Agents and Actions, vol. 16, 5 (1985)

0065-4299/85/050446-06$2.50

Carrageenin-induced thrombosis in rats and mice: A model for testing antithrombotic substances?

H. BEKEMEIER, R. HIRSCHELMANN and A.J. GIESSLER

Department of Pharmacology, Section of Pharmacy, Martin Luther University Halle-Wittenberg, DDR-4020 Halle (S.), Weinbergweg 15

Abstract

Among different carrageenins, kappa-carrageenins were found to be thrombogenic, whereas lambda-carrageenins were inactive in this respect, although the latter substances exerted a stronger edemogenic activity. Kappa-carrageenin (Sigma) was the most potent thrombogen. As the consequence of thrombosis tail infarction became visible some minutes after i.v. administration, but it was delayed for about 3 hours after the i.p. route and for about 6 hours after subplantar injection. Infarction frequency as well as extent of infarction was inhibited by heparin, cyproheptadine, phentolamine and dibenamine. Other substances like aspirin and dipyridamole showed no or only weak effects.

Advantages of the carrageenin-induced thrombosis model in rats and mice are: (i) simple induction in small laboratory animals, (ii) easy observation and quantification all the time without killing the animals, and (iii) possible external testing of antithrombotic agents by applicating substances on the tail.

Introduction

Some years ago when we occasionally used carrageenin from Serva (Heidelberg) for inducing rat paw edema, sometimes infarction and subsequent sloughing of the distal portion of the tail was observed, whereas this could never be found if the carrageenin Viscarin® (Marine Colloids) was used. The Serva carrageenin consists of kappa-carrageenin (personal information by Dr Renate Loewe, Serva GmbH & Co. Heidelberg), and Viscarin® is lambda-carrageenin. Our observation is in line with the findings of THOMSON et al. [1] and BICE et al. [2], according to which carrageenins, particularly the kappa-form, can induce disseminated intravascular coagulation.

Here we report on dose–response relationships and potency of different carrageenins in rats, mice, guinea-pigs and rabbits, on test procedures for systemic testing of coagulation-active substances in rats, and on first results obtained with some respective substances.

Materials and methods

(1) Animals

Male or female rats of an outbred Wistar strain were used, mainly of 90–150 g BW. Mice were outbred strains of AB mice (Halle), AB mice (Berlin) or AB mice (Berger), mainly of 18–28 g BW (both sexes). Guinea-pigs (both sexes) were of 350–400 g BW, and rabbits, mainly Chinchilla strain, were of about 3 kg BW.

(2) Carrageenins:

Kappa-carrageenin (lot No. C-1263, from Eucheuma cottonii, type III, Sigma Chem. Comp., St Louis, USA), kappa-carrageenin (Serva, Heidelberg, FRG; Carraghenin, purest quality, No. 16,245; MW 500,000); kappa-carrageenin (Satiagel GS 350, lot No. L-2267, Satia SA, Neuilly s./Seine, France), lambda-carrageenin (Viscarin®, lot Nos. 5515, RE 8984 and RE 8985, resp.; kindly supplied by Mr D. Stancioff, FMC Corp., Marine Colloids Division, Rockland Maine, USA), lambda-carrageenin (lot No. C-3889, from aciculaire and pistillata of the genus Gigartina, type IV, Sigma Chem. Comp., St. Louis, USA), lambda-carrageenin (Satiagum B, lot No. L-1915, Satia SA, Neuilly s./Seine, France).

(3) Tested substances

Aprotinin (Contrykal®, VEB AWD, GDR), aspirin (AB 2-DDR, GDR), dipyridamole (Curantyl®, VEB AWD, GDR), benzydamine (Troponwerke, FRG), imidazole (Fluka AG, Switzerland), levamisole (Richter AG, Hungary), dibenamine (Serva, FRG), phentolamine (Regitin®, Ciba-Geigy AG, Switzerland), carteolol (Endak®; kindly supplied by Dr D. Lorenz, Madaus & Co., FRG), cyproheptadine (EGYT, Hungary), streptokinase (Awelysin, VEB AWD, GDR), dihytamine (VEB AWD, GDR), *p*-aminomethylbenzoic acid (PAMBA®, VEB AWD, GDR), indalpine (Pharmuka, France), sulfin-pyrazone (Anturan®, Ciba-Geigy AG, Switzerland), Solcoseryl® (Solco Basel AG, Switzerland), heparin (Richter AG, Hungary).

(4) Thombosis

Thrombosis was induced by bolus injection of carrageenins at different doses and routes (see Tables and Figures). Thrombosis was evaluated by determining frequency and length of tail infarction, respectively.

(5) Paw edema

Edema was induced by injecting 0.1 ml of the 1% carrageenin solution into the pad of the left hind paw of rats. Edema volume was plethysmometrically determined.

Results

1. Thrombogenic and edemogenic potency of several carrageenins

With respect to the thrombotic activity, kappa-carrageenin (Sigma) exhibited highest potency in the rat whereas kappa-carrageenin (Serva) and kappa-carrageenin (Satiagel GS 350) hold the second and third rank, respectively (Table 1). Blueing and swelling of the paws and/or toes was sometimes seen after i.v. or i.p. injection of kappa-carrageenin (Sigma) or Satiagel GS carrageenin at doses of 1.5–20 mg/kg if thrombotic reaction was strong. Kappa-carrageenin (Serva) only occasionally gave blueing and swelling of the paws and/or toes. In mice, kappa-carrageenin (Sigma) likewise seems to produce a stronger thrombotic reaction than kappa-carrageenin (Serva) (Table 1), however, different strains can considerably differ in their thrombotic response. Whereas activity of kappa-carrageenin (Sigma) was high in the AB (Halle) strain, it was low in the AB (Berlin) and AB (Berger) strain (Fig. 1).

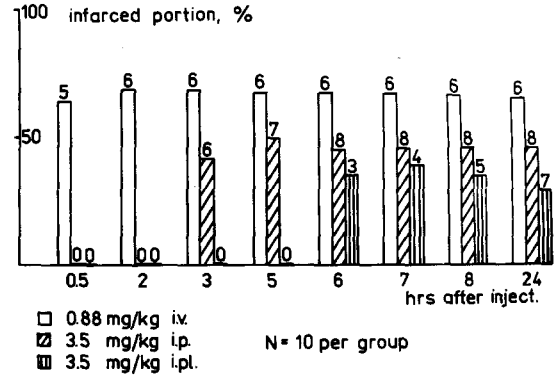


Figure 1
Time of appearance of tail infarction after i.v., i.p. and intraplantar injection of kappa-carrageenin (Sigma) in rats, given as the infarced portion (%) of the whole tail length (≙ 100%). Numbers on the top of the columns indicate number of animals with tail infarction per group ($N = 10$/group).

Lambda-carrageenin (Viscarin®), lambda-carrageenin (Sigma) and lambda-carrageenin (Satiagum B) caused no thrombotic reaction in rats and mice (Table 1). No thrombosis could be found after i.v. kappa-carrageenin in guinea-pigs and rabbits (Table 1). The thrombotic activity of the carrageenins does not parallel their

Table 1
Thrombotic activity of different carrageenins in rats, mice, guinea-pigs and rabbits.

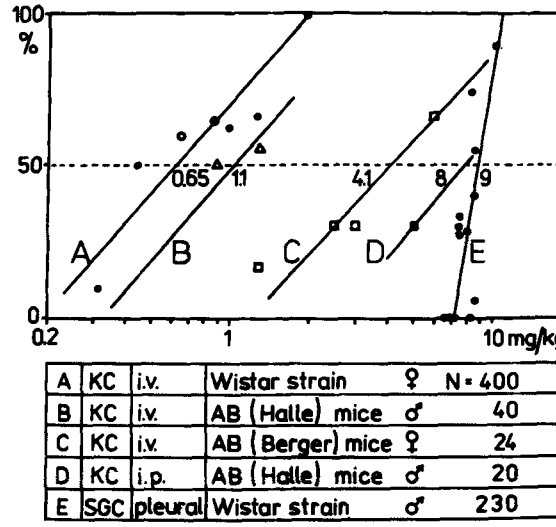
Carrageenin	Rats		Mice[a]	
	(mg/kg)	Frequency/length (%)	(mg/kg)	Frequency/length (%)
Kappa-carrageenin (Sigma)	0.3–20 i.v.	10–100/70	0.88–2 i.v.	40–65/44
	1–2.5 i.pl.	50–90/75		
	1–5 i.p.	30–90/46	5 i.p.	30–50/16
Kappa-carrageenin (Serva)	20 i.v.	0–80/5–50	10–20 i.v.	0–80/55
	10–15 i.pl.	0–50/n.m.	40 i.pl.	50/50
	20 i.v. +20 i.p.	50–1000/n.m.	40 i.pl.	0/0 (?)
			15 i.v. +50 i.p.	15/24
Kappa–carrageenin (Satiagel GS 350)	5–15 i.pl.	0/0		
	7–10 pleural	5–90/n.m.		
Lambda-carrageenin (Viscarin®)	10–45 i.v.	0/0		
	10 i.pl.	0/0		
	50–250 i.p.	0/0		
Lambda-carrageenin (Sigma)	3–7 pleural	0/0		
	5 i.v.	0/0		
Lambda-carrageenin (Satiagum B)	5–15 i.pl.	0/0		
	5–10 i.p.	0/0		

Guinea-pigs and rabbits after 30 mg/kg kappa-carrageenin (Sigma) i.v.: 0/0 (pads of animals).
[a] AB (Halle) mice or AB (Berlin) mice; i.pl. = intraplantar; frequency = percent animals with tail infarction in relation to number of animals per group (24 hours after injection); length = percent of length of tail infarction in relation to total tail length; n.m. = not measured.

Table 2
Edemogenic potency of different carrageenins in the carrageenin rat paw edema after intraplantar injection of 1 mg/paw. $N = 8–15$ per group.

Carrageenin	% increase of paw volume					
	1	2	3	4	5	24
	(hours after injection)					
Kappa-carrageenin (Sigma)	32	47	49	50	36	18
Kappa-carrageenin (Serva)	30	42	44	33	30	11
Kappa-carrageenin (Satiagel GS 350)	39	27	48	57	51	35
Lambda-carrageenin (Viscarin®, No. 5515)	29	39	75	83	80	69
Lambda-carrageenin (Viscarin®, No. RE 8985)	40	57	66	70	66	70
Lambda-carrageenin (Satiagum B)	29	31	62	69	74	69

Standard deviation, s, was in the range of 16% (higher values of increase) to 34% (lower values of increase).



A	KC	i.v.	Wistar strain ♀	N = 400
B	KC	i.v.	AB (Halle) mice ♂	40
C	KC	i.v.	AB (Berger) mice ♀	24
D	KC	i.p.	AB (Halle) mice ♂	20
E	SGC	pleural	Wistar strain ♂	230

Figure 2
Dose–response relationship and ED_{50} values of different carrageenins, routes of administration as well as species and strains, respectively. KC = kappa-carrageenin (Sigma); SGC = kappa-carrageenin (Satiagel GS 350). Response is given as the percentage of animals with infarction per group.

edemogenic potency. According to Table 2, the lambda-carrageenins, which cause no thrombosis, produce stronger paw edemas than the thrombogenically active kappa-carrageenins.

2. Time course of thrombosis, route of administration and dose–response relationships

According to Fig. 1, the time course of the appearance of tail infarction depends on the route of administration. Infarction becomes visible already some minutes after i.v. injection and a sharp demarcation can be seen after 30 minutes to 1 hour. After i.p. injection, infarction is evident at 3 hours. It occurs only at 6 hours, occasionally even after days, when injection was performed subplantarly. After becoming visible, only little change of demarcation could be observed. However, little blueing or reddening of the tail tip disappeared sometimes, but sometimes it exacerbated within several hours.

Dose–response relationships and ED_{50} values for some carrageenins are shown in Fig. 2. In this case, frequency of tail infarction was taken as the effect parameter since the infarced portion of the tail correlated with the dose to a lesser degree. As can be seen from Fig. 2, i.v. kappa-carrageenin (Sigma) has by far the highest activity in rats ($ED_{50} \sim 0.65$ mg/kg) and in AB (Halle) mice ($ED_{50} \sim 1.1$ mg/kg). When the i.p. route was used, the ED_{50} is only about 8 mg/kg in AB (Halle) mice. After intrapleural administration of kappa-carrageenin (Satiagel GS 350), the ED_{50} was found to be 9 mg/kg in rats. The ED_{50} of kappa-carrageenin (Serva), the third investigated kappa-carrageenin, was in the range of 15–20 mg/kg after i.v. injection in AB (Halle) mice.

3. Effect of substances on frequency and extent of thrombosis

Frequency of thrombosis in rats was significantly reduced by certain doses of the anti-serotoninergic cyproheptadine, the α-anti-adrenergics phentolamine and dibenamine, and by heparin (Table 3). The cyclo-oxygenase inhibitor aspirin, streptokinase and dipyridamole were without effect as were the proteinase inhibitors aprotinin and PAMBA® (Table 3). Using the length of the infarced tail portion as parameter, again cyproheptadine, phentolamine, dibenamine and heparin were active. Furthermore, the anti-serotoninergic indalpine, dipyridamole and aprotinin seem to cause inhibition now (Table 4).

After i.v. injection of kappa-carrageenin (Sigma), the number of platelets is lowered in rats. However, other carrageenins are likewise effective in this respect (Fig. 3), so that the high thrombotic activity of kappa-carrageenin (Sigma) can

Table 3
Effect of some substances in the carrageenin thrombosis test on tail infarction frequency (animals with tail infarction to total animals per group) in rats; 0.88, 1.25 or 1.76 mg kappa-carageenin (Sigma)/kg i.v.

Substance (mg/kg)	Control 24	Test group			
		0.5	1	3	24
		(hours after injection of carrageenin)			
Heparin					
1250 IU/kg††	11/20	0/20**	0/20**	0/20**	0/20**
Cyproheptadine					
1 p.o.†	10/10	4/10*	4/10*	4/10*	4/10*
1.25 p.o.†	6/10	1/10	1/10	1/10	1/10
2.5 p.o.†	14/30	3/30**	1/30**	1/30**	1/30**
5 p.o.†	8/20	1/20*	0/20**	0/20**	0/20**
10 p.o.†	8/10	8/10	5/10	5/10	3/10
Phentolamine					
6.25 i.v.	7/20	1/19	0/19*	0/19	0/19**
12.5 i.v.	17/30	6/27*	5/27**	4/27**	4/27*
25 i.v.	20/40	10/40*	9/40**	9/40**	9/40**
Dibenamine					
7.5 i.v.	9/9	3/10**	3/10**	3/10**	4/10*
15 i.v.	18/19	12/18	11/18*	11/18*	11/18*
Aspirin					
20 p.o.†	10/10	10/10	10/10	10/10	10/10
250 p.o.†	9/10	8/10	8/10	8/10	8/10
500 p.o.†	9/10	7/9	7/9	8/9	8/9
Dipyridamole					
5 i.v.	6/10	8/10	8/10	7/10	7/10
20 i.v.	9/10	9/10	9/10	5/10	5/10
Sulfinpyrazone					
10 p.o.†	7/20	7/20	5/20	5/20	8/20
30 p.o.†	7/20	3/20	3/20	3/20	8/20
45 p.o.†	7/10	3/10	3/10	3/10	2/10
60 p.o.†	5/10	3/10	2/10	2/10	2/10
90 p.o.†	7/10	1/8*	1/8*	1/8*	1/8*
Indalpine					
5 p.o.†††	7/10	10/10	10/10	10/10	10/10
10 i.p.	5/10	8/10	7/10	5/10	4/10
25 i.p.	8/12	12/12	12/12	12/12	12/12
Dihytamine					
5 i.v.	9/10	7/10	7/10	8/10	8/10
Carteolol					
0.2 i.v.	5/10	5/10	4/10	4/10	5/10
0.4 i.v.	9/10	3/10*	3/10*	3/10*	3/10*
0.8 i.v.	4/10	4/10	4/10	4/10	n.d.
Benzydamine					
100 p.o.†	10/10	9/10	10/10	10/10	10/10
Imidazole					
12.5 p.o.†	8/10	9/10	9/10	9/10	9/10
25 p.o.†	14/19	8/20	6/20*	7/20*	7/20*
50 p.o.†	8/10	7/10	7/10	7/10	7/10
100 p.o.†	16/28	9/29	9/29	9/29	8/29*
Streptokinase					
125,000 IU i.v.	8/19	7/18	7/18	6/18	6/18
250,000 IU i.v.	8/10	7/10	7/10	7/10	7/10
PAMBA®					
100 p.o.†	5/10	5/10	4/10	4/10	4/10
Aprotinin					
25,000 IU i.v.	9/9		9/9	9/9	9/9
Solcoseryl®					
1 ml/kg i.v.	8/9		8/10	8/10	8/10
Levamisole					
100 p.o.†	8/10	9/10	9/10	9/10	9/10

† 0.5 hour prior to carrageenin; †† heparin was i.v. injected 0.5 hour prior to carrageenin and it was i.p. administered 0.5 hour after carrageenin; ††† two times daily during 4 days before carrageenin; *, ** $p < 0.05$; 0.01 (χ^2-test).

Table 4
Effect of some substances in the carrageenin thrombosis test on the length of portion of tail infarction (% of whole tail) in rats; 1.5, 1.76, 2 or 4 mg kappa-carrageenin (Sigma)/kg i.v.

Substance (mg/kg)	Control (range)	Test group			
		0.5	1	3	24
		(hours after carrageenin injection)			
Aspirin					
20 p.o.	71–77	56	54	57	57
250 p.o.	28–41	22	17	17	17
Sulfinpyrazone					
10 p.o.	43–51	41	40	37	49
30 p.o.	43–51	50	46	44	44
45 p.o.	29–35	36	37	32	33
60 p.o.	28–41	23	23	17	21
90 p.o.	29–35	7	8	7*	6*
Cyproheptadine					
1.25 p.o.	47–54	10*	10*	12*	10*
2.5 p.o.	66–86	27**	23**	23*	0
5 p.o.	66–86	50	15	24	25
10 p.o.	62–64	29**	32**	30**	30**
Phentolamine					
6.25 i.v.	20–59	0	0	0	0
12.5 i.v.	20–59	0	0	0	0
25 i.v.	20–59	0	0	0	0
Indalpine					
10 i.p.	78–87	49**	46**	56**	60*
Carteolol					
0.1 i.v.	45–51	20*	20*	21*	28
0.2 i.v.	49–69	23**	47	47	48
0.4 i.v.	81–84	20**	20**	21**	16**
Benzydamine					
150 p.o.	60–70	22*	41*	41*	40
Dipyridamole					
5 i.v.	84–97	90	91	91	91
20 i.v.	61–65	41	33**	36**	37**
Aprotinin					
25,000 IU i.v.	83–94		37 (2 h)**	37 (4 h)**	46**

*,** $p < 0.05$; 0.01 (t-test).

hardly be explained by a strong aggregatory or thrombopenic activity.

Discussion

Our results in Wistar rats regarding thrombotic activity of carrageenins (Table 1, Fig. 2) agree with findings of THOMSON et al. [1] according to which kappa-carrageenins of different origin or preparation can differ in their thrombotic activity, and lambda-carrageenin (Marine Colloids) did not produce intravascular coagulation in Sprague–Dawley rats even at high doses. Iota-carrageenin (Marine Colloids) was found to be inactive, too [1]. The edemogenic potency of lambda-carrageenins is rather high,

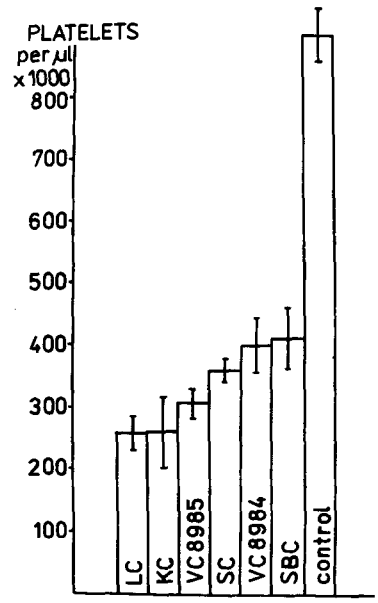


Figure 3
Thrombocytopenia ($\bar{x} \pm s$) 30 minutes after i.v. injection (2.5 mg/kg) of different carrageenins in the rat. $N = 5$/group. LC = lambda-carrageenin (Sigma); KC = kappa-carrageenin (Sigma); VC = lambda-carrageenin (Viscarin®); SC = kappa-carrageenin (Serva); SBC = lambda-carrageenin (Satiagum B). Thrombocytopenia after carrageenins is statistically significant from control in any case ($p < 0.01$; t-test).

while that of kappa-carrageenins is relatively low (Table 2). The rat paw edema after iota-carrageenin is 2.5-fold weaker than the lambda-carrageenin rat paw edema [4]. Thus, there seems to be no positive or negative correlation between thrombogenic and edemogenic activity of various carrageenins.

Whereas NEVEU and THIERRY [5] described ischaemic lesions of foot pad in guinea-pigs after high i.p. doses (200–300 mg/kg) of kappa-carrageenin (Sigma), we could not find such lesions after i.v. 30 mg/kg of the same type of carrageenin in guinea-pigs and rabbits. The weak thrombogenic activity of kappa-carrageenin in these species could be due to the relatively high plasmin activity in guinea-pigs and rabbits, and probably, to the high antithrombin III level in these animals.

The question was, whether carrageenin thrombosis can be influenced by antithrombotic drugs. For testing this, rats were usually given i.v. doses of 0.88–1.76 mg/kg kappa-carrageenin (Sigma). These doses are near the ED_{50} (Fig. 2),

so that thrombosis inhibiting and increasing substances can be detected. We found indeed significant antithrombotic effects after single administration of cyproheptadine, phentolamine, dibenamine and heparin (Tables 3 and 4). Cyproheptadine and phentolamine were also somewhat effective in mice [3]. These results might refer to an involvement of serotonin and, possibly, of catecholamines in the mechanism of carrageenin thrombosis. However, the α-antiadrenergics could also act by their vasodilating effects, only. The question is whether the length of the thrombotic tail portion in rats might be a better parameter than thrombosis frequency for valuation of antithrombotic effects, because aprotinin and dipyridamole were also inhibitory if the length of the infarced tail portion was the measure (Table 4).

Regarding the thrombotic activity of kappa-carrageenins, THOMSON et al. [1] have mentioned activation of Hageman factor which is followed by intravasal coagulation. Histologically, disseminated intravasal coagulation with participation of thrombocyte aggregates as well as leukostasis can be seen [1, 6]. Induction of platelets aggregation by carrageenins can hardly explain the thrombogenic activity of kappa-carrageenins, since thrombocyte aggregation is induced by all carrageenins tested (Fig. 3). There is at present no convincing explanation for the different thrombogenic potency of various carrageenins in rats and mice.

Further substances have to be included in our investigations and more detailed work has to be done in order to more reliably discuss the mechanisms of thrombotic action of kappa-carrageenins. However, some advantages of the kappa-carrageenin thrombosis model are already evident:

(1) Thrombosis can easily be induced in mice and rats, i.e. small laboratory animals can be used.
(2) Thrombosis can be observed, easily quantified and followed up all the time without killing the animals.
(3) Thrombosis occurs in the tail so that external antithrombotic substances can be tested.

Although standard test substances gave results similar to those obtained in other thrombosis models, the significance of the carrageenin thrombosis model has yet to be further cleared up, particularly with respect to man.

Received 10 September 1984

References

[1] A.W. THOMSON, A.R. WILSON, W.J. CRUICKSHANK and C.H.W. HORNE, *Evaluation of carrageenan as an immunosuppressive agent and mediator of intravascalur coagulation*, Biomedicine *24*, 102–106 (1976).
[2] E.D. BICE, D.G. GRUWELL, J.E. SALVAGGIO and E.O. HOFFMAN, *Suppression of primary immunization by carrageenan – a macrophage toxic agent*, Immunol. Communic. *1*, 615–625 (1972).
[3] H. BEKEMEIER, R. HIRSCHELMANN and A.J. GIESSLER, *Carrageenin-induced thrombosis in the rat and mouse as a test model of substances influencing thrombosis*, Biomed. Biochim. Acta *43*, 347–350 (1984).
[4] S. LEYCK, E. ETSCHENBERG, U. HADDING AND J. WINKELMANN, *A new model of acute inflammation: Cobra venom factor induced paw edema*, Agents and Actions *13*, 437–438 (1983).
[5] P.J. NEVEU and D. THIERRY, *Effects of carrageenan – a macrophage toxic agent – on antibody synthesis and on delayed hypersensitivity in the guinea pig*, Int. J. Immunopharmac. *4*, 175–179 (1982).
[6] U., FUCHS, personal communication.