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Rat paw edema and leukocyte immigration induced by plant lectins

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Abstract

Lectins from *Dioclea grandiflora* (DG) and *Canavalia brasiliensis* (CB) were compared with Concanavalin A (ConA) for their ability to induce paw edema and peritoneal cell immigration in rats. ConA caused a slight edema with a peak at 1 h after injection, while DG or CB induced a pronounced and long-lasting edema that reached a maximum at about 6 h. Different antiinflammatory drugs partially inhibited the edema. α-D-glucose (GLU) partially blocked the edema caused by ConA and markedly inhibited that due to CB, but had no effect on the edema induced by DG. α-Methyl mannoside (α-MM) blocked the edema caused by DG and ConA, but did not affect that caused by CB. At doses much lower than those used to induce paw edema, the lectins promoted an intense accumulation of neutrophil and mononuclear cells in the rat peritoneal cavity. CB and DG were more potent than ConA, which also presented a different profile of cell immigration. GLU significantly inhibited leukocyte accumulation caused by all lectins. α-MM impaired ConA- and DG-induced cell immigration, but only partially inhibited CB. Thus, despite their physicochemical similarities with ConA, DG and CB have more powerful pro-inflammatory effects. This difference seems to be related to their sugar-binding properties. However, while ConA- and DG-induced effects were inhibited more by α-MM than by GLU, CB-induced effects were inhibited more by glucose.

Introduction

Lectins have been defined as proteins of non-immunological origin that can bind selectively to carbohydrates, without inducing any chemical change in them [1]. Since virtually all cells are coated with sugar residues, lectins bind readily to them and can cause a variety of biological effects, including agglutination [2]. Binding of many lectins to leukocytes serves as a signal for mitogenic stimulation of lymphocytes [2, 3], can mediate phagocytosis of target cells [4] and may induce cytotoxic activity in macrophages [5], among other effects [2].

Lectins are prominent constituents of many biological materials, especially in plant seeds. Concanavalin A (ConA), the lectin of *Canavalia ensiformis* seeds, is without doubt the most celebrated of the plant lectins, and it has been widely used as a tool in biomedical research [2].

Like ConA, *Dioclea grandiflora* (DG) and *Canavalia brasiliensis* (CB), two lectins purified from seeds

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of Leguminosae from the tribe *Diocleae*, belong to the group of mannose/glucose-binding lectins [6, 7]. They also resemble ConA in many physicochemical properties, but despite these similarities, both lectins have been shown to differ from ConA in their potency for inducing certain biological effects [8–10].

Here we have compared the proinflammatory activity of CB, DG and ConA in inducing rat hind-paw edema and cell immigration into the rat peritoneal cavity.

Material and methods

Lectins

Lectins from *Dioclea grandiflora* (100 kDa) and *Canavalia brasiliensis* (100 kDa) seeds were purified by affinity and molecular-sieve chromatography as described earlier [6, 7]. Concanavalin A (type IV, 96 kDa) was kindly provided by Dr. C. R. Carlini (Dept. de Bioquimica, ICB, Universidade Federal do Rio de Janeiro).

Rat paw edema

Edema was induced in the left hind paw of Wistar rats (180–220 g) under light ether anesthesia by a single subplantar injection of ConA, DG or CB, diluted in 0.1 ml sterile saline (NaCl 0.9%). The right paw, used as control, received the same volume of saline alone. Edema was measured plethysmographically according to Ferreira [11], at the indicated time intervals after injection. The results are expressed as the difference ($\Delta\mu l$) between the values obtained from paws injected with lectin and those injected with saline.

In some cases rats were pretreated with antiinflammatory drugs (s.c.), 1 h before the lectin intraplantar injection. The results are expressed in absolute values ($\Delta\mu l$) as well as in percentage of inhibition of the edema in comparison with non-pretreated animals.

In vivo polymorphonuclear and mononuclear cell immigration

Immigration of phagocytic cells in *vivo* was induced by intraperitoneal injection of 3 ml of sterile saline containing different concentrations of lectins. At various time intervals after injection, the cells were harvested by lavage of the cavity with 10 ml of phosphate-buffered saline containing heparin (5 IU/ml) and bovine serum albumin (0.1% w/v). Usually, 70–80% of the lavage fluid was recovered. Total and differential cell counts were performed as described elsewhere [12]. The results are reported as the number of cells per ml of collected fluid. Rats injected with 3 ml of sterile saline were used as controls.

Influence of carbohydrates on lectin-induced inflammatory responses

For some experiments, lectins were dissolved in a solution of α-D-glucose or α-methyl mannoside before injection into the paw or the peritoneal cavity. Carbohydrates, unless otherwise stated, were used at concentrations 100 times greater than that used for the lectins, on a molar basis. Animals treated with carbohydrates alone were used as control.

Statistical analyses

Results are presented as means ± SD of experiments in at least six rats. The significance of the results at the 5% level ($P < 0.05$) was determined by analysis of variance (ANOVA), followed by Bonferroni's *t*-test.

Results

Lectin-induced hind-paw edema

Figure 1 shows the edematogenic effect of DG and CB lectins, as well as of ConA, at doses varying from 25 to 500 μg/paw.

All lectins induced rat hind-paw edema in a dose-dependent manner, although their effects differed in potency and in time course. The edema induced by Con A (Fig. 1A) reached its maximum (280 μl) 1 h after injection, returning to basal levels after 6–8 h for doses from 75 to 250 μg, and after 24 h for the dose of 500 μg/paw.

When compared to ConA, DG and CB showed more powerful edematogenic effects, as well as different profiles of edema development. One hour after injection, paw swelling induced by DG (Fig. 1B) reached values similar to those caused by ConA. However, in DG-treated animals the edema continued to increase, achieving its maximum at 6–8 h. At this time, the paw volume (500 μl) was about twice that observed for ConA after 1 h. The

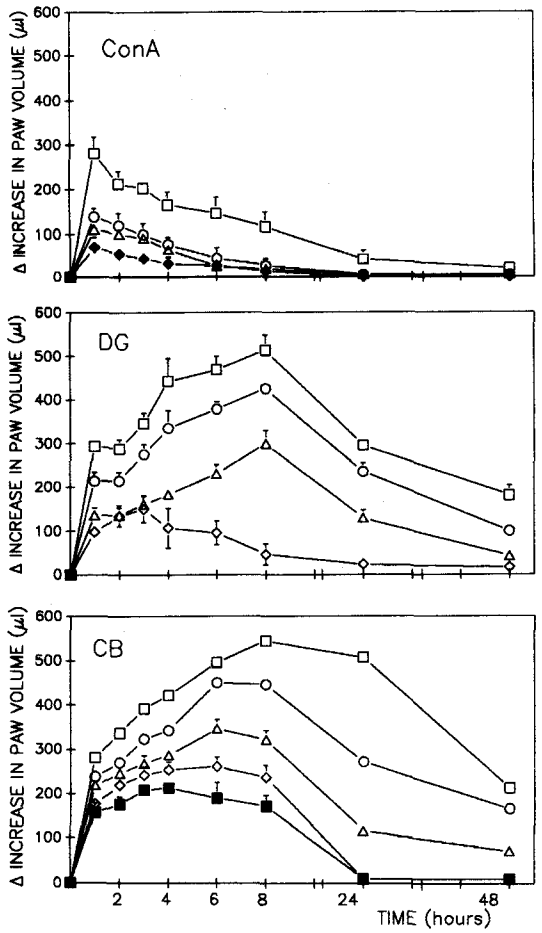

Figure 1
Time course of rat hind-paw edema induced by lectins. Concanavalin A (ConA-Panel A), *Dioclea grandiflora* lectin (DG-Panel B) or *Canavalia brasiliensis* lectin (CB-Panel C) was injected into the left hind paw in a final volume of 0.1 ml; the right paw received vehicle (0.9% NaCl). The doses used were 25 μg (■); 50 μg (◇); 75 μg (◆); 125 μg (△); 250 μg (■) and 500 gμg (■). Results are expressed as the mean ± SD ($n = 10$) of the difference in volume (Δ μl) between control (saline) and treated paws. Where no error bars are shown, the SD was smaller than the size of the symbol.

time course of CB-induced rat paw edema (Fig. 1C) was similar to that of DG (Fig. 1B). However, CB proved to be more effective than DG during the first few hours after injection, for all doses tested. On injection of 500 μg/paw, CB induced a more persistent edema; even 24 h later, paw swelling was equivalent to that observed at 6–8 h. For both lectins, at the lowest doses used (25–100 μg/paw), the edema resolved and disappeared after 48 h. However, in animals that received 250–500 μg of DG or CB, significant paw swelling still persisted at this time.

Effect of carbohydrates on lectin-induced paw edema

Intraplantar injection of carbohydrates alone (5 m*M*) did not cause significant paw swelling. When 500 μg ConA plus glucose (5 m*M*, GLU) or α-methyl mannoside (5 m*M*, α-MM) were conjointly injected into rat hind paws, the edematogenic effect was markedly reduced compared to the effect of the lectin alone. The maximum edema, 1 h after ConA injection, decreased by 53 ± 7% ($n = 7$; $P < 0.05$) in the presence of GLU and decreased by 80 ± 9% ($n = 7$; $P < 0.01$) in the presence of α-MM. In contrast to their effects on ConA-induced paw swelling, α-MM, but not GLU, was able to reduce the edematogenic effect induced by 500 μg DG (Fig. 2A). Even when the doses of the lectin was reduced to 100 μg/paw, GLU had no effect, in contrast to the strong inhibitory effect of α-MM (Fig. 2A). With CB, on the other hand, only GLU inhibited the edema (Fig. 2B). α-MM had no effect on CB-induced paw swelling, even when the dose of the lectin was reduced (Fig. 2B).

Effect of antiinflammatory drugs on lectin-induced paw edema

Table 1 shows the effect of different antiinflammatory drugs on paw edema induced by ConA, DG and CB. The possible involvement of serotonin and histamine in lectin-induced paw edema was evaluated in rats pretreated with methysergide or meclizine, respectively. Both drugs significantly inhibited the edema induced by all lectins 1 h after intraplantar injection. However, the peak effect of CB or DG, 6 h after injection, was not altered by the antagonists. On the other hand, dexamethasone, a glucocorticoid, and mepacrine, a phospholipase inhibitor, significantly inhibited the edema induced by ConA, at 1 h and by DG or CB at 1 and 6 h. Dexamethasone showed a more pronounced inhibitory effect than mepacrine, mainly at 6 h. Pretreatment with the cyclooxygenase inhibitor indomethacin inhibited the edema induced by ConA by 43% and reduced the extent of the edema

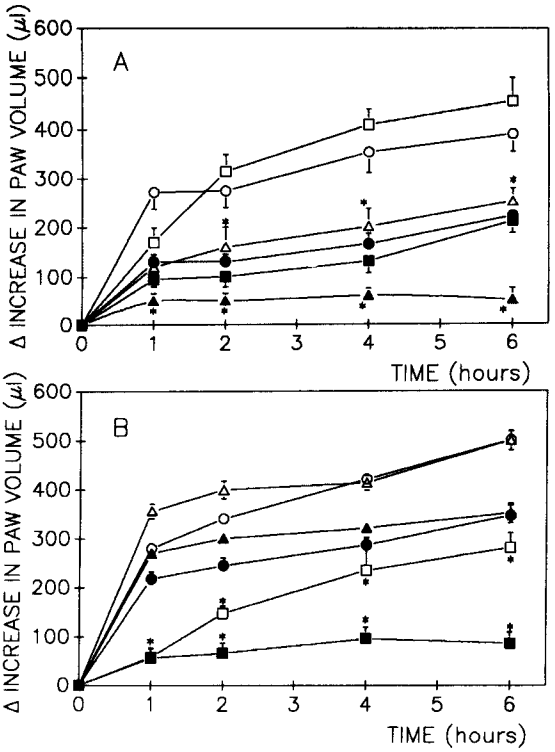

Figure 2
Influence of carbohydrates on edema formation. *Dioclea grandiflora* lectin (Panel A) or *Canavalia brasiliensis* lectin (Panel B) was injected alone (circles) or conjointly with 5 m*M* of D-glucose (squares) or α-methyl mannoside (triangles). The doses used for lectins were 100 μg (filled symbols) and 500 μg (open symbols). Carbohydrates injected alone did not cause any significant paw swelling. Results are expressed as the mean ± SD ($n = 7$) of the difference in volume (Δ μl) between control (carbohydrates alone) and treated paws. Where no error bars are shown, the SD was smaller than the size of the symbol. $^*P < 0.05$ (ANOVA followed by Bonferroni's *t*-test) in comparison to animals injected with lectins alone.

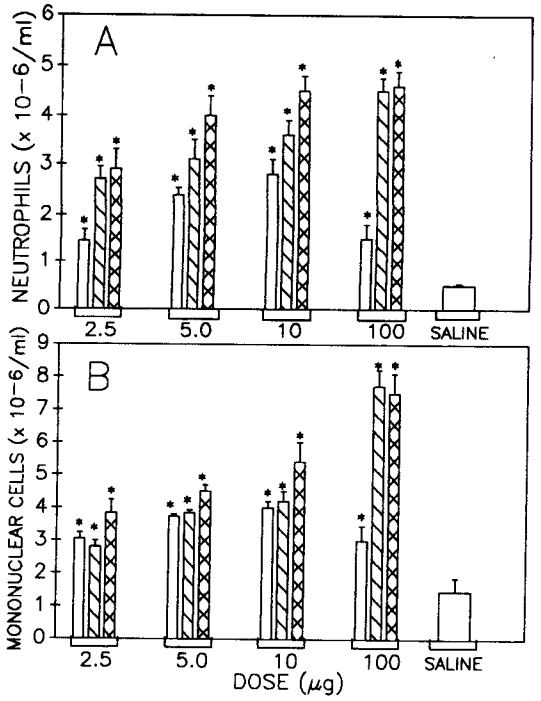

Figure 3
Peritoneal cell immigration induced by lectins. Different doses of Concanavalin A (Open Bars), *Dioclea grandiflora* lectin (Diagonal Bars) and *Canavalia brasiliensis* lectin (Cross Hatched Bars) were injected i.p. in rats. Neutrophil immigration (Panel A) was evaluated at 4 h and mononuclear cells (Panel B) were counted 48 h after i.p. injection of each lectin diluted in sterile saline. Results are expressed as the mean ± SD ($n = 7$). $^*P < 0.05$ (ANOVA followed by Bonferroni's *t*-test) in comparison to animals injected with the vehicle (Last Open Bar).

induced by DG and CB by about 30%, at any time studied (Table 1)

Lectin-induced peritoneal cell immigration

Figure 3 shows the effect of ConA, DG and CB in inducing neutrophil and mononuclear cell immigration into rat peritoneal cavity after i.p. injection of doses varying from 2.5 to 100 μg.
Neutrophil accumulation, evaluated 4 h after lectin injection, was a dose-related event for both DG and CB (Fig. 3A). Of the three lectins, CB exhibited the most powerful migratory effect for polymorphonuclear cells (PMNL). ConA, besides being less effective, reached a peak at 10 μg and then decreased.
Mononuclear cells (MNC) immigration was evaluated 48 h after lectin intraperitoneal injection (Fig. 3B). All lectins significantly increased the number of MNC, although CB was somewhat more effective than the other two lectins. At a dose of 100 μg, CB- and DG-treated animals showed a sharp increase in cell number, whereas the MNC count in animals treated with 100 μg of ConA decreased from the values found after injection of 10 μg (Fig. 3B).

Table 1
Effect of antiinflammatory drugs on lectin-induced paw edema.

	Lectin				
	ConA	DG		CB	
Pretreatment	(1 h)	(1 h)	(6 h)	(1 h)	(6 h)
None	274 ± 20	256 ± 22	434 ± 24	247 ± 20	402 ± 15
Meclizine (40 mg/kg)	84 ± 14* (69%)	124 ± 15* (51%)	367 ± 29 (16%)	132 ± 20* (46%)	343 ± 17 (15%)
Methysergide (5 mg/kg)	133 ± 19* (51%)	87 ± 9* (66%)	438 ± 22 (0%)	120 ± 25* (51%)	407 ± 16 (0%)
Dexamethasone (0.5 mg/kg)	102 ± 15* (62%)	88 ± 6* (65%)	195 ± 18* (55%)	98 ± 8* (60%)	166 ± 20* (59%)
Mepacrine (40 mg/kg)	112 ± 12* (59%)	104 ± 16* (59%)	243 ± 40* (44%)	105 ± 8* (57%)	256 ± 29* (36%)
Indomethacin (5 mg/kg)	154 ± 19* (43%)	170 ± 12* (33%)	314 ± 30* (27%)	159 ± 27* (35%)	278 ± 22* (31%)

Paw swelling was measured 1 h after intraplantar injection of ConA (500 µg) or 1 h and 6 h after injection of DG (250 µg) or CB (250 µg). Drugs were administered (s.c.) at indicated doses, 1 h before lectin injection. Results are expressed as the mean ± SD ($n = 7$) of the difference in volume (µl) between saline- and lectin-treated paws. Percentage of inhibition in relation to non-pretreated group (None) is shown in parentheses. *$P < 0.05$; ANOVA followed by Bonferroni's t-test.

Effect of carbohydrates on lectin-induced cell immigration

In this experiment, the lectins (10 µg) were diluted in a solution of α-methyl mannoside or D-glucose before injection into the rat peritoneal cavity. These sugars were used at concentrations 100 times greater than that used for the lectins, on a molar basis. They did not cause a significant increase in cell number when injected alone, at any time evaluated.

Table 2 shows that both carbohydrates significantly inhibited both PMNL and MNC immigration, but the extent of inhibition depended on the lectin and on the type of cell investigated. GLU inhibited PMNL accumulation into peritoneal cavities induced by CB and DG, by 85% and 88%, respectively. ConA-induced immigration was reduced by about 66% by GLU. However, with mononuclear cells, GLU was more effective in inhibiting ConA-induced immigration than that promoted by DG or CB. The migratory effect on both PMNL and MNC induced by either ConA or DG was substantially or totally blocked by α-MM, whereas CB-induced cell immigration was only partially inhibited.

Discussion

The lectins from *Dioclea grandiflora* [6] and *Canavalia brasiliensis* [7] seeds have been described to have similar physicochemical properties to those of ConA, the well-known lectin from the seeds of *Canavalia ensiformis* [2]. In spite of these similarities, CB and DG were far more efficient than ConA in inducing lymphocyte proliferation [8], IFN-τ production by blood mononuclear cells [9] and macrophage activation [10] (CB > DG > ConA).

In our studies, ConA also proved to be less effective than the other two lectins in inducing either rat paw edema or peritoneal leukocyte immigration. In addition, the concentration dependence and the time course of these ConA-induced effects were quite different from those exhibited by DG or CB. Upon intraplantar injection, all three lectins induced a dose-dependent edema. However, while CB or DG induced a long-lasting paw swelling, with a peak of effect at about the sixth hour, ConA administration caused a less pronounced edema which reached its maximum after 1 h and disappeared within 24 h. The edema induced by the other lectins, depending on the dose, was still significant even after 48 h. It has been demonstrated that

Table 2
Effects of carbohydrates on lectin-induced leukocyte migration.

Treatment[a]	PMN[b]	Percentage inhibition[c]	MNC[d]	Percentage inhibition[c]
Saline	0.5±0.2	—	1.6±0.3	—
GLU	0.7±0.3	—	2.0±0.4	—
α-MM	0.9±0.3	—	2.4±0.7	—
ConA (10 μg)	3.0±0.6	—	4.2±0.6	—
ConA + GLU	1.6±0.3*	66	2.4±0.3*	86
ConA + α-MM	0.8±0.2*	100	2.0±0.2*	100
DG (10 μg)	3.5±0.3	—	4.3±0.7	—
DG + GLU	1.2±0.5*	85	3.5±0.3*	44
DG + α-MM	1.2±0.3*	90	3.1±0.4*	73
CB (10 μg)	4.5±0.3	—	5.3±0.6	—
CB±GLU	1.2±0.2*	88	3.3±0.7*	66
CB±α-MM	3.0±0.3*	34	4.3±0.6*	48

[a] Animals were injected (i.p.) with lectin (10 μg) diluted in a solution (10 μ *M*) of α-glucose (GLU) or α-methyl mannoside (α-MM). Carbohydrate injection alone did not induce any significant cell migration, when compared to saline. Results are expressed as mean ±SD.
[b] PMNL ($\times 10^6$ /ml), neutrophil accumulation evaluated 4 h after injection.
[c] MNC ($\times 10^6$ /ml), mononuclear cell immigration evaluated 48 h after injection.
[d] Percentage inhibition: Percentage of inhibition in relation to animals treated with lectins alone.
*$P<0.05$; ANOVA followed by Bonferroni's *t*-test.

phagocytic cells are potentially able to contribute to the development of the edema by releasing several mediators of an acute inflammatory response [13]. Thus, the difference in the potency of the edematogenic effect induced by ConA and the other lectins, may be related to the ability of DG and CB to induce a more intense cellular mobilization, mainly polymorphonuclear leukocytes, to the site of injection within the first few hours after lectin administration.

The pretreatment of animals with inhibitors of the classical mediators of inflammatory responses demonstrated that, for all lectins, the edematogenic effect is probably a multimediated phenomenon. The data suggest that histamine as well as serotonin, probably plays a major role in the initial edema (1 h). This suggestion is supported by the observation that ConA, DG and CB induced *in vitro* an intense mast cell degranulation and histamine release (unpublished data). On the other hand, arachidonic acid metabolites may participate during the prolonged development of paw swelling induced by DG and CB. The partial inhibition induced by the pretreatment with indomethacin and the strong inhibition caused by the phospholipase A2 inhibitors, dexamethasone and mepacrine, at 1 and 6 h, pointed to this. Thus, prostaglandins as well as other metabolites, such as the leukotriene B4, a potent chemotactic agent [14], could play an important role in the development of the edema induced by DG and CB.

At doses that were ineffective in inducing paw edema (2.5–10 μg), the three lectins caused a significant leukocyte accumulation in rat peritoneal cavities. The ability of CB to induce PMNL and MNC immigration was greater than that of either of the other lectins. This effect was dose-dependent and levelled off at 10 μg. ConA was the least effective, presenting at the range of dose from 2.5 to 100 μg/cavity, a bell-shaped curve for either PMNL or MNC migration. Interestingly, in animals treated with doses higher than 10 μg of ConA, the maximum PMNL immigration occurred 8 h after ConA injection and the number of MNC was significantly higher at 96 h (not shown).

The binding of lectins to specific carbohydrate sites is probably the signal for triggering an inflammatory reaction, which involves release or synthesis of several mediators that together account for the responses observed. The inhibitory effect of carbohydrates injected conjointly with lectins shows that the proinflammatory actions of these proteins are closely related to their specific sugar-binding properties. However, although all three lectins show similar specificities for mannose and glucose in hemagglutinating assays [6, 7], in both experimental models studied they differed markedly in potency and their effects were differently inhibited

by these carbohydrates. While ConA and DG effects were markedly inhibited by α-methyl mannoside, the powerful proinflammatory effects of CB were significantly reduced by glucose. The data may suggest that, the differences between the biological activity of ConA, DG and CB may be related to their different affinities for the glycosyl groups present on the cell surface.

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