Glycoconjugate Journal 15, 531–533 (1998)

EGC2
An intravascular chemoattractant lectin inhibits neutrophil migration

Marcelo Sakamoto[1], Marcelo Dias-Baruffi[3], Ricardo Santos-de-Oliveira[1], Fernando Queiroz Cunha[2] and Maria Cristina Roque-Barreira[1]*

[1]*Department of Parasitology, Microbiology and Immunology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, S.P., 14049-900, Brazil*
[2]*Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, S.P., 14049-900, Brazil*
[3]*Department of Clinical Analysis, Faculty of Pharmaceutic Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, S.P., 14040-903, Brazil*

KM^+, a lectin purified from *Artocarpus integrifolia* seeds, is an attractant for neutrophils, and has properties similar to fMLP, IL-8 and MNCF. The endogenous lectin MNCF, inhibits carrageenan-induced neutrophil migration when intravenously administered in rats. In an attempt to mimic the activity of MNCF with KM^+, we determined the effect of intravenous (*iv*) injection of KM^+ (5 μg) on neutrophil migration to the peritoneal cavity of Wistar rats induced by KM^+ (50 μg, intraperitoneal, *ip*), fMLP (5 ng, *ip*) and carrageenan (300 μg, *ip*). Initially we evaluated the effect of the time interval between intravenous and intraperitoneal administration of KM^+. The intervals ranged from 20 to 120 min and progressively stronger inhibition was observed with increasing time intervals up to a maximum of 60 min, with effect decreasing thereafter. With injections at the optimum interval of 60 min, we observed that KM^+ inhibited KM^+- and carrageenan-induced neutrophil migration by 72%, and fMLP-induced migration by 56%. White cell counts for Wistar rats that only received KM^+ *iv*, performed at 0 to 120 min intervals after injection, revealed early neutropenia lasting 60 min, followed by a marked increase in circulating neutrophils that reached a maximum of twice the initial levels within 90 min and after 120 min returned to levels near to that observed before intravenous administration of KM^+. These results indicate that when KM^+ is present in the intravascular space, it produces an inhibitory effect on neutrophil migration similar to that caused by the intravenous administration of other chemoattractants, regardless of whether they act through a mechanism independent of carbohydrate recognition, as does IL-8, or are dependent on carbohydrate recognition, like MNCF.

***Keywords*: lectin, neutrophil, chemoattractant, anti-inflammatory**

Introduction

Localized neutrophil migration is the cellular hallmark of an acute inflammatory response [1]. Circulating neutrophils adhere to the endothelial lining of venules and then migrate through endothelial junctions into extravascular tissues directed by a chemoattractant gradient [2, 3]. Neutrophil migration rapidly increases following local application of a chemoattractant but then subsides to low levels suggesting that neutrophil migration at sites of acute inflammation is a self-limited process [3]. Intravenously administered chemoattractants, such as IL-8 or fMLP, reduce neutrophil recruitment to inflammatory sites, apparently as a consequence of transient granulocyte trapping in the pulmonary microcirculation and inhibition of neutrophil functions necessary for transmigration [4]. Recently, we described a neutrophil recruitment inhibitory activity exerted by intravenous administration of a chemoattractant released by macrophages (MNCF), which acts through a D-galactose recognition site [5]. This observation motivated us to investigate the effect of intravenous administration of KM^+, a neutrophil migration-inducing lectin extracted from *Artocarpus integrifolia* seeds [6], on neutrophil migration to the rat peritoneal cavity after *ip* stimulation by KM^+ itself or by other chemoattractants. We also determined the effect of intravenous KM^+ administration on the number of circulating neutrophils.

*To whom correspondence should be addressed.

Materials and methods

Purification of KM^+

A crude extract of *Artocarpus integrifolia* seeds was prepared as described in reference 7. The lectin KM^+ was purified to apparent electrophoretic homogeneity from a saline extract of *Artocarpus integrifolia* seeds by sugar affinity chromatography as previously reported [6].

Neutrophil migration into the peritoneal cavity of rats

Male Wistar rats weighing 180–200 g were injected with KM^+ (5 μg in 0.3 ml PBS) into the penial venous sinus 20, 40, 60, 80, 100 or 120 min before the intraperitoneal administration of KM^+ (50 μg in 0.3 ml PBS) or 60 min before the intraperitoneal administration of carrageenan (300 μg in 3 ml per animal) and fMLP (5 ng in 3 ml PBS per animal). Four hours after the intraperitoneal stimuli, the cavity was washed with 10 ml PBS containing 0.2% (w/v) bovine serum albumin (BSA) and 5 U ml^{-1} heparin. Five ml of the fluid was removed for total and differential cell counting. The positive control group received an intravenous injection of PBS 40 or 60 min before the intraperitoneal stimuli. The negative control group received PBS injections by both intravenous and intraperitoneal routes.

Circulating neutrophil number

Male Wistar rats weighing 180–200 g received an intravenous injection of KM^+ (5 μg in 0.3 ml PBS). The number of circulating neutrophils was determined in blood venous samples collected 0, 5, 10, 30, 60, 90 and 120 min after the injection. The negative control group received 0.3 ml PBS intravenously.

Results

Effect of intravenous KM^+ administration on neutrophil migration to the peritoneal cavity induced by inflammatory stimuli

KM^+ (5 μg per animal) was injected *iv* into Wistar rats 20, 40, 60, 80, 100 and 120 min before the administration of KM^+ (50 μg/animal) into the peritoneal cavity. Four hours after the *ip* stimulus the number of exudate neutrophils in the peritoneal cavity was determined. Neutrophil migration induced by *ip* injection of KM^+, when no chemoattractant was *iv* administered, is indicated by a gray bar in Figure 1. This neutrophil migration was progressively inhibited by previous *iv* administration of the lectin. A maximal inhibition of 58% was observed when the time between the two injections was 60 min, with the anti-inflammatory effect progressively decreasing at longer times between injections, although it was still 42% less than the migration observed for the positive control. The table shows that at the ideal time interval of 60 min, the intravenous administration of KM^+, in contrast to PBS *iv* injection, inhibited the neutrophil migration caused by three different inflammatory stimuli (Table 1). The migration induced by KM^+ itself (50 μg/animal) or carrageenan (300 μg/animal) was inhibited by 72% and the migration induced by fMLP (5 ng/animal) was inhibited by 56%.

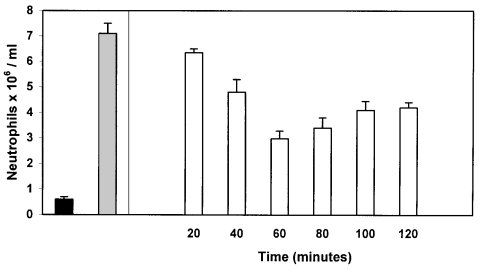

Figure 1. Effect of intravenous injection of KM^+ on the neutrophil migration induced by intraperitoneal stimulus with KM^+. KM^+ was intravenously injected in rats before the intraperitoneal administration of KM^+. The time between the two injections is indicated below each open bar. The positive control group (gray bar) received intravenous injection of PBS 40 min before intraperitoneal injection of KM^+. The negative control group (black bar) received PBS injection by both intravenous and intraperitoneal routes, with a time interval also of 40 min. The bars indicate the number of neutrophils detected in the peritoneal cavity, reported as mean ± SEM neutrophils × 10^6/ml wash for six rats per group.

Table 1. Inhibition of neutrophil migration into the peritoneal cavity by intravenous administration of KM^+.

Intraperitoneal stimulus	*Intravenous injection*		*Inhibition*
	PBS	*KM^+*	
KM^+	3.65 ± 0.28	1.02 ± 0.10	72%
CAR	3.50 ± 0.40	0.98 ± 0.10	72%
fMLP	3.00 ± 0.30	1.32 ± 0.09	56%
PBS	0.80 ± 0.09	ND	–

KM^+ or vehicle (PBS) was intravenously injected in Wistar rats 60 min before the intraperitoneal administration of KM^+, carrageenan (CAR), fMLP or vehicle. Four hours later the number of exudate neutrophils in the peritoneal cavity was determined. The data are reported as mean ± SEM neutrophils × 10^6/ml wash for six rats per group.

Effect of intravenous KM^+ administration on the number of circulating neutrophils

Cells counts carried out 0 to 120 min after intravenous KM^+ administration (5 μg/animal) showed an early decrease in neutrophil number lasting 60 min when compared to the number of cells of rats injected *iv* with the vehicle (PBS) (Figure 2). This was followed by an increase in neutrophil number that was maximal at 90 min after *iv* KM^+ administration. After 120 min the number of circulating neutrophils was similar to that before injection.

Discussion

The ability of KM^+, a D-mannose binding lectin from *Artocarpus integrifolia* seeds, to induce neutrophil migration

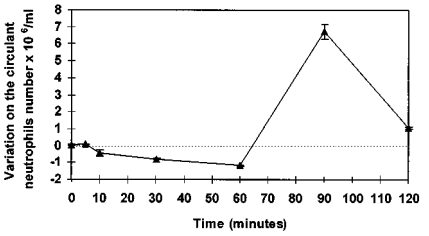

Figure 2. Effect of intravenous injection of KM^+ on circulating neutrophil number. KM^+ was intravenously injected in rats and the total number of circulant neutrophils was determined in blood samples collected at different times after the injection. The negative control group received PBS *iv*. The data indicate the absolute variation on the circulant neutrophil number using the values obtained on the negative control as reference and are reported as mean for six rats per group. Representative data from one of three experiments are presented. The dotted horizontal line (zero variation on the ordinate) corresponds to the number of circulating neutrophils at zero time when KM^+ was injected.

in vivo and *in vitro* [6], has led to its characterization as a chemoattractant which can mimic endogenous chemokines. The present observations show that intravascular KM^+ has an anti-inflammatory effect (Figure 1), as reported for IL-8 [4] and MNCF [5], which are primarily described as inflammatory mediators. An intravenous bolus of KM^+ (5 μg) in rats inhibits the neutrophil migration to the sites of acute inflammation induced by 50 μg KM^+ itself. The inhibition of migration was maximal, (from 58 to 72%, Figure 1 and Table), when there was a 60 min interval between the *iv* bolus and the intraperitoneal stimulus, which is close to the interval observed with intravascular IL-8 in rabbits [4] or with intravascular MNCF in rats [5]. The neutrophil migration observed with time intervals of more than 60 min between *iv* and *ip* administration of KM^+ has discretely increased (Figure 1). The inhibition of neutrophil accumulation at the site of acute inflammation may be associated with the reduction of circulating neutrophils, detected at 60 min after the *iv* administration of KM^+ (Figure 2). Subsequently the number of circulating neutrophils exceeded the preinjection levels. Intravenous administration of IL-8 also caused neutropenia, which was normalized 30 min after injection and 90 min after the injection neutrophilia was demonstrable. Neutrophilia has been attributed to the release of neutrophils sequestered in the lung and to the release of immature forms of neutrophils from the bone marrow [4]. We suggest that these phenomena may also be occurring in our experiments utilizing intravascular KM^+. The differences in the time required to reverse the initial neutropenia induced by KM^+ or IL-8 may be due to differences in the clearance of those chemoattractants from the circulation [8]. As the neutrophil migration inducing activity of KM^+ is dependent on the formation of a haptotactic gradient, we suggest, as previously hypothesized for intravascular IL-8 [9], that the anti-inflammatory activity of intravascular KM^+ is a consequence of the formation of a negative haptotactic gradient, with a greater concentration of KM^+ in the vascular lumen than in the endothelial and perivascular tissue, blocking the directed migration of neutrophils through the acute inflammation site. This negative haptotactic gradient has been shown to prevent neutrophil migration induced *in vitro* by IL-8 [10], MNCF [11] or KM^+ [6]. Present data suggest that KM^+ can be a useful tool to mimic endogenous mammalian mediators of inflammation and to study the role of protein-carbohydrate interactions in this process.

Acknowledgments

This work was funded by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and by the Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), Financiadora de Estudos e Projetos (FINEP), Brazil. The authors thank Sandra Maria de Oliveira Thomaz and Daniel Marques Mota for expert technical assistance and Dr Lewis Joel Greene for his critical review of the manuscript.

References

1 Arfors KE, Lundberg C, Lindbom L, Lundberg K, Beatty PG, Harlan JM (1987) *Blood* **69**: 338–40.
2 Snyderman R, Goetzl EL (1981) *Science* **213**: 830–7.
3 Colditz IG, Movat HZ (1984) *J Immunol* **133**: 2169–73.
4 Hechtman DH, Cybulsky MI, Fuchs HJ, Baker JB, Gimbrone MA Jr (1991) *J Immunol* **147**: 883–92.
5 Tavares-Murta BM, Cunha FQ, Dias-Baruffi MD, Roque-Barreira MC, Ferreira SH (1996) *Mediators Inflammation* **5**: 116–20.
6 Santos-de-Oliveira R, Dias-Baruffi MD, Thomaz SMO, Beltramini LM, Roque-Barreira MC (1994) *J Immunol* **153**: 1789–807.
7 Roque-Barreira MC, Campos-Neto A (1985) *J Immunol* **134**: 1740–3.
8 Harada A, Mukaida N, Matsushima K (1996) *Molecular Medicine Today* **2**: 482–9.
9 Ley K, Baker JB, Cybulsky MI, Gimbrone MAJr, Luscinskas FW (1993) *J Immunol* **151**: 6347–57.
10 Rot A (1993) *Eur J Immunol* **23**: 303–6.
11 Dias-Baruffi M, Roque-Barreira MC, Cunha FQ, Ferreira SH (1995) *Mediators Inflammation* **4**: 263–9.

Received 9 September 1996, revised 9 June 1997, accepted 29 July 1997