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Assessment of Pharmaceutical Excipient-Induced Gastrointestinal Mucosal Damage in Rats *in Vivo* by Measuring the Permeation of Phenolsulfonphthalein

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The effect of pharmaceutical excipients on the gastrointestinal mucosa in rats was examined by measuring the permeation of phenolsulfonphthalein (phenol red), a poorly absorbed drug. The urinary recovery after oral administration of phenol red was significantly increased by pretreatment with sodium lauryl sulfate and Triton X-100 in rats. However, pretreatment with polysorbate 80, lactose, fructose, mannitol, calcium citrate, acacia, or polyethylene glycol 4000 had no apparent effect.

Keywords——phenolsulfonphthalein; phenol red; mucosal damage; membrane permeability; membrane transport; gastrointestinal absorption; excipient; surfactant; screening test

Most solid oral formulations will be composed of one or more medicaments plus pharmaceutical excipients of various types such as diluents, fillers, disintegrants, absorbents, flavoring agents, colorants, binding agents, granulating fluids, antifrictional agents, and surfactants as wetting agents. It is highly desirable that these pharmaceutical excipients should be nontoxic and inert.

In the previous reports,[1-3] we examined the permeation of phenolsulfonphthalein (phenol red), a poorly absorbed drug, as an index for the assessment of gastrointestinal mucosal damage *in vivo*. The urinary recovery after oral administration of phenol red was significantly increased in rats with indomethacin-induced mucosal damage.[1,2] A similar result was obtained in rats with ulcers induced by restraint and water immersion stress.[1,3]

The present study was undertaken to investigate urinary recovery after oral administration of phenol red as an index for the assessment of pharmaceutical excipient-induced gastrointestinal mucosal damage *in vivo*.

Experimental

Materials——Phenol red, sodium lauryl sulfate (SLS), Triton X-100, polysorbate 80, lactose, fructose, mannitol, calcium citrate, acacia, and polyethylene glycol 4000 (PEG 4000) were of reagent grade. All other reagents were of the finest grade available.

Animal Experiments——Male Wistar albino rats, weighing approximately 200 g, were used in these studies. The rats were fasted, then SLS, Triton X-100, polysorbate 80, lactose, fructose, mannitol, calcium citrate, acacia, or PEG 4000 was administered by gastric intubation under light ether anesthesia, and the animals were allowed free access to water only. The pharmaceutical excipients were dissolved in distilled water, except for calcium citrate, which was suspended in distilled water. Fifteen hours after the pharmaceutical excipient treatment, experiments on the urinary excretion were carried out. Phenol red (2 μmol in 2 ml of saline) was administered by gastric intubation under light ether anesthesia. 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 each 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 the dose. Statistical analyses were performed by using Student's *t*-test.

Analytical Method——Spectrophotometric determination of phenol red was used. The urine was made up to 10 ml with distilled water and centrifuged for 10 min at 2500 rpm. One ml of sample solution was alkalinized with 5 ml of 1 N NaOH and determined spectrophotometrically at 560 nm.

Results and Discussion

Phenol red, which is 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, as well as its rapid renal tubular secretion and ease of assay.

The effect of oral pretreatment with various pharmaceutical excipients on the urinary excretion of phenol red administered orally was examined in rats. The results are summarized in Table I. The mean recoveries (percent) of phenol red in control animals were 1.6 and 2.3% of the dose in 4 and 8 h, respectively. The urinary recovery of phenol red was significantly increased by oral pretreatment with either 10% SLS solution or 10% Triton X-100 solution, while no effect was found in the case of 1 or 5 % SLS solution. Engel and Riggi reported that the intraduodenal administration of SLS (25 mg/kg) facilitated the intestinal absorption of heparin, a poorly absorbed mucopolysaccharide, to a certain extent in the dog.[4] Nadai *et al.* have shown that the perfusion of SLS solution (1%) gives rise to a remarkable histological change in rat intestinal tissue, employing scanning electron and light microscopy.[5,6] Because of this histological change, the permeability of marker compound from the blood vessels to the intestinal lumen was observed to increase. SLS and Triton X-100 are widely used as surfactants in the study of membranes, both to isolate particular proteins and to probe the structure of the membrane itself. As shown in Table I, polysorbate 80, lactose, fructose, mannitol, calcium citrate, acacia, and PEG 4000 did not increase the urinary recovery after oral administration of phenol red. Feldman and Reinhard observed an effect of SLS and polysorbate 80 on the transfer of salicylate across the everted intestinal preparation.[7] Large increases in salicylate transfer occurred after exposure of the intestinal segments to micellar concentrations of SLS, but no increase was noted after the intestinal segments were exposed

TABLE I. Urinary Recovery after Oral Administration of Phenol Red in Rats Orally Pretreated with Pharmaceutical Excipients

Excipient	Urinary recovery (%)		
	0—4 h	4—8 h	0—8 h
Control	1.6±0.7	0.8±0.4	2.3±0.8 (9)
SLS (1%)	2.0±0.3	0.7±0.3	2.7±0.2 (5)
SLS (5%)	2.0±0.6	0.7±0.6	2.7±0.8 (15)
SLS (10%)	4.2±2.1[a]	1.6±1.2	5.7±2.9[a] (13)
Triton X-100 (10%)	3.6±1.9[b]	3.3±1.8[a]	6.8±3.4[a] (10)
Polysorbate 80 (10%)	1.8±0.7	0.7±0.4	2.5±0.9 (15)
Lactose (10%)	2.2±0.5	0.8±0.3	3.0±0.7 (14)
Fructose (10%)	1.7±0.7	0.9±0.4	2.7±0.7 (15)
Mannitol (10%)	1.5±0.6	0.8±0.4	2.3±0.6 (14)
Calcium citrate (10%)	1.8±0.7	0.6±0.5	2.4±0.8 (14)
Acacia (10%)	1.8±0.4	0.7±0.4	2.6±0.6 (15)
PEG 4000 (10%)	2.0±0.5	0.7±0.3	2.8±0.5 (15)

Two milliliters of pharmaceutical excipient solution or suspension was administered by gastric intubation. Values each represent the mean±S.D. Numbers in parentheses represent the number of experiments. Statistical significance: *a*) $p<0.01$, *b*) $p<0.02$.

to micellar concentrations of polysorbate 80. It is well known that polysorbate 80, a non-ionic surfactant, has a mild action on the gastrointestinal mucosa compared to SLS.[8] The exact mechanism of the surfactant-induced change in the membrane permeability is still unknown.

These findings suggest that this method may be utilized as a simple, useful, and noninvasive screening test for the assessment of pharmaceutical excipient-induced gastrointestinal mucosal damage *in vivo*. The simplicity of the experimental procedure and lack of any requirement for surgical treatment are convenient for a screening test.

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