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An assessment of indomethacin-induced gastrointestinal mucosal damage in-vivo: enhancement of urinary recovery after oral administration of phenolsulfonphthalein in rats

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The permeability of phenolsulfonphthalein(phenol red), a poorly absorbed drug, was examined as an index of an assessment of gastrointestinal mucosal damage in-vivo. The urinary recovery after oral administration of phenol red was significantly increased in rats with indomethacin-induced ulcers. However, the urinary recovery of phenol red after its intravenous administration was not affected by the ulcers. Gastric absorption of phenol red from the stomach was examined by means of the in-situ loop technique. A significant increase in disappearance of phenol red from the luminal solution was observed in rats orally pretreated with indomethacin. These findings suggest that the increase in urinary recovery of phenol red is due to increased gastrointestinal absorption. This method may be utilized as a simple, useful and non-invasive screening test for an assessment of gastrointestinal mucosal damage in-vivo.

The damaging effects of drugs on the gastrointestinal tract are considered in terms of the gastrointestinal mucosal barrier, gastrointestinal erosions and microbleeding and whether peptic ulcers are caused. It is usually assessed by macroscopical and microscopical examination. Measurement of gastrointestinal blood loss is also used extensively. However, few studies have investigated ulcer and gastrointestinal mucosal damage using the permeability of marker compounds. An attempt has been made to examine the intestinal mucosa in coeliac disease by permeability of cellobiose and mannitol in man (Cobden et al 1980).

We postulated that the gastrointestinal mucosa in drug-induced mucosal damage would be permeable to a poorly absorbed compound, phenolsulfonphthalein (phenol red). The present study was undertaken to investigate urinary recovery after oral administration of phenol red as an index of assessment of gastrointestinal mucosal damage in-vivo.

MATERIALS AND METHODS

Materials

Phenol red, indomethacin and carboxymethyl cellulose sodium salt (CMC) were of reagent grade. All other reagents were the finest grade available.

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Animals and experimental ulcer

Male Wistar albino rats, 200 g, were fasted, then indomethacin suspended in 1% CMC solution was administered by gastric intubation under light ether anaesthesia and the animals were allowed free access to water only. Fifteen h later, the ulcer index for the stomach was estimated as follows. The stomachs were removed under ether anaesthesia, inflated with 10 ml of 0·9% NaCl (saline) and placed in 1% formalin solution for 5 min to fix the muscular outer surface (Brodie & Hanson 1960). A cut was made along the greater curvature and the lesions were measured. The ulcer index was expressed as the sum total of the length (mm) of individual lesions in each animal. Results were compared statistically using Student's *t*-test.

Urinary excretion of phenol red

Fifteen h after the indomethacin-treatment, experiments on the urinary excretion were made in another group of rats. Phenol red, 2 μmol in 2 ml of saline, was administered by gastric intubation under light ether anaesthesia. Following the intubation, the animals were placed in a metabolic cage. The urine was collected at 4 h intervals, and the phenol red content of the voided sample was determined. Blank determinations were made in the same manner, except that phenol red for oral administration was

replaced by saline. The urinary recovery of phenol red was expressed as percent of dose. Statistical analyses were performed using Student's *t*-test.

Absorption experiments

The procedure of the in-situ absorption experiment in the rat stomach was similar to that reported by Schanker et al (1957). Animals were anaesthetized with pentobarbitone, given intraperitoneally, and the stomach was cannulated to prepare an in-situ loop. Four ml of drug solution dissolved in saline was placed in the gastric lumen. At the end of an absorption period (2 h), the drug solution in the gastric lumen was withdrawn as completely as possible, and the gastric lumen was washed with saline. The washings were combined with the drug solution and made up to 50 ml with saline. The amount that disappeared from the lumen was calculated as the difference between the amount of the dye in the initial and the final solutions.

Analytical methods

Spectrophotometric determination of phenol red was applied. The urine was made up to 10 ml with distilled water and centrifuged for 10 min at 2500 rev min^{-1}. One ml of sample solution was made alkaline with 5 ml of 1 M NaOH and determined spectrophotometrically at 560 nm.

RESULTS

Phenol red, almost completely ionized at pH above 1, was chosen as a marker compound due to its poor absorbability at any physiological pH of the gastrointestinal tract, rapid renal tubular secretion and ease of assay. Schanker et al (1957, 1958) have shown that phenol red was poorly absorbed from the rat stomach and small intestine. Also, we have shown that the poor absorbability of phenol red is due to its very low affinity to the intestinal mucosa in addition to its poor lipoid solubility (Nakamura et al 1976a,b).

The effects of oral pretreatment with indomethacin on the urinary excretion of phenol red administered orally was examined in rats. The result is summarized in Fig. 1. The urinary recovery of phenol red and the ulcer index were significantly increased by oral pretreatment with either 20 or 100 mg kg^{-1} of indomethacin, while no effect was found in the case of 2 mg kg^{-1} drug. It is evident that the effect of indomethacin on the gastrointestinal mucosa is dose-dependent. Studies in which phenol red (0·1 μmol) was injected intravenously indicated that the oral pretreatment with 100 mg kg^{-1} of indomethacin had no influence on the urinary

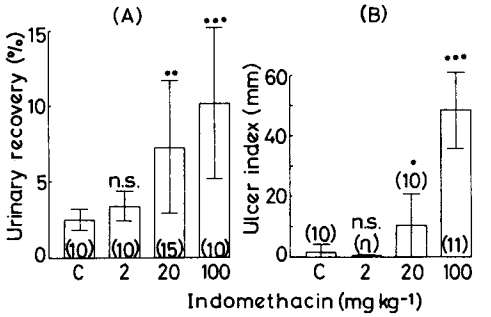

FIG. 1. Urinary recovery in 8 h of phenol red administered orally (A) and ulcer index (B) in rats orally pretreated with indomethacin. Vertical bars indicate ± s.d. Numbers in parentheses represent the number of experiments. Statistical significance: n.s. not significant; (*) $P < 0.05$; (**) $P < 0.005$; and (***) $P < 0.001$.

recovery of the dye. The mean percent of phenol red recovered in the urine after intravenous administration to indomethacin-treated rats was 42·1 ± 16·8% (mean ± s.d., ten rats) in 4 h, essentially identical to the control value of 43·9 ± 11·4% (mean ± s.d., ten rats). This finding rules out an effect of indomethacin on the net distribution and elimination patterns of phenol red and suggests that the increase in urinary recovery of phenol red is due to the increased gastrointestinal absorption of the compound.

In order to examine the duration of the increased urinary recovery of phenol red, urine was collected from 8 to 12 and 12 to 16 h in rats orally pretreated with 100 mg kg^{-1} of indomethacin. The results are shown in Fig. 2. The urinary recovery of phenol red was significantly increased in 16 h. From these results, it seems that the alteration of the permeability of the intestinal mucosa in addition to the gastric mucosa causes the increase in the urinary recovery of phenol red.

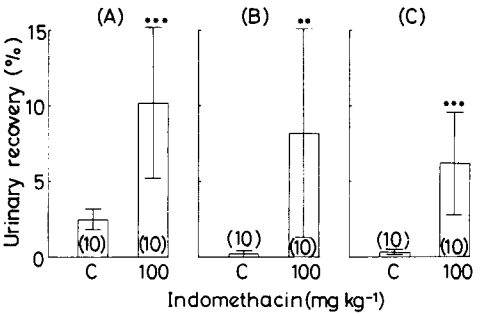

FIG. 2. Urinary recovery of phenol red after oral administration with indomethacin-pretreatment. Urine was collected from 0 to 8 (A), 8 to 12 (B) and 12 to 16 h (C). Vertical bars indicate ± s.d. Numbers in parentheses represent the number of experiments. Statistical significance: (**) $P < 0.005$; and (***) $P < 0.001$.

Fig. 3 shows the effect of healing period on the urinary recovery of phenol red and the ulcer index in rats orally pretreated with 20 mg kg^{-1} of indomethacin. The healing period of 10 days was enough to restore to the level of control both the urinary recovery of phenol red and the ulcer index.

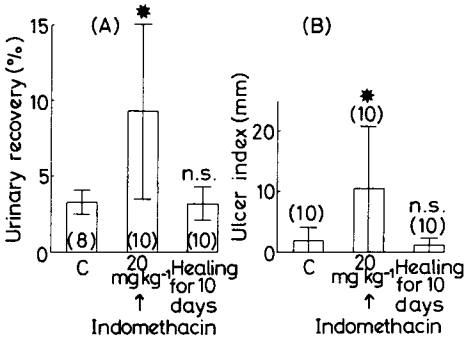

FIG. 3. Effect of healing period on urinary recovery in 8 h of phenol red administered orally (A) and ulcer index (B) in rats orally pretreated with indomethacin. Vertical bars indicate ± s.d. Numbers in parentheses represent the number of experiments. Statistical significance: n.s. not significant; and (*) $P < 0.05$.

Absorption experiments on the stomach were carried out using in-situ loop technique. The results are summarized in Fig. 4. The increased disappearance of phenol red from the luminal solution was observed in rats orally pretreated with 100 mg kg^{-1} of indomethacin. However, the pH of luminal solution did not change compared with the control. Consequently, the increase in gastric absorption of phenol red was not due to a change in gastric pH, but from changes in the permeability of the gastric mucosa.

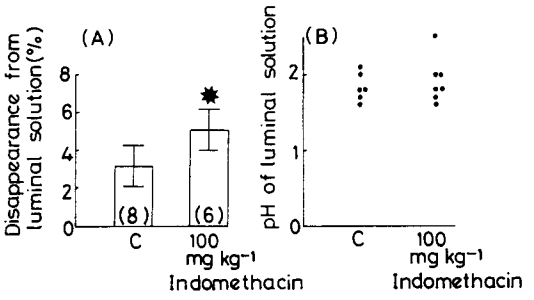

FIG. 4. Absorption of phenol red in 2 h from in-situ gastric loops (A) and the pH of luminal solutions (B). Concentration of phenol red: 1 mM. Vertical bars indicate ± s.d. Numbers in parentheses represent the number of experiments. Statistical significance: (*) $P < 0.05$.

DISCUSSION

It is known that administration of large doses of steroidal as well as non-steroidal anti-inflammatory agents causes gastrointestinal ulceration. The purpose of the present study was to determine whether the urinary recovery of phenol red after oral administration could be utilized to assess indomethacin-induced gastrointestinal mucosal damage in-vivo. It is generally recognized that length, area and depth of the ulcerated area may be used for the quantitative assessment of ulceration. Croft & Lubran (1965) reported a method of determining human gastric mucosal cell exfoliation, based on measurement of the DNA concentration in gastric washings. Garner (1977) has shown that gastric mucosal damage in the guinea-pig was assessed by measuring the concentrations of blood and DNA in gastric washings and by morphological examination of the mucosa. Estimation of the DNA concentration in gastric washing was found to be a sensitive index of mucosal damage, detecting superficial as well as focal cell loss. It also has the advantages of being quantitative and allowing the time course of lesion development to be studied. The fact that a wide variety of methods exists for assessing the deleterious effect of drugs on the gastrointestinal mucosa is indicative of the lack of an entirely suitable laboratory model.

As shown in Fig. 1, the urinary recovery of phenol red and the ulcer index were significantly increased by oral pretreatment with indomethacin. On the other hand, the urinary recovery of the dye administered intravenously did not change significantly in rats pretreated with indomethacin. In addition, phenol red absorption from in-situ gastric loops was increased by the indomethacin-treatment (Fig. 4). These results suggest that the increase in urinary recovery of phenol red is due to the increased gastrointestinal absorption. Feldman et al (1970) have studied the influence of sodium deoxycholate on the absorption of phenol red in the rat by urinary excretion data after oral administration to intact animals. Similarly, Malik et al (1975) and Khalafallah et al (1975) reported that urinary excretion data after oral administration of phenol red to intact rats and humans were utilized to assess the effect of surfactants on the absorption of the marker.

In Fig. 3, the healing period of 10 days was enough to restore the urinary recovery of phenol red to the level of control, which is correlated well with the data of the ulcer index. From the results described above, this method may be utilized as a simple, useful and non-invasive screening test for an assessment of gastrointestinal mucosal damage in-vivo. Phenol red

given by intramuscular or intravenous injection is excreted predominantly by tubular secretion and is widely used in the testing of the renal function in man. Therefore, this test may be helpful both in diagnosis and in assessment of responses to the treatment of mucosal damage and the ulcer.

The oral route of drug administration is preferred in most cases. In the preformulation studies and preclinical stages of drug development, it is important to have defined the potential for gastrointestinal mucosal damage in addition to biopharmaceutical, pharmacological and toxicological considerations. Further studies are needed to investigate the urinary recovery after oral administration of phenol red as an index of quantitative assessment of gastrointestinal mucosal damage in-vivo.

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