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Acceleration of large intestine transit time in rats by sennosides and related compounds

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Sennosides A + B and their natural metabolites, sennidins A + B, rheinanthrone and rhein, as well as the synthetic laxative danthron, were investigated for their influence on small and large intestine transit time in rats. Carmine red, as a marker, was administered through a gastric tube for small intestine transit or intracaecally by a chronically implanted catheter for colon transit. High doses of sennosides (250–500 mg kg^{-1}) given orally from 20 min or up to 6 h before marker administration had no effect on small intestine transit time. The metabolites and danthron (10–100 mg kg^{-1} p.o.) also did not accelerate upper gastrointestinal passage. Intracaecal administration at the same time as carmine red, however, reduced the time for the appearance of the first coloured faeces from more than 8 h in the controls to 46 ± 9 min after sennosides, 34 ± 11 min after sennidins, 53 ± 83 min after rhein and 16 ± 4 min after rheinanthrone (50 mg kg^{-1} of each). Danthron was ineffective. Thus, sennosides and their natural metabolites specifically influence large intestinal motility. Acceleration of colonic transport seems to be a major component of the laxative action whereas for danthron motility changes are not responsible for its laxative action. Indomethacin partly inhibited the acceleration of large intestine transit induced by sennosides. An involvement of endogenous prostaglandins may therefore be possible, although a local bolus administration of $PGF_{2\alpha}$ or PGE_2 into the caecal lumen neither influenced transit time nor induced diarrhoea.

The main active components of sennoside laxatives are the stereoisomers sennosides A and B which are prodrugs that pass, chemically unchanged and without being absorbed in the upper gastrointestinal tract, into the large intestine where microbial digestion takes place. There the sennoside molecule is split into two rheinanthrone molecules with the aglycone sennidin as an intermediate product. Subsequent oxidation may lead to some rhein (Lemmens 1979; Sasaki et al 1979; Lemli & Lemmens 1980; Dreessen et al 1981; Hattori et al 1982). Danthron, which is structurally similar to rhein, is the only synthetic anthraquinone used as a laxative. It is known to be effective only in much higher doses than the sennosides (Ferguson 1956; Fairbairn & Moss 1970; Dufour & Gendre 1984).

Sennosides given orally affect fluid absorption in the large intestine (Beubler & Juan 1979; Donowitz et al 1984). Local administration into the small intestine or into the rinsed colon does not produce any effect, but the metabolites are active in both (Lemmens 1976; Lemmens & Borja 1976; Leng-Peschlow 1980). Anthraquinones also influence intestinal motility but there is little information on this. Therefore, the effect of the pure sennosides and their natural metabolites, as well as of danthron, on small and large intestine transit time, has been studied in rats. As prostaglandins are thought to mediate the laxative action, pretreatment with indomethacin and the effect of exogenous prostaglandins were also investigated.

METHODS

Animals

Female Wistar rats, ca 200 g, were fed with a commercial pellet food which was withdrawn 24 h before measurement of small intestine transit. In the large intestine transit studies, food was available all the time.

Measurement of small intestine transit

Carmine red (1%) suspended in a 1% tragacanth solution was administered through a stomach tube at a fixed time after administration of the test substance. 20 min after application of this marker, the animals were killed by an overdose of ether. The stomach and the whole small intestine were removed and the length of the coloured part of the small intestine was measured and calculated as percent of its total length.

Measurement of large intestine transit

Under ether anaesthesia, a PVC-catheter was chronically implanted into the caecum with the distal end fixed on the animal's neck. During the experiment the animals were kept individually in a wire

meshed cage to enable the faeces to fall through onto blotting paper. Carmine red (10 mg in 0·4 ml distilled water per animal) was injected into the caecum through the catheter immediately after administration of the test substance. The time until appearance of the first coloured faeces was registered.

Test substances

Formulae of the test drugs are shown in Fig. 1. A mixture of sennosides A + B (each about 50%), sennidins A + B, rhein-Na and rhein-9-anthrone-Na were obtained from the Chemical Department of Madaus. The purity of each substance was more than 96% with the exception of rheinanthrone (purity 72%) which is unstable and contained approximately 12% sennidins, 7% rhein and 8% water. The anthraquinones were dissolved in $NaHCO_3$ (0·5%) and administered in a volume of 10 ml kg^{-1} orally and 2·5 ml kg^{-1} intracaecally. Danthron was obtained from Sigma Chemical Co., St Louis, and suspended in a 0·5% tragacanth solution.

PGE_2 and $PGF_{2\alpha}$ (Sigma Chemical Co., St Louis) were injected intracaecally (0·01–5·0 mg kg^{-1}) or intravenously (0·01–1·0 mg kg^{-1}, only $PGF_{2\alpha}$) in 2 ml physiological saline. Indomethacin (MSD Pharma, München) was administered intraperitoneally in a dose of 4 mg kg^{-1} 1 h before intracaecal injection of carmine red.

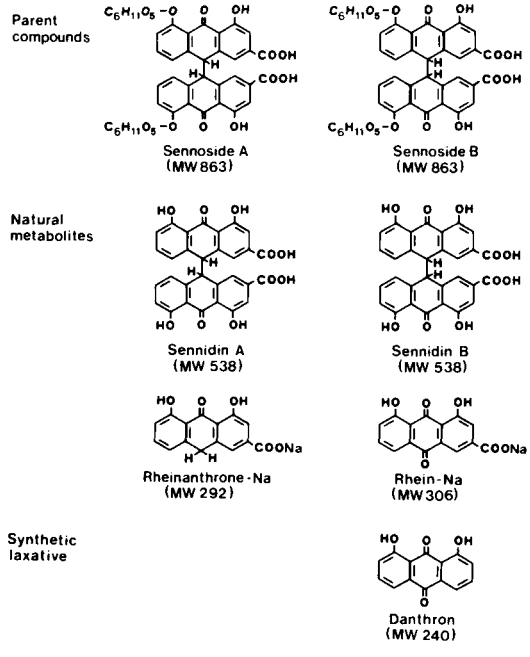

FIG. 1. Chemical structures and molecular weights of the anthraquinones used in the experiments.

Statistical evaluation

All values are presented as mean ± standard deviation. Statistical significance was assessed with Student's *t*-test or the U-test of Wilcoxon et al.

RESULTS

Small intestine transit time

Sennosides A + B did not accelerate small intestine transit when administered orally from 20 min until up to 6 h before the administration of the transport marker (Table 1). A slight retardation occurred when the pretreatment time was prolonged to include the time when active metabolites were being released in the large intestine. The doses of sennosides chosen were rather high, since 50 mg kg^{-1} p.o. are sufficient to induce a nearly maximal laxative effect in the rat.

Table 1. Small intestine transit shown as a percentage of the total small intestine length. The sennosides were administered at various times before the carmine red marker.

Time of treatment (h before carmine)	Sennosides A + B p.o. (mg kg^{-1})	No. of animals	Small intenstine transit (%)
0·3	0	15	56 ± 5
	250	15	54 ± 9
	0	15	54 ± 5
	500	15	52 ± 8
2	0	15	54 ± 5
	250	15	51 ± 10
	0	15	57 ± 8
	500	15	50 ± 6*
3·5	0	15	56 ± 7
	250	15	48 ± 10**
	0	15	52 ± 6
	500	15	45 ± 6**
6	0	10	61 ± 8
	50	10	51 ± 12*
	100	10	55 ± 9

*$P \leq 0{\cdot}05$, **$P \leq 0{\cdot}01$ as compared with the control.

Oral administration of the pharmacologically active metabolites, rhein and rheinanthrone, or of the synthetic laxative danthron 20 min before the marker also did not influence small intestine transit time (Table 2) thus excluding a direct effect on small intestinal motility.

Large intestine transit time

In controls, coloured faeces appeared only in 5% of the animals within 5 h after intracaecal administration of the marker substance. Intracaecal treatment with sennosides (10 mg kg^{-1}) immediately before

Table 2. Small intestine transit shown as a percentage of the total small intestine length. All anthraquinones were administered 20 min before the carmine red marker.

Test compound	Dose p.o. (mg kg^{-1})	No. of animals	Small intestine transit (%)
Rheinanthrone-Na	0†	15	53 ± 6
	50	15	46 ± 31
	0*	19	58 ± 9
	100	8	56 ± 21
Rhein-Na	0†	15	53 ± 6
	50	15	52 ± 8
	0*	19	58 ± 9
	100	7	57 ± 13
Danthron	0	10	72 ± 6
	100	10	74 ± 7
	200	10	71 ± 11

†, * Identical control groups.

carmine red induced faecal loss of the marker within 5 h in 58% of the animals. A dose of 50 mg kg^{-1} caused faecal excretion of the marker within 46 min in 100% of the rats. An increase to doses up to 250 mg kg^{-1} did not produce further reduction in transit time (Table 3).

The aglycones, sennidins A + B accelerated large intestine transit slightly more than the sennosides when compared on a weight for weight base.

The metabolite rheinanthrone was the most effective compound. A dose of 10 mg kg^{-1} resulted in a mean transit time of 60 min and a dose of 50 mg kg^{-1} in marker excretion after only 16 min. After rhein (50 mg kg^{-1}), as a second laxative metabolite of the sennosides, mean intestine transit time was about 30 min with a high individual variation.

The synthetic laxative, danthron, was far less effective in influencing large intestine transit. Only half of the animals responded with marker excretion within 5 h after a dose of 200 mg kg^{-1}. Only normal faecal pellets could be seen with this substance, whereas, with the other drugs, marker excretion usually occurred in soft or fluid faeces.

Preliminary data (3–7 animals per dose) with prostaglandins, the possible mediators of the laxative action, showed that neither $PGF_{2\alpha}$ nor PGE_2 administered intracaecally or $PGF_{2\alpha}$ injected intravenously reduced large intestine transit time. There was also no laxative effect in these studies. Pretreatment with indomethacin significantly prolonged transit time after 50 mg kg^{-1} of sennosides A + B from 42 ± 10 to 82 + 19 min (n = 10), but these values were still far below control values (>8 h).

DISCUSSION

The present data clearly show that neither the sennosides themselves, nor their metabolites affect the motility of the upper gastrointestinal tract by increasing transit rate. On the other hand, large intestinal motility is very effectively influenced resulting in a reduced retention time leading to the rapid excretion of soft or, dose-dependently, of fluid faeces.

Table 3. Large intestine transit time after simultaneous intracaecal administration of the test compounds and the marker substance.

Test compound	Dose (mg kg^{-1})	No. of rats per group	Rats with marker excretion within 5 h (%)	Large intestine transit (min)	
				Median	Mean ± s.d. (n)
Control groups (combined)	0	128	5	>300	
Sennosides A + B	1	13	0	>300	
	10†††	12	58	206	
	50†††	14	100	47	46 ± 9 (14)
	125†††	13	100	44	44 ± 12 (13)
	250†††	13	100	35	38 ± 20 (13)
Sennidins A + B	50†††	15	100	30	34 ± 11 (15)**
Rheinanthrone-Na	1	13	7	>300	
	10†††	14	100	57	59 ± 20 (14)
	50†††	13	100	17	16 ± 4 (13)***
Rhein-Na	1	13	7	>300	
	10	13	7	>300	
	50†††	12	92	23	30 ± 23 (11)
Danthron	50	15	7	>300	
	100	5	7	>300	
	200†	15	47	>300	

**$P \leq 0{\cdot}01$, ***$P \leq 0{\cdot}001$, compared with sennosides A + B (50 mg kg^{-1}) according to Student's t-test.
†$P \leq 0{\cdot}05$, †††$P \leq 0{\cdot}001$, difference in transit time compared with the corresponding control group according to the U-test of Wilcoxon, Mann & Whitney.

The sennosides are normally classed among the stimulant laxatives, but this term is often misunderstood. It is true that they stimulate large intestine transit but motility in terms of contraction frequency is reduced. Electromyographic measurements in dogs and rats have shown that there is a pronounced decrease in the number of caecal and colonic contractions after oral or intracolonic administration of senna preparations. Upper gastrointestinal motility is altered, too, but in the sense that the migrating myoelectric complexes, normally present in the small intestine of fasted animals, disappeared and a continuous irregular activity, like a fed state, is established (Garcia-Villar et al 1980). As our results show, these electromyographic changes in the small intestine are not accompanied by an increase in transit rate.

The present findings, together with the electromyographic evidence, are consistent with the concept of Connell (1962) that the normal segmenting contractions in the colon represent a resistance to peristaltic forces and delay the distal transport of the stool. If the number of contractions is reduced, intraluminal pressure decreases and the flow of intestinal contents is facilitated. Thus, diarrhoea is normally associated with hypomotility and constipation with hypermotility, but atonic forms of constipation also exist where the gut tone is too weak to produce any important peristaltic waves. In man it has been shown that treatment with senna reduces intraluminal pressure in the constipated patient (Waller 1975) and that it stimulates colonic peristalsis (Hardcastle & Wilkins 1970). Recent experiments in cats suggest a shift in the equilibrium between propulsive spike bursts and segmenting ones in favour of peristaltic movements without changing the overall activity (Wienbeck et al 1985). From experiments with rats and dogs (Garcia-Villar et al 1980), it seems more justified to conclude that there is no specific stimulation of peristaltic waves, but that the efficiency of the resting peristaltic activity is improved by the diminution in the number of segmental contractions. In this point, however, further clarification is necessary.

Under the experimental conditions used here, where the test drugs were added to the normal caecal contents containing all bacterial enzymes necessary to break down the dianthrone glycosides to the laxative active metabolites, the prodrugs sennosides and sennidins rapidly reduce colon transit time. A nearly maximal effect is obtained with a dose of 50 mg kg^{-1} of sennosides. An increase in the dose does not further accelerate the passage, indicating that the metabolic capacity is exhausted. Rheinanthrone is the most active among the investigated drugs in accelerating colonic transit. This is also the case when molar proportions are considered. Rheinanthrone has also been shown to be the most active in reducing net water absorption in a tied-off, bacteria-free rat colonic segment in-situ, whereas dianthrones (sennosides, sennidins) or anthroneglycosides have no effect on water absorption (Lemmens 1976). When considering the low purity of rheinanthrone, which is extremely unstable especially in aqueous, aerobic solutions and is transformed preferentially into the inactive sennidins at a neutral or alkaline pH (Grimminger, personal communication 1984), its activity seems to be much more important than the available data may show.

The synthetic laxative danthron is greatly inferior to the sennosides and their metabolites in its efficacy in influencing colon transit. It is well known that, orally, it must be given in doses more than 10 times higher than the sennosides to induce a comparable laxative effect (Dufour & Gendre 1984). This is ascribed to its easy absorbability in the small intestine preventing a local action in the colon (Breimer & Baars 1976; Moireau et al 1985).

Our own investigations show that the laxative effect of danthron in mice after oral administration is only 1/3 of that of rhein and that rhein, too, is a weak laxative as compared with sennosides. A considerable amount of rhein is also absorbed by the small intestine (Dobbs et al 1975). Direct administration into the colon should avoid such absorption problems, but, here again, an even more pronounced difference was evident between danthron and the other anthraquinones with respect to large intestine transit acceleration. This suggests that danthron exerts its laxative effect not predominantly by changing motility, but by affecting fluid absorption mechanisms which may take place in the small intestine as well.

PGE-synthesis is increased in the colon of senna- or danthron-treated rats (Cohen 1982; Capasso et al 1983) and PGE_2 has been shown to be involved in fluid secretion induced by sennosides (Beubler & Kollar 1985). Prostaglandins play a role in gastrointestinal motility, too, but it is not known whether the motility effects of these laxatives are also mediated by prostaglandins. Colon transit is enhanced by subcutaneous 16,16-dimethyl PGE_2 in rats and is associated with diarrhoea (Rush & Ruwart 1984). In man, intraluminal colonic pressure is reduced by intravenous $PGF_{2\alpha}$, but not by PGE_2 (Hunt et al 1975). In our preliminary experiments

with local administration of high doses of $PGF_{2\alpha}$ and PGE_2 into the caecal lumen of the rat, there is no effect on large intestinal transit time nor induction of diarrhoea. These results do not exclude the possibility that endogenous prostaglandins may, nevertheless, be involved in colonic motility changes, since the intraluminally administered exogenous prostaglandins could be rapidly destroyed by the caecal microflora. Intravenous $PGF_{2\alpha}$, however, also did not show any effect. On the other hand, pretreatment with indomethacin significantly reduces the effect of sennosides on large intestine transit, but it remains far from normal. Compared with the pronounced inhibition of senna-induced fluid secretion in the rat colon by indomethacin (Beubler & Kollar 1985), endogenous prostaglandins seem to contribute less to the motility effect of the sennosides.

Since constipation usually is a specific colon motility problem and the production of a hard stool is the consequence of a prolonged retention time in the large intestine, a laxative preferentially should normalize colon motility and not only stimulate fluid secretion, at least not in the small intestine. According to our results, sennosides specifically accelerate colon transit. As sennosides induce motility changes very quickly after reaching the colon, motility seems to be an important factor in their laxative action and to be largely independent of fluid accumulation. As seen for danthron, other anthraquinones may act differently and not as specifically as the sennosides.

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