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EVALUATION OF AN ANIMAL MODEL FOR THE SCREENING OF COMPOUNDS POTENTIALLY USEFUL IN HUMAN ULCERATIVE COLITIS: EFFECT OF SALICYLAZOSULFAPYRIDINE AND PREDNISOLONE ON CARRAGEENAN - INDUCED ULCERATION OF THE LARGE INTESTINE OF THE GUINEA PIG

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ABSTRACT

Irritation of the cecum of guinea pigs was induced by providing them with drinking water containing 5% degraded carrageenan for a period of 14 days. The current study was conducted to test the procedure as a useful model for the evaluation of compounds potentially useful in the treatment of ulcerative colitis. The criterion used was improvement in the carrageenan induced adverse symptomatology following treatment with either salicylazosulfapyridine or prednisolone. Both of these latter compounds are somewhat effective in relieving the symptomatology of ulcerative colitis in humans. On the basis of the following: (1) fecal occult blood; (2) occult blood in the cecum upon autopsy; and (3) weight loss; neither compound showed effectiveness. The finding that two drugs known to be effective in human ulcerative colitis failed to protect against a carrageenan induced irritation of the large intestine of the guinea pig indicates that this model may not be useful as a screening procedure.

INTRODUCTION

Ulceration of the colon of guinea pigs produced by the oral administration of degraded carrageenan was first reported in 1969 by Watt and Marcus (1) and subsequently verified in numerous experiments (2-6). Feeding animals a 5% solution results in weight loss, bloody diarrhea, and colonic ulceration, particularly in the cecum (7). Morphologically, the lesions are comparable to those of human ulcerative colitis (8). Presently, a satisfactory animal model does not exist to screen compounds for this disease state. It has therefore been suggested (3,5,7-9) that carrageenan ulceration in the guinea pig provides an experimental model for studying the pathology of ulcerative lesions in the large intestine as well as for assessing the effects of therapeutic agents.

It was the purpose of this study to investigate the feasibility of utilizing this animal model to screen compounds potentially useful in the treatment of human ulcerative colitis. In this regard, the influence of co-administration of salicylazosulfapyridine and prednisolone on carrageenan-induced ulceration was investigated. These two drugs are effective in ameliorating the inflammation associated with human ulcerative colitis, although they apparently act by different mechanisms (10). Therefore, if these drugs were to prove efficacious by reducing inflammation in the guinea pig model, this system could provide a model for the screening of new chemical entities potentially useful for the treatment of human ulcerative colitis. Unfortunately, it will be reported herein that these two drugs failed to protect animals in this particular experiment.

EXPERIMENTAL

Materials

Prednisolone (Sigma Chem. Co., St. Louis, Mo.), salicylazosulfapyridine (Matheson Coleman and Bell, Norwood, Ohio) and degraded carrageenan (Pierrefitte-Auby, 92202 Neuilly-sur-Seine, France) were used without further purification. The degraded carrageenan (C-16) was derived from the red seaweed Eucheuma Spinosum and degraded by mild acid hydrolysis so as to retain 28.6% ester sulfate.

Methods

Male Hartley strain guinea pigs (Charles River, Wilmington, Mass.) were used. The animals were housed individually and placed on a diet of Purina ground guinea pig chow for a period of eight days prior to the initiation of the test procedures. The animals were then divided into four groups of five animals each, still being maintained in individual caging. Groups A-C were placed on drinking water containing 5% degraded carrageenan. Group D, the controls, received normal drinking water. The animals in Group B received guinea pig chow containing salicylazosulfapyridine, amd Group C received ground guinea pig chow containing prednisolone. The drugs were mixed into the diets to yield a dosage level of 100 mg/kg/day of salicylazosulfapyridine and 2 mg/kg/day of prednisolone. Food and fluid consumption was measured on a daily basis except during the weekend intervals. The concentration of salicylazosulfapyridine and prednisolone was adjusted periodically in order to achieve the desired dosage levels. The carrageenan solutions were prepared biweekly and kept at 4°C until used. Each day the animals received a new solution of carrageenan or water. The animals were maintained on the previously described schedules for a period of two weeks. During the first week, the feces of any animals which exhibited diarrhea or showed a pallid body color were tested for occult blood using guaiac paper (Hemocult slide, Smith-Kline Diagnostics, Philadelphia, Pa.). During the last five days of the study, the feces of all animals were tested for occult blood. On day fourteen of the study, the surviving animals were killed, and the large intestine examined macroscopically. In addition, the cecum was opened and the contents tested for occult blood. The data were analyzed for statistical significance by means of the Student t-test.

RESULTS AND DISCUSSION

Table 1 shows the mean body weight of each treatment group of animals. As shown, the carrageenan group consistently lost weight throughout the treatment period in contrast to the controls which gained weight. At the termination of the study, the four surviving animals in the carrageenan group had lost 22% of their initial body weight while the controls showed a 20% weight gain. The salicylazosulfapyridine plus

TABLE 1

Body Weight of Guinea Pigs Drinking Water Containing 5% Degraded Carrageenan: Effect of Salicylazosulfapyridine or Prednisolone Treatment

Body Weight (grams) Mean ± S.E.

Drug Group	Days 0	3	4	5	6	7	10	11	12	13	14
A. Carrageenan[a]	307 ± 7.5	295 ± 11.7*	301 ± 14.0	298 ± 13.0*	294 ± 13.3*	286 ± 14.2**	263 ± 20.2*	253 ± 23.8**	250 ± 28.1**	242 ± 31.2**	239 ± 34.9**
B. Salicylazosulfapyridine + Carrageenan	307 ± 7.6	307 ± 7.9	310 ± 6.1	310 ± 9.5	305 ± 13.1*	324 ± 17.2	279 ± 16.9	271 ± 30.8*	262 ± 29.6**	273 ± 13.5**	259 ± 2.0***
C. Prednisolone + Carrageenan	311 ± 7.6	285 ± 10.9**	280 ± 13.9**	315 ± 8.1	319 ± 7.5*	317 ± 8.5	293 ± 7.4	290 ± 9.4*	291 ± 13.2*	283 ± 14.5**	267 ± 12.8**
D. Control	312 ± 8.4	333 ± 9.2	332 ± 8.2	336 ± 8.8	344 ± 7.4	348 ± 8.4	338 ± 22.2	360 ± 12.7	370 ± 11.0	379 ± 13.4	376 ± 11.1

Statistically different from the control group
* $p < .05$
** $p < .01$
*** $p < .001$

[a]There were not statistically significant differences ($p > 0.05$) at all times between groups A and B as well as groups A and C.

carrageenan and prednisolone plus carrageenan treated groups also showed a reduction in body weight which was somewhat less than that observed with the untreated carrageenan group. On day 14 of the study, these animals had lost 16 and 14%, respectively, of their initial body weights.

Table 2 shows the food consumption of each group. As can be noted, the three groups which received drinking water containing the 5% carrageenan ate considerably less than the controls. The average food consumption over the entire 14 day experimental period showed that the carrageenan group and the carrageenan plus salicylazosulfapyridine treated group consumed food at 45 and 42% of the control group. The food consumption of the prednisolone plus carrageenan treatment group was reduced by 38% as compared to the average control. The average intake over the 14 day experimental period of salicylazosulfapyridine was found to be 109 ±6.8 (Mean ± S.E.) mg/kg and that of prednisolone 1.90 ± 0.12 mg/kg.

Fluid consumption of all four groups is shown in Table 3. This was most depressed in the carrageenan and carrageenan plus salicylazosulfapyridine groups, being on the average 44 and 42% of the control intake. The administration of prednisolone partially counteracted the carrageenan induced decrease, in that fluid consumption in this group was 60% of the control values.

Prior to the termination of the study, one animal died in the carrageenan group on the tenth day and three animals died in the salicylazosulfapyridine plus carrageenan group. The deaths in this group occurred on the tenth, eleventh, and thirteenth days. In each case, the cecum was found to be full of bloody looking fluid which when tested for occult blood with guaiac paper gave a strong positive reaction. The result of fecal occult blood testing and observations on the coloration of the animals are shown in Table 4. All three groups receiving carrageenan showed a high incidence of fecal occult blood. The controls were consistently negative. Upon autopsy at the termination of the experiment, the content of the ceca were tested for the prescence of occult blood. Again positives were detected in each of the groups receiving carrageenan while the controls were completely negative (Table 4).

TABLE 2

Food Consumption

Daily Food Consumption (Grams) Mean ± S.E.

Treatment Group	Days 3	4	5	6	7	10	11	12	13	14	Mean Days 1-14
A. Carrageenan[a]	12.7 ± 1.01***	15.9 ± 1.06*	8.5 ± 3.34**	11.9 ± 2.92**	9.4 ± 1.91**	10.7 ± 2.56**	10.8 ± 2.76*	7.2 ± 3.54**	8.4 ± 4.48**	7.4 ± 3.84**	10.3
B. Salicylazo-sulfapyridine + Carrageenan	13.2 ± 1.14***	10.6 ± 1.10***	11.9 ± 1.62***	11.1 ± 3.42**	7.8 ± 2.40**	9.2 ± 3.96**	10.8 ± 4.99	7.0 ± 2.39**	7.6 ± 1.50***	7.1 ± 1.25***	9.6
C. Prednisolone + Carrageenan	11.4 ± 1.14***	1.20 ± 1.60**	17.5 ± 0.83**	19.9 ± 1.49*	11.6 ± 1.08***	13.5 ± 2.47**	15.4 ± 2.79	13.5 ± 3.26*	14.1 ± 2.95**	11.7 ± 3.93*	14.1
D. Control	22.4 ± 0.72	20.0 ± 1.41	22.5 ± 0.91	24.6 ± 1.27	17.6 ± 0.24	24.5 ± 0.96	22.1 ± 2.11	23.0 ± 1.63	28.4 ± 1.03	22.4 ± 1.40	22.8

Statistically different from the control group * $p < .05$
** $p < .01$
*** $< .001$

[a]Using group A as the standard, there were statistically significant differences compared to group B on day 4 ($p \leq 0.01$) and group C on days 5 and 6 ($p \leq 0.05$).

TABLE 3

Fluid Consumption

Daily Fluid Consumption (ml) Mean ± S.E.

Treatment Group	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean Days 1-14
A. Carrageenan[a]	27 ± 3.2**	23 ± 5.2***	42 8.1*	43 ± 7.7	38 ± 11.5	46 ± 10.0**	38 ± 6.3*	41 ± 8.6***	27 ± 11.9**	36 ± 13.5*	41 ± 12.6*	27 ± 8.5**	31 ± 13.4**	35 ± 12.2**	35.3
B. Salicylazo-sulfapyridine + Carrageenan	30 ± 2.7*	26 ± 5.1***	44 ± 6.9*	36 ± 5.3	45 ± 5.6	45 ± 7.9**	36 ± 7.8*	40 ± 13.3**	25 ± 4.8**	31 ± 7.5*	40 ± 10.7**	28 ± 5.2**	22 ± 7.5*	18 ± 6.0***	33.5
C. Prednisolone + Carrageenan	33 ± 3.7*	22 ± 5.7***	19.8 ± 5.5***	45 ± 7.2	62 ± 4.5	61 ± 10.8	53 ± 12.7	60 ± 10.1**	33 ± 10.8**	52 ± 11.5*	74 ± 12.4	50 ± 11.3	58 ± 12.1	55 ± 14.9*	48.4
D. Control	54 ± 6.9	56 ± 2.2	66 ± 6.2	65 ± 13.2	70 ± 10.1	87 ± 6.9	74 ± 10.4	109 ± 7.7	90 ± 12.2	100 ± 16.3	87 ± 3.8	73 ± 4.0	90 ± 8.9	101 ± 7.1	80.1

Statistically different from the control group

* $p < .05$
** $p < .01$
*** $p < .001$

[a]There were no statistically significant differences ($p > 0.05$) at all times between groups A and B as well as groups A and C.

TABLE 4

No. Showing Physical Signs/No. of Animals Surviving

	Pallid Body Color				Fecal Occult Blood			
	Group #				Group #			
Day Treatment*	A	B	C	D	A	B	C	D
5	1/5	1/5	0/5	0/5	-	-	-	-
6	1/5	2/5	0/5	0/5	1/1[a]		1/2[a]	- -
7	2/5	3/5	0/5	0/5	2/2[a]		3/3[a]	- -
10	2/4	2/4	0/5	0/5	5/5		5/5	4/5 0/5
11	2/4	1/3	0/5	0/5	4/4		4/4	5/5 0/5
12	3/4	1/3	1/5	0/5	4/4		3/3	4/5 0/5
13	3/4	0/2	2/5	0/5	4/4		1/2	2/5 0/5
14	2/4	0/2	2/5	0/5	4/4		1/2	3/5 0/5
					Occult Blood in Contents of Cecum			
Autopsy					3/4		1/2	4/5 0/5

*A-Carrageenan
B-Carrageenan plus Salicylazosulfapyridine
C-Carrageenan plus Prednisolone
D-Control

[a]Only those animals which exhibited pallid skin coloration or diarrhea were tested for fecal occult blood.

Upon autopsy and macroscopic examination of the cecum and remainder of large intestine, the following was observed:

Carrageenan Group: One animal had a slight hemorrhage along the cecum vessels. A second animal had congested vessels on the mucosal

surface which were studded with black spot of less than 1 mm diameter. The cecum of the other animals in this group appeared normal.

Carrageenan - Salicylazosulfapyridine Group: Both surviving animals in this group showed congested blood vessels and several 2-3 mm diameter hemorrhages

Carrageenan - Prednisolone Group: congested cecum vessels. Tiny black spots less than 1 mm diameter were observed in two of these animals. The cecum of the two remaining animals appeared normal. The remainder of the large intestine appeared normal for all four groups.

Therefore, on the basis of the following observations: Fecal occult blood, occult blood in the cecum upon autopsy, and weight loss; neither compound showed effectiveness. On the basis of mortality, salicylazosulfapyridine increased carrageenan's adverse effect in that only two out of five animals survived the 14 day treatment period in the salicylazosulfapyridine group while four out of five animals survived when only carrageenan was administered. The finding that two drugs known to be effective in human ulcerative colitis failed to protect against a carrageenan induced irritation of the large intestine of the guinea pig indicates that this model may not be useful as a screening procedure for therapeutic agents potentially useful in the treatment of human ulcerative colitis.

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