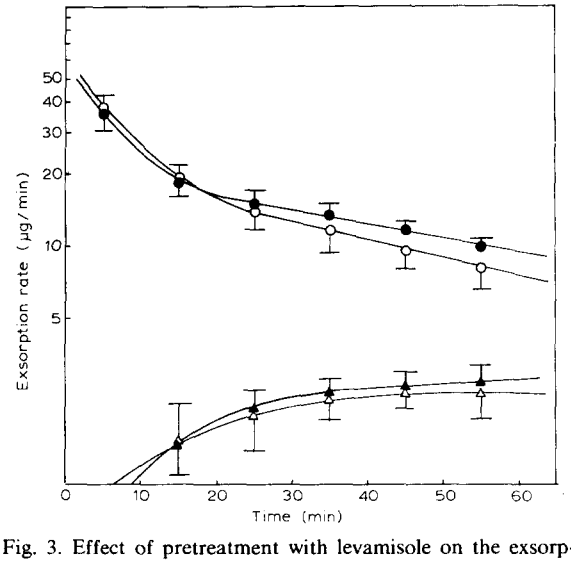

Fig. 3. Effect of pretreatment with levamisole on the exsorption rate of sulfanilamide and its metabolites from the rat small intestine. Levamisole (2 mg) dissolved in 0.5 ml of saline was administered intraperitoneally 1 day before the experiments. Sulfanilamide (10 mg/0.25 ml in saline) was administered intravenously into the right femoral vein, and the small intestine was perfused with pH 6.5 buffer solution at 3 ml/min. The perfusate was collected every 10 min for 1 h, and the exsorption rates of sulfanilamide and its metabolites were calculated. Results are expressed as the mean ± S.D. of 4 rats. Key: ○, control (sulfanilamide); ●, levamisole (sulfanilamide); △, control (metabolites); ▲, levamisole (metabolites).

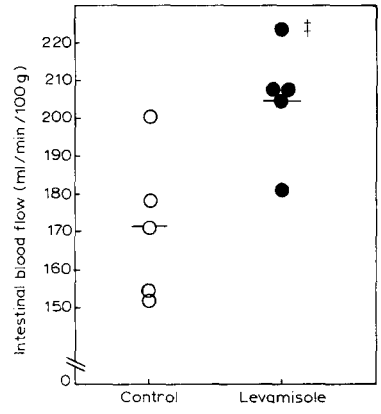

Fig. 4. Effect of pretreatment with levamisole on intestinal blood flow. Intestinal blood flow in control (○) and levamisole-treated (●) rats was measured by means of a hydrogen gas clearance method. Levamisole (2 mg) dissolved in 0.5 ml of saline was administered intraperitoneally 1 day before the experiments. Each circle represents the value of intestinal blood flow in each animal and horizontal bars show the mean values of intestinal blood flow in each group. Key: ‡ $P < 0.02$, compared with the control.

Effect of pretreatment with levamisole on intestinal blood flow

Fig. 4 shows the intestinal blood flow in levamisole-pretreated and control rats. Intestinal blood flow measured by means of a hydrogen gas clearance method was significantly increased by the pretreatment with levamisole. The mean values of intestinal blood flow in control and levamisole-pretreated rats are 171.4 ± 19.9 (ml/min/100 g), and 204.8 ± 15.0 (ml/min/100 g), respectively, and the difference is statistically significant ($P < 0.02$).

Effect of dibutyryl cyclic-AMP on levamisole-induced enhancement of drug absorption from the rat small intestine

Fig. 5 illustrated the effect of dibutyryl cyclic AMP on the levamisole-induced enhancement of sulfanilamide absorption from the rat small intestine. As shown in Fig. 5, enhanced absorption of sulfanilamide by the pretreatment with levamisole was restored to the control level by the intraperitoneal administration with 0.2 mg of dibutyryl cyclic AMP just before the absorption studies. No statistical significance was obtained

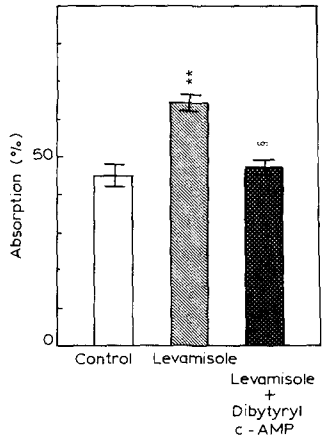

Fig. 5. Effect of dibutyryl cyclic AMP on the levamisole-induced enhancement of drug absorption from the rat small intestine. Dibutyryl cyclic AMP (0.2 mg) was administered intraperitoneally just before starting the absorption experiments, and intestinal absorption of sulfanilamide was examined by means of an in situ recirculation technique. Horizontal bars indicate ± S.D. of at least 3 rats. Key: ** $P < 0.001$; ns not significantly different, compared with the control.

between control group and levamisole and dibutyryl cyclic AMP-treated group.

Discussion

We have previously demonstrated that intestinal absorption of salicylic acid, sulfanilamide and aminopyrine was significantly enhanced by intraperitoneal administration of the synthetic immunomodulator, levamisole (Utsumi et al., 1987; Yamamoto et al., 1987). In these reports, it was also suggested that the effect of levamisole on salicylic acid absorption 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. These results are consistent with our present finding that a dose- and time-dependent increase in sulfanilamide absorption was observed in levamisole-pretreated rats (Tables 1, 2). However, enhancement effect of sulfanilamide absorption is greater than that of salicylic acid absorption since the ratios (levamisole/control) of salicylic acid and sulfanilamide absorption are 1.27 and 1.43, respectively.

The enhancement effect of levamisole on sulfanilamide absorption was confirmed by means of the measurement of blood concentration (Fig. 1) and the urinary excretion of sulfanilamide (Fig. 2). The finding that enhancement effect of levamisole was observed in the initial phase of the experiments suggested the reversibility of its action.

Although we found that intraperitoneal administration of levamisole influenced the absorption of some low molecular weight drugs from the gastrointestinal tract, the mechanism has not been demonstrated. Therefore, in the present study, we have examined its mechanisms from various physiological viewpoints such as changes in pH value of intraluminal solution, the amount of protein and mucus in the perfusate, mucosal permeability of drugs, intestinal blood flow, and cyclic nucleotide levels.

The absorption of sulfanilamide, which was known to be transported across the gastrointestinal membrane by a passive diffusion process (Koizumi et al., 1964), was affected by intraluminal pH. However, we found no significant change in intraluminal pH, suggesting that increased absorption of sulfanilamide might not be due to changes in pH value of intraluminal solution. No significant decrease was observed on the intraluminal protein and mucus which are known to interfere with the intestinal absorption of various drugs (Yamamoto et al., 1984b; 1986; Mayer et al., 1985; Schurgers et al., 1985; Kearney et al., 1986). Consequently, it was indicated that changes in macromolecular substances in the perfusate do not contribute to the enhanced absorption of sulfanilamide by levamisole.

Another possibility is that levamisole may cause the structural changes in the intestinal mucosa and may alter the membrane permeability of sulfanilamide. However, only small change was observed in an everted sac experiment suggesting that levamisole might not influence the membrane permeability of drugs (Table 3). This result was also confirmed by the in situ exsorption experiments (Fig. 3).

On the other hand, intestinal absorption of sulfanilamide is known to be affected by the alteration of intestinal blood flow (Komuro et al., 1975). Therefore, in the present study, we have

measured intestinal blood flow by means of a hydrogen gas clearance method and clarified the increased blood flow (Fig. 4). Thus, it seems likely that the blood flow may contribute to the increased absorption of sulfanilamide. This finding also supports the result that no dramatic effect was observed by means of an in vitro everted sac experiment, which can escape from the contribution of the blood flow.

Moreover, the enhancement effect of levamisole pretreatment was almost restored to the control level when 0.2 mg of dibutyryl cyclic AMP was given intraperitoneally just before the absorption experiments. In previous reports, Nakamura et al. (1979) demonstrated that the intestinal absorption of Phenol red and Bromphenol blue was significantly increased when the intestinal lumen was pretreated with theophylline, caffeine and isoproterenol, which are known to produce intracellular accumulation of cyclic AMP. Kim et al. (1982) also indicated that dibutyryl cyclic AMP enhanced the gastric absorption of salicylamide and sulfanilamide. In addition, it was known that an increase in cyclic GMP and a decrease in cyclic AMP were observed in vitro in the presence of levamisole (Hadden et al., 1975). These findings suggest that changes in the cyclic nucleotide level induced by levamisole may also play an important role in the enhancement of drug absorption. The relationship between mesenteric blood flow and changes in cyclic nucleotide level was not clearly understood. Further investigation is required to clarify its possible relation to the cyclic AMP system.

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