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Influence of 1,8-dihydroxyanthraquinone and loperamide on the paracellular permeability across colonic mucosa

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Administration of 1,8-dihydroxyanthraquinone (DHA) markedly increases the permeability of guinea-pig colonic mucosa. In 1 h 25% of the administered dose of ^{99m}Tc-EDTA complex leaks through the mucosa. Orally administered loperamide blocks the ^{99m}Tc-EDTA transfer after DHA administration. Loperamide injected in situ in the ligated colon segment shows the same blocking properties of the transfer rate of the complex. These findings suggest that the opposing action on fluid transport of the laxative DHA and the antidiarrhoeal loperamide could be due to these drugs affecting the permeability of the colonic mucosa. The minimal dose of loperamide, able to restore normal permeability, was as low as 0·01 mg kg^{-1}.

Laxatives of the diphenolic type not only inhibit the absorption of water and sodium from the intestine but cause a net transfer of fluid and electrolytes into the intestinal lumen. This was shown by Ewe & Hölker (1974) for bisacodyl in the human colon and by Forth et al (1966) for oxyphenisatin in the rat colon. Natural and synthetic anthraquinone laxatives exert their action in the manner described by Lemmens (1974) and Lemmens & Borja (1976). In contrast, the inhibitory effect of opiate agonists on the stimulated fluid secretion caused by several substances was recently reported by Beubler & Lembeck (1979). The antidiarrhoeal agent loperamide reduced the effect of bisacodyl on water net flux in the ligated colon of the rat in situ. We observed the same antisecretory effect of loperamide on the stimulation of fluid secretion after in vivo administration of rhein and phenophthalein to rats (unpublished results). Loperamide was also found to counteract castor oil-induced diarrhoea in rats (Awouters et al 1975) and prostaglandin-induced fluid accumulation in rat and man (Karim & Adaikan 1977). Therefore the antidiarrhoeal action of the opiate agonists is caused not only by affecting intestinal motility but mainly by inhibiting net water secretion in the gut (Beubler & Lembeck 1979).

During the last few years, the existence of two distinct pathways across epithelia has been confirmed (Ussing & Windhager 1964; Schultz et al 1974), a high and a low conductance pathway. The shunt pathway is an extracellular route through the mucosal epithelium across the intercellular spaces and the tight junctions, a route which is of particular importance in low resistance epithelia like the intestinal mucosa. In order to discriminate between these two pathways, Nell et al (1976) measured the transfer through the colonic mucosa of well-established markers of the extracellular space; they found that the diphenolic laxative oxyphenisatin raises the permeability of the mucosa for these test substances ^{51}Cr-EDTA and [^{14}C]inulin, which do not enter living cells. Beside having a laxative effect, 1,8-dihydroxyanthraquinone (DHA) was found to increase the permeability of guinea-pig colonic mucosa when a non-absorbable marker ^{99m}Tc-EDTA was used (Verhaeren et al 1981).

To find a connection between the opposing effects of the non-osmotic laxatives and the antidiarrhoeal opiate agonists on the water secretion in the intestine, we investigated the effect of loperamide on increased paracellular permeability, following the transfer of the ^{99m}Tc-EDTA complex across the colonic mucosa of guinea-pigs in vivo.

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MATERIALS AND METHODS

1,8-Dihydroxyanthraquinone, Dantron Merck AG; loperamide, Imodium Janssen Pharmaceutica, Beerse.

Preparation of the ^{99m}Tc-EDTA complex in situ

The ^{99m}Tc-EDTA complex was prepared by a modification of the instant kit method as described by Eckelman & Richards (1970) for the ^{99m}Tc-DTPA complex. A kit of $SnCl_2$-EDTA at pH 5·1 was prepared by passing a 1 ml aliquot of a solution containing 5 mg Na_2-EDTA and 0·5 mg $SnCl_2.2H_2O$ per ml through a 0·22 μm membrane filter into empty 3 ml serum vials and was stored at −20 °C. Just

before use 1 ml of sterile eluate of a ^{99m}Tc generator (Stercow ^{TM}99m Byk-Mallinckrodt) containing 2–10 μCi ^{99m}Tc was added to a thawed $SnCl_2$-EDTA kit and mixed thoroughly. The labelling efficiency was determined by ascending paper chromatography on Whatman no. 1 paper with either 0·9% NaCl or methylethylketone as solvent. It was found to be more than 98%. The final solution had physiological osmolarity as measured with an osmometer.

Biological assay method

To estimate the effect of chronic oral administration of DHA on the permeability of the colonic mucosa, we used male guinea-pigs, of about 450 g. They were kept for one week on a diet of pellet food containing 0·1% DHA (a daily intake of about 20 mg per animal). A group of control animals received the same food without added DHA.

Sixteen hours before the experiment food was withdrawn, but there was free access to water. Four hours before the beginning of the experiment, loperamide was orally administered by stomach tube to some of the animals in the group on DHA diet. During the experiment each animal was anaesthetized with diethylether. The abdomen was opened and two ligatures were tied around the colon at 5 and 15 cm distance from the caecum. Care was taken to obtain an intact segment. Then 0·25 ml of the ^{99m}Tc-EDTA solution was injected into the colon segment by passing a needle through the ligature before tightening it, to prevent the solution from flowing back.

One hour after the administration of the labelled complex, the animal was overdosed with chloroform. The kidneys, urine and ligated colon segment were isolated and their radioactive content measured in a scintillation well counter (Berthold Gamma sample changer BF 5300) at 140 $\pm$ 10 keV. Control animals were similarly treated. The identity of the recovered radioactivity in the urine was investigated by using ^{99m}Tc-Gel Chromatography Column Scanning on Sephadex G-25 M, following the method of Persson (1975). The route followed by the ^{99m}Tc-EDTA complex, after DHA treatment, was identified as the paracellular route, as the protonated form of triaminopyrimidine (TAP) (pH 6·0) was able to retain the radioactive complex in the ligated colon segment (Verhaeren et al 1981).

In order to assess the effectiveness of loperamide when administered to the mucosal side, the drug was directly injected in the ligated colon segment of DHA-pretreated animals. Half an hour later, the radioactive compound was similarly injected, through a new ligature to avoid loss of activity. The procedure was performed as described for the DHA-treated animals.

RESULTS

When administered into the ligated colon of control guinea-pigs, the ^{99m}Tc-EDTA complex essentially does not pass across the intestinal wall. In consequence of manipulation a very small amount of radioactivity is transferred to the blood and filtered through the kidneys. Nearly all radioactivity remains in the colon, demonstrating the tightness of the paracellular pathway and the tissue impermeability to the ^{99m}Tc-EDTA complex.

Pretreatment of guinea-pigs with DHA for one week markedly increased the permeability of the intestine to the labelled complex. Within 1 h of administration of the complex nearly 25% of the radioactivity leaks through the colon and can be recovered from kidneys and urine.

However when loperamide is administered to the DHA-pretreated animals, the permeability of the mucosa returns to that of the non-treated animals. The radioactivity is practically totally concentrated in the colon.

The results are summarized in Fig. 1.

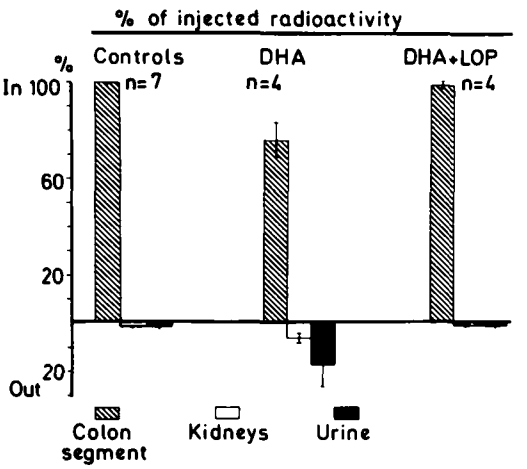

FIG. 1. Biodistribution of the ^{99m}Tc-EDTA after 1 h incubation (mean with s.d.). The influence of orally administered loperamide on DHA pretreated animals.

No significant difference was observed between the colonic permeability of normal non-treated animals and animals treated with DHA and loperamide ($P < 0·05$) using the *t*-test.

The minimal oral active dose of loperamide was assessed by administering increasing doses (starting from 0·64 μg kg^{-1}) of drug. Doses from 0·01 mg kg^{-1} showed maximal effectiveness as illustrated in Fig. 2.

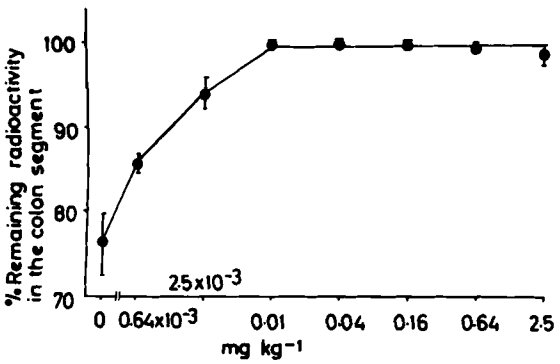

FIG. 2. The influence of increasing doses of orally administered loperamide on the permeability of the colon of DHA pretreated animals for ^{99m}Tc-EDTA.

Even the minimal dose of 0·64 μg kg^{-1} exerted a blocking effect. Loperamide was also effective when directly applied by injection on the mucosal side of the colon segment (Fig. 3).

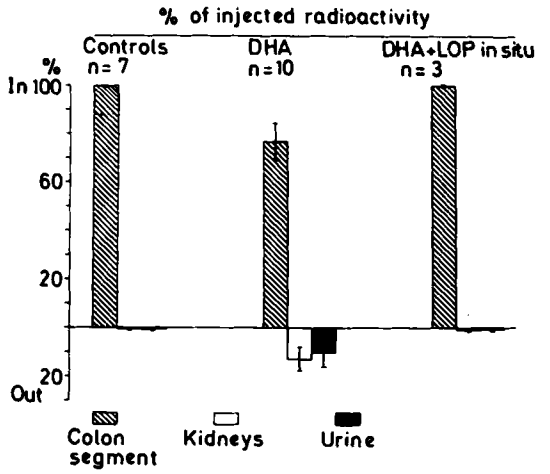

FIG. 3. Biodistribution of the ^{99m}Tc-EDTA after direct administration of loperamide to the mucosal side of a colon segment (mean with s.d.).

DISCUSSION

Under normal circumstances the labelled complex cannot enter past the mucosa but after pretreatment of the guinea-pigs with DHA, the paracellular permeability increased significantly. This phenomenon could be responsible for the water and electrolyte movement across the intestine under influence of anthraquinone laxatives. Our results are in good agreement with earlier findings of Nell et al (1976) in experiments with oxyphenisatin and ^{51}Cr-EDTA.

The effect of the anthraquinones seems to be reversible: when the antidiarrhoeal agent loperamide is administered in combination with DHA, the junctions of the colonic mucosa 'close' again. The initial impermeability of the intestinal wall is restored and the ^{99m}Tc-EDTA complex is no longer transferred from lumen to blood. The opposing effect of loperamide towards the laxative may explain the antisecretory action of this opiate agonist observed by Beubler & Lembeck (1979). The authors attribute the inhibitory effect, at least in part, to the ability of the opiates to counteract the antiabsorptive action of prostaglandins (E_1 and E_2), released after administration of diphenolic laxatives. We observed the same prostaglandin E mediated effect of the anthraquinone laxatives rhein and DHA in the rat in vivo (unpublished results). The laxative effect however was never totally abolished.

In the present report it is postulated that the antisecretory action of the opiate agonist loperamide and the stimulation of fluid secretion by non-osmotic laxatives such as DHA may also be attributed to opposing effects on the paracellular permeability of the colonic mucosa. These findings indicate that these drugs may have several modes of action.

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