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Degraded and undegraded carrageenans and experimental gastric and duodenal ulceration

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The prevention of histamine-induced gastric and duodenal ulceration in the guinea-pig has been examined using a series of undegraded and degraded carrageenans. Undegraded carrageenans were active at lower doses than degraded carrageenans. The high viscosity of the undegraded carrageenans in solution prevented their use in larger doses. Degradation of carrageenan without serious loss of sulphate, gives a product which allows the dose to be increased to an extent that its effect more than offsets the slight loss in activity caused by the degradation. No single feature of carrageenan structure can be related to anti-ulcer activity although degradation, and hence reduction of molecular size, generally reduces activity. Sulphate contents over 30% have little apparent effect on activity; κ-carrageenans were not consistently different in anti-ulcer activity from λ-carrageenans. This contrasts with the antipeptic activity of carrageenans where κ-carrageenans are less active than their λ-counterparts. As with antipeptic activity, the degree of anti-ulcer activity is probably determined by a combination of structural features which includes molecular size and polyanionic properties.

PREVENTION of experimental histamine gastroduodenal ulceration in guinea-pigs by carrageenan (Anderson & Watt, 1959) has been confirmed using several animal species (Houck, Bhayana & Lee, 1960; Misaki, Okita, & others, 1965; Lambelin, 1966). It is also known that carrageenan has antipeptic activity (Anderson & Watt, 1959; Bonfils, Dubrasquet & Lambling, 1959, 1960; Anderson, 1961; Anderson & Harthill, 1967), but neither the role of the proteolytic component of gastric secretion in peptic ulceration, nor the relation between antipeptic and anti-ulcer effects of sulphated polysaccharides is understood. It is likely, however, that the elucidation of one of these actions could help our understanding of the other.

The examination of a series of different carrageenans (Anderson & Harthill, 1967) revealed differences in antipeptic activity. The present report deals with differences between the anti-ulcer activities of selected carrageenans of the series.

Experimental

MATERIALS AND METHODS

Carrageenans. Selection was made on the basis of antipeptic activity and availability. The code names correspond to those used by Anderson (1967). A degraded κ-carrageenan from *C. crispus* was also included, CRF-κ-D2 (SO_3Na, 28·6%; η_{inh}, 0·90; antipeptic activity 30, 11 by methods A and B, respectively). Antipeptic activities, determined at the lower carrageenan concentrations by methods A and B (Anderson, 1967) showed that, of the undegraded carrageenans, CNS-κ was less active than GP-λ and CY-λ ($P < 0{\cdot}01$). Differences in antipeptic activity did not exist ($P > 0{\cdot}1$) between the degraded carrageenans.

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Gastric ulceration. Gastric ulceration was produced in the pylorus-ligated guinea-pig of suitable strain (Anderson & Soman, 1963). Guinea-pigs, fasted 24 hr, with water *ad lib.*, were kept individually in cages with raised grids of suitable mesh to minimize coprophagy. They were anaesthetized with pentobarbitone (30 mg/kg) intraperitoneally and the gastroduodenal junction ligated. Saline (1 ml) alone (control) or containing half the dose of carrageenan in solution was injected intraduodenally 0·5 hr before, and repeated immediately after, the injection of histamine acid phosphate (5 mg/kg, subcutaneously in saline, 1 ml/kg). The stomach was not emptied since the resting volumes were small and reasonably uniform, and complete empyting without disturbance of, and the risk of mucosal damage to, the stomach is very difficult; the procedure may also stimulate secretion. The animals were killed 1 hr after administration of histamine; ulceration was scored on a 4+ scale (Anderson & Soman, 1965a) and averaged for each group. Reduction in average ulceration in the test groups was expressed as a percentage of the average ulceration for the control group. Secretion volumes were measured and converted to volume per kg body weight; free and total acidities were titrated using Topfer's reagent and phenolphthalein respectively.

Duodenal ulceration. Histamine acid phosphate (10 mg/kg), suspended in a beeswax–arachis oil (10:90) base (1 mg/ml), was administered intramuscularly to intact fasted guinea-pigs. This consistently produced duodenal ulceration (incidence = 100%) at the end of 24 hr. Ulceration was assessed on a 4+ scale and averaged for each group (Anderson & Soman, 1965b). In this experiment, the carrageenan was administered in the drinking water. In the test groups, carrageenan solution was offered in place of the usual drinking water during the 24 hr preceding the histamine and again during the 24 hr following the histamine. The volumes consumed were recorded. Degraded carrageenans were used at 1% or 5% concentration in the drinking water; undegraded carrageenans had to be used at 0·5% because of their viscosity in solution.

Reduction in average duodenal ulceration in test groups was expressed as a percentage of the ulceration occurring in the control groups, which were offered normal drinking water.

Results and discussion

The results are in the Tables. For both gastric and duodenal ulceration a 30% reduction in ulcer score is considered to be the smallest acceptable for anti-ulcer activity.

Concerning the aetiology of the two types of experimental ulceration used in this work, we have contended (Anderson & Soman, 1966a) that the lesion appearing in the duodenum is caused by simple exposure to the passing of the copious, acid gastric secretion resulting from histamine stimulation, whereas the ulceration appearing in the stomach is caused by a lesser secretion acting on an exhausted, devitalized mucosa. Evidence of devitalization is that gastric secretion increases on increasing the dosage of histamine without the appearance of ulcers until a stage is reached when

further increase in histamine dosage results in lowered volumes of secretion. It is at this stage that ulceration appears. The lowered volumes of gastric secretion are taken as evidence of a devitalized mucosa. In this condition, together with the angiotoxic effects of histamine, the mucosa is susceptible to the erosive gastric secretion.

We have used both types of ulceration to demonstrate ulcer prevention. For duodenal ulcers we have made use of the antisecretory effects of carrageenan (Anderson, Marcus & Watt, 1962; Anderson & Soman, 1965c). But for gastric ulcers, the protection is of a different (and unknown) nature from the simple protection by surface contact postulated earlier (Anderson & Watt, 1959) although the mucosa is obviously protected when ulceration is prevented. In the present antigastric ulcer experiment carrageenan does not appear on the mucosal surface.

Comparison of antiduodenal ulcer activities in the present experiments involves the assumption that the animals follow the same drinking pattern throughout the 48 hr. Drinking carrageenan solutions before histamine stimulation confers greater protection than drinking after (Anderson & Soman, 1965b); drinking before and after is better still. In general, there was a tendency to drink more before the histamine than after it. Carrageenan solution was offered only before histamine in the case of CY-λ and CNS-κ and this probably accounts for the slightly lower protection compared with GP-λ where carrageenan solutions were offered both before and after the histamine injections.

The undegraded carrageenans showed anti-ulcer activity by both methods even at the low doses used. These doses were the highest practicable. In the duodenal ulceration experiments the guinea-pigs were reluctant to drink solutions more viscous than those used although they appeared to drink non-viscous carrageenan solutions at least as avidly as they drink water; in the gastric ulceration experiments highly viscous solutions could not easily be injected into the duodenum.

UNDEGRADED CARRAGEENANS

No evidence of marked difference between the activities of undegraded κ- and λ-carrageenans in the anti-ulcer experiments was found. This contrasts with the anticoagulant and antipeptic activities of undegraded κ- and λ-carrageenans where λ-carrageenans are generally more active than the corresponding κ-carrageenans (Hawkins & Leonard, 1962; Anderson & Duncan, 1965; Anderson & Harthill, 1967).

Because the use of higher doses of undegraded carrageenans was prevented by the high viscosity of their solutions, there remains the possibility that greater activity might be shown if more substance could be administered. Even oral administration in solid dosage form is unsatisfactory because of the low solubility, poor dispersibility and the slow rate of dissolution of undegraded carrageenans after administration.

DEGRADED CARRAGEENANS

Degradation of the carrageenans allows higher and more frequent dosage to be administered conveniently, and the results show that higher

TABLE 1. ANTI-GASTRIC ULCER ACTIVITY AND THE EFFECTS ON GASTRIC SECRETION OF UNDEGRADED AND DEGRADED CARRAGEENANS

Carrageenan*	Number of animals	Reduction in gastric ulceration % score	Total dose of carrageenan, intraduodenally mg	Gastric secretion		
				Average volume mg/kg	Average acidity m-equiv./litre	
					Free	Total
Undegraded						
CY-λ	16	46	20	31	96	104
CNS-κ	11	57	20	32	99	107
GP-λ	8	38	20	32	103	111
Degraded						
C16	8	12	200	29	97	105
,,	14	60	400	21	83	101
GP-λ-D2	4	36	50	31	105	113
,,	4	47	150	29	96	104
CRF-κ-D2	4	20	50	35	101	110
,,	6	60	150	30	116	124
CY-λ-D5	4	36	150	33	91	100
CY-λ-D6	4	89	,,	40	115	124
CY-λ-D7	5	55	,,	35	101	110
CY-λ-D8	5	22	,,	27	89	97
CY-λ-D9	4	4	,,	27	72	82
CY-λ-D10	6	73	,,	42	109	116
CY-λ-D11	4	57	,,	29	98	106
GP-λ-D3	4	47	150	32	100	108
GP-λ-D4	4	0	,,	21	80	90
GP-λ-D5	4	49	,,	36	99	109
GP-λ-D6	4	39	,,	45	103	111
GP-λ-D7	4	49	,,	44	101	109
ED3	6	44	150	37	83	93
ED4	5	6	200	40	89	98
ED5	6	0	200	33	92	100
ED6	6	4	200	36	89	98
Controls	27	Average ulceration = 3·74 + (±0·10)	1 ml saline intraduodenally, twice	33 (±2)	99 (±3·6)	107 (±3·4)

* See Anderson & Harthill (1967), Table 1, for the code.

TABLE 2. ANTI-DUODENAL ULCER ACTIVITY OF UNDEGRADED AND DEGRADED CARRAGEENANS

Carrageenan*	Number of animals	Reduction in duodenal ulceration % score	Dose of carrageenan (from drinking water) mg		
			Pre-histamine	Post-histamine	Total
Undegraded					
CY-λ	12	27	115	—	115
CNS-κ	12	36	100	—	100
GP-λ	12	44	110	85	195
Degraded					
C16	11	39	380	280	660
,,	6	52	1150	900	2050
,,	21	44	—	2150	2150
,,	21	73	2400	—	2400
GP-λ-D2	10	73	330	260	590
CRF-κ-D2	5	0	300	180	480
CY-κ-D10	4	44	150	150	300
GP-κ-D4	4	47	220	230	450
GP-λ-D6	4	47	220	270	490
GP-λ-D7	4	39	250	310	560
Controls	60	Average ulceration = 3·76 + (±0·07)			

For undegraded carrageenans, 0·5% solutions were used; for degraded carrageenans, 1% solutions were used, with the exception of the three highest doses of C16, where a 5% solution was used.
CY-λ-D7, CY-λ-D8, CY-λ-D9, CY-λ-D11, GP-λ-D3 and GP-λ-D5, all gave less than 10% protection.
* See Anderson & Harthill (1967), Table 1, for the code.

activity can thereby be obtained. Thus C16, CRF-κ-D2, and probably also GP-λ-D2, have increasing anti-gastric ulcer activity with increasing dosage (Table 1); a similar response is seen for the anti-duodenal ulcer activity of C16 (Table 2). Although the increased dosage made possible by degradation is valuable in therapeutic use, degradation, even without sulphate hydrolysis, appears to result in substances with lower activity than the parent substance. In some instances degradation causes a loss of all anti-ulcer activity.

SULPHATE CONTENT

It is noteworthy that sulphate content remained fairly constant through the degraded series (Anderson & Harthill, 1967: Table 1). This suggests that even a content of 30–40% sulphate does not assure anti-ulcer activity, although we have never encountered (in an unreported screening programme) anti-ulcer, or indeed marked antipeptic, activity, either in non-sulphated polysaccharides from a variety of vegetable and animal sources, or in carrageenans with sulphate of less than 25%. It would appear that sulphate content is not a lone determinant of the quantity of biological activity although the possession of adequate sulphate appears necessary for some activity. A resolution of the relation between sulphate content and biological activity is therefore not even possible amongst carrageenans, although the polysaccharide contains only one principal sugar—galactose, either in the undegraded state [compare CY-λ, CNS-κ, GP-λ, Tables 1 and 2; sulphate contents are in Table 1 (Anderson & Harthill, 1967)] or in the degraded state.

ANTI-ULCER ACTIVITY

Anti-ulcer activity was sufficiently indiscriminate to preclude the use of one or other ulcerous condition to differentiate between undegraded κ- and λ-carrageenan, the only differentiation being between a degraded κ- and a degraded λ-carrageenan for antiduodenal ulcer activity (GP-λ-D2 and CRF-κ-D2, Table 2).

Eucheuma carrageenan, a κ-type carrageenan, yielded a degraded series of which only ED3 has activity (Table 1); ED4-6 although having similar percentages of sulphate, have lower viscosities (Table 1, Anderson, 1967), Degradation in this series was essentially by mild acid treatment which Black, Blakemore & others (1965) found sufficient to split the acid labile 3,6-anhydrogalactosidic link. However, the anti-ulcer activity of λ-carrageenans, with their naturally low content of 3,6-anhydrogalactose links, discounts dependence on this link for activity, a conclusion also reached by Anderson & Harthill (1967) for antipeptic activity.

It is not clear at present why only certain of the degraded CY-λ and GP-λ series should possess antiduodenal ulcer activity. The results suggest that sulphate content and molecular characteristics (and antipeptic activity) cannot be taken as unfailing indication of anti-ulcer activity of both types.

EVALUATION OF THE RESPONSE

If percentage reductions in gastric ulceration scores (Table 1) are grouped into those below 30% (8) and those above 50% (7) and the corresponding means for volume and free acidity of secretion compared, no significant difference emerges between the mean volumes for the two groups, but the free acid is higher for the group experiencing greater protection (103 m-equiv./litre) than for the group where protection was less than 30% (89 m-equiv./litre) ($P < 0{\cdot}01$). Thus the protection afforded by carrageenan against ulceration appears to be accompanied by a fully functional secretory mechanism which fails to some extent if protection is less than 30%. A similar effect is also seen when degraded carrageenans are administered intravenously (Anderson & Soman, 1967).

THE RELATION OF ANTIPEPTIC AND ANTI-ULCER ACTIVITY

We conclude that for the antipeptic and antiulcer activities of carrageenan, whether in the undegraded or degraded state, there is a combination of as yet undefined molecular features, including sulphate, which determines the amount of activity. On the other hand, activity appears to accompany not only the high molecular weight carrageenan but (in smaller degree) even relatively small molecules, such as C16 and GP-λ-D2 which have weight average molecular weights of around 25,000 and possess 30–40% sulphate.

While there is, in the undegraded series, a systematic difference between the antipeptic activities of the κ- and λ-components, there is no real differentiation between the anti-ulcer activity of κ- and λ-carrageenans. However, it is not clear whether such a differentiation is to be expected; nor is it clear that refinement in method (if that proves to be possible) will reveal differences.

In the case of degraded carrageenan, where absorption from the gastrointestinal tract appears to occur, only a small fraction of the administered dose appears to be absorbed (Anderson & Soman, 1966b). Intravenous administration of an amount equivalent to the fraction absorbed gives anti-ulcer activity (Anderson & Soman, 1967) but, again, κ- and λ-carrageenans are not differentiated. It may well be that anti-ulcer activity can be indirectly mediated, by some humoral mechanism, which can be triggered by κ- or λ-carrageenan (degraded or undegraded) in the duodenum. This would also explain the anti-ulcer activity of undegraded carrageenan (at even smaller dosage than certain degraded carrageenans) which is apparently not absorbed from the intestine of the guinea-pig. If this hypothesis is correct there is, as yet, no apparent reason why any differences between the antipeptic activity of κ- and λ-fractions, as demonstrated *in vitro*, should correlate with corresponding observed differences in anti-ulcer activity.

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