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Renal effects induced by the lectin from *Vatairea macrocarpa* seeds

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Abstract

Lectins are glycoproteins that interact reversibly and specifically with carbohydrates. The renal effects of the galactose-binding lectin from the seeds of *Vatairea macrocarpa* were investigated. Isolated kidneys from Wistar rats (240–280 g) were perfused with Krebs–Henseleit solution containing 6% bovine serum albumin. The *V. macrocarpa* lectin (10 μg mL^{-1}) increased the perfusion pressure, renal vascular resistance, urinary flow and glomerular filtration rate. However, *V. macrocarpa* lectin did not change the percentage sodium, potassium or chloride tubular transport. Pre-treatment with lectin–galactose complex significantly blocked the increase in perfusion pressure, renal vascular resistance, urinary flow and glomerular filtration rate. The control group showed a small amount of a proteinaceous material in the urinary space, although no alteration in the renal tubules was detected. The administration of galactose alone did not modify the functional parameters of the kidney. Kidneys perfused with *V. macrocarpa* lectin showed moderate deposits of a proteinaceous material in the tubules and urinary space. Those pre-treated with lectin–galactose complex had only small amount of a proteinaceous material in the urinary space. No abnormalities were seen in renal tubules. The results suggest that lectin from *V. macrocarpa* seeds has important effects on the carbohydrate-binding sites of the renal system, given the reversal of renal effects with the use of that specific inhibitor.

Introduction

Lectins are glycoproteins that interact reversibly and specifically with carbohydrates (Yamazaki et al 2000). These proteins are widely distributed in nature and have been found in microorganisms, plants and animals (Lis & Sharon 1986; Moreira et al 1991). The possibility of purification and characterization of their molecular structure makes them potentially useful for therapeutic use. Lectins are well suited to act in many biological processes, including lymphocyte function, cell communication and signal transduction, host defence and fertilization. They have also been used for glycoconjugate purification and characterization, as well as specific reagents for biomedical research (Weis & Drickamer 1996).

Vatairea macrocarpa (Leguminosae: Dalbergieae) lectin is a galactose-binding protein that contains a mixture of doubly (28 525 Da) and singly (27 354 Da) glycosylated alpha chains, present in the family (Calvete et al 1998). The anti-inflammatory and pro-inflammatory activity of *V. macrocarpa* lectin has been reported (Alencar 2001).

The ability of some lectins to bind kidney cells has been demonstrated (Holthofer 1983). We previously demonstrated the renal effects of the lectin from *Canavalia brasiliensis* seeds (Teixeira et al 2001). Its recognition of the cellular carbohydrate is a central event triggering several biological effects and it is supposedly important to various processes of cell-to-cell interaction. The study of lectins is justified by the

important role played by carbohydrates in the biochemical processes involved in the interactions among cells. In the present study, we investigated the effects of the lectin from the seeds of *V. macrocarpa* in isolated rat kidney.

Materials and Methods

Materials

All chemical reagents were purchased from Sigma Chemical Co. (St Louis, MO, USA). *V. macrocarpa* lectin was obtained from the Biomol-lab, Department of Biochemical and Molecular Biology, Federal University of Ceara, Brazil.

Kidney perfusion

Adult Wistar rats of both sexes (240–280 g) were fasted with free access to water for 24 h before each experiment. The animals were anaesthetized with sodium pentobarbital (50 mg kg^{-1}). The perfusion fluid was a modified Krebs–Henseleit solution with the following composition (mmol L^{-1}): Na^+ 147, K^+ 5, Ca^{2+} 2.5, Mg^{2+} 2, Cl^- 110, HCO_3^- 2.5, SO_4^{2-} 1, PO_4^{2-} 1. Bovine serum albumin (6 g%; fraction V), urea (0.075 g), inulin (0.075 g) and glucose (0.15 g) were added to the solution, resulting in a final perfusate volume of 100 mL. Bovine serum albumin was previously dialysed for 48 h at 4°C in 1.5 L of Krebs solution, which was changed in the solution every 24 h (Hanson & Ballard 1968; Pegg 1971). The pH was adjusted to 7.4 and the perfusion system, based on Bowman's technique (Bowman 1970; Fonteles et al 1998), was modified (Walson et al 1955) by the addition of an artificial lung to improve oxygenation (Hamilton et al 1974) and a 1.2-mm Millipore filter (Pegg 1971). Flow calibration and the resistance of the system were determined before each experiment. Perfusion pressure was determined at the tip of the stainless steel cannulae. The right renal artery was cannulated through the upper mesenteric artery and the kidney was isolated (Nishiitsutji-Uwo et al 1967; Ross 1978; Fonteles et al 1983), allowing uninterrupted perfusion. After an equilibration period of 15–20 min, the experiments were carried out, with a total experimental time of 120 min. The *V. macrocarpa* lectin (10 μg mL^{-1}) was added 30 min after the start of the experiment. To investigate if the renal effects of the lectin from *V. macrocarpa* depend on carbohydrate ligant, we evaluated the action of galactose (6.25 mM; specific sugar of lectin from *V. macrocarpa* seeds) and that of the lectin incubated with galactose, which we termed the lectin–galactose complex. Galactose or lectin–galactose complex was administered to the kidney 30 min after the start of the experiment. The perfusion pressure was measured at 5-min intervals. Samples of the perfusate were collected every 10 min for the determination of sodium, potassium, chloride, inulin levels and osmolality. Urine flow was also measured at 10-min intervals. Sodium and potassium concentrations were determined by flame photometry (flame photometer Model 445; Micronal, Brazil). The analysis of chloride was made using a LabTest kit (LABTEST, São Paulo, Brazil) and inulin levels were determined by direct hydrolysis (Walson et al 1955). The osmolality of the samples was measured using a WESCOR 5100c vapor pressure osmometer (WESCOR, Needham Heights, MA, USA).

After the experiment, both right and left kidneys were removed and fixed in 10% formaldehyde for histological processing. The kidney tissue was embedded in paraffin, cut into 3–5-μm sections, stained with haematoxylin–eosin and processed further for light microscopy.

The experiment followed the methodology recommended by International Ethical Standards in animal research and was approved by the Scientific and Ethical Committee Federal University of Ceara, Brazil.

Statistical analysis

The data were evaluated using analysis of variance followed by the Bonferroni test. Results are expressed as mean ± s.e.m. with the level of significance set at 5% ($P < 0.05$; $n = 6$).

Results and Discussion

Lectins isolated from seeds exert a wide variety of effects and can be used as tools in biological research and also therapeutically (Yamazaki et al 2000; Teixeira et al 2001). Perfusion of isolated kidneys has been extensively used as a model for studying the renal effects of biologically active substances, thus avoiding the interference of systemic action.

Lectins with different carbohydrates specificities were able to bind distinct renal cells (Holthofer 1983; Murata et al 1983). It has been demonstrated that jacalin, a galactose-binding lectin, strongly binds to renal cells of the distal convoluted tubules and collecting ducts, but does not bind to the cells of proximal convoluted tubules (Engel et al 1997).

We previously demonstrated the renal effects induced by the lectin from *C. brasiliensis* seeds, which included an increase in perfusion pressure, vascular renal resistance, urinary flow and glomerular filtration rate (Teixeira et al 2001). On the other hand, we observed a decrease in electrolyte transport. In the present study, using a similar approach, lectin from *V. macrocarpa* seeds (10 μg mL^{-1}) caused a significant increase in perfusion pressure and renal vascular resistance, with a maximal effect at 120 min (Figure 1). The urinary flow and glomerular filtration rate were also significantly increased after infusion of *V. macrocarpa* lectin, again with a maximal response at 120 min (Figure 2). The *V. macrocarpa* lectin did not change the percentage of sodium, potassium or chloride tubular transport (Table 1). The selected parameters were also evaluated in kidneys perfused with the modified Krebs–Henseleit solution in the absence of *V. macrocarpa* lectin under stable experimental conditions during the experimental time (Figures 1 and 2; Table 1).

In order to determine if the renal effects of the *V. macrocarpa* lectin (a galactose-binding lectin) depended

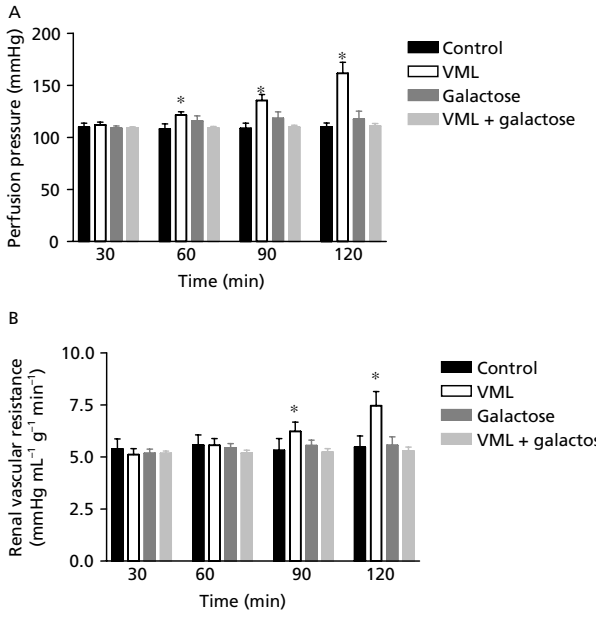

Figure 1 Effects of *Vatairea macrocarpa* lectin (VML) on perfusion pressure (A) and renal vascular resistance (B) in isolated rat kidney. Data are presented as the mean ± s.e.m. of six rats in each group. $^*P < 0.05$ compared with the corresponding control group.

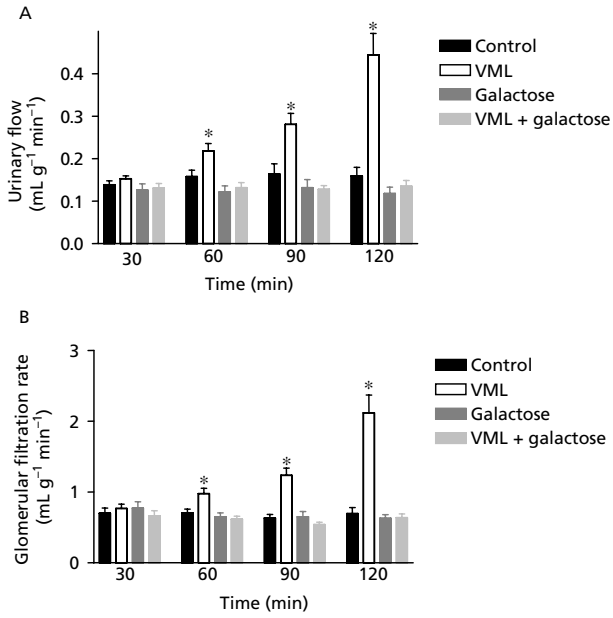

Figure 2 Effects of *Vatairea macrocarpa* lectin (VML) on renal urinary flow (A) and glomerular filtration rate (B) in isolated rat kidney. Data are presented as the mean ± s.e.m. of six rats in each group. $^*P < 0.05$ compared with the corresponding control group.

on the binding sites of the lectins, we administered the lectin–galactose complex and found that the increase in perfusion pressure and renal vascular resistance was significantly blocked. The treatment with lectin–galactose complex also reversed the effects on urinary flow and glomerular filtration rate (Figures 1 and 2). Galactose (6.25 mM) alone did not modify the kidney functional parameters when compared with the internal control group (Figures 1 and 2).

Histological evaluation of the control group, in which kidneys were perfused with Krebs–Henseleit solution only, revealed a small amount of a proteinaceous material in the urinary space of some glomeruli; no alteration in the renal tubules was detected. After treatment with *V. macrocarpa* lectin, the kidneys exhibited a moderate deposit of a proteinaceous material in the tubules and urinary spaces. Eosinophilic casts were seen in the renal tubules; no abnormalities were seen in the renal vessels. Kidneys pre-treated with the lectin–galac-

Table 1 Effects of lectin from *Vatairea macrocarpa* seeds (10 μg mL^{-1}) on ion transport in isolated perfused rat kidney

Parameter	Treatment	Time (min)			
		30	60	90	120
% Sodium tubular transport					
	Control	78.4 ± 1.48	78.1 ± 1.33	78.4 ± 1.22	79.6 ± 1.07
	Vaitarea macrocarpa lectin	79.9 ± 1.18	79.3 ± 1.16	78.7 ± 0.63	79.2 ± 0.92
	Galactose	81.4 ± 1.26	80.3 ± 1.39	78.8 ± 1.79	79.7 ± 2.16
	Lectin–galactose complex	79.3 ± 1.51	78.8 ± 1.66	76.9 ± 1.06	79.2 ± 1.53
% Potassium tubular transport					
	Control	72.0 ± 2.75	71.5 ± 3.76	73.0 ± 4.15	71.7 ± 5.53
	Vaitarea macrocarpa lectin	74.0 ± 1.22	74.1 ± 1.43	75.3 ± 1.22	78.3 ± 0.89
	Galactose	68.3 ± 2.03	62.9 ± 2.91	59.1 ± 3.21	59.6 ± 5.04
	Lectin–galactose complex	71.5 ± 2.80	72.6 ± 2.28	68.3 ± 2.07	72.7 ± 1.86
% Chloride tubular transport					
	Control	79.9 ± 1.03	81.3 ± 2.44	77.3 ± 2,22	78.5 ± 2.33
	Vaitarea macrocarpa lectin	76.4 ± 2.84	74.2 ± 3.30	73.6 ± 1.74	76.2 ± 2.61
	Galactose	79.6 ± 2.16	79.8 ± 3.75	77.1 ± 3.42	79.7 ± 3.33
	Lectin–galactose complex	80.6 ± 2.82	81.2 ± 3.84	78.9 ± 3.07	79.8 ± 3.16

Data are presented as the mean ± s.e.m of six rats in each group. The treatments were given 30 min after the start of each perfusion.

tose complex had only small amount of proteinaceous material in the urinary space (Figure 3).

These findings suggest that *V. macrocarpa* lectin preferentially constricts the glomerular efferent arteriole. Thus, the increase in the perfusion pressure was probably responsible for the increase in glomerular pressure and, consequently, the increase in urinary flow. Tubuloglomerular feedback is a complex process that primarily regulates glomerular filtration rate; any changes in renal blood flow are a secondary effect (Vander 1995). We presume that the differences found regarding the renal effects of lectin from *C. brasiliensis* seeds and *V. macrocarpa* lectin were due to the carbohydrate-binding activity, the lectin from *C. brasiliensis* being a glucose/mannose-specific lectin and that from *V. macrocarpa* being a galactose-binding lectin.

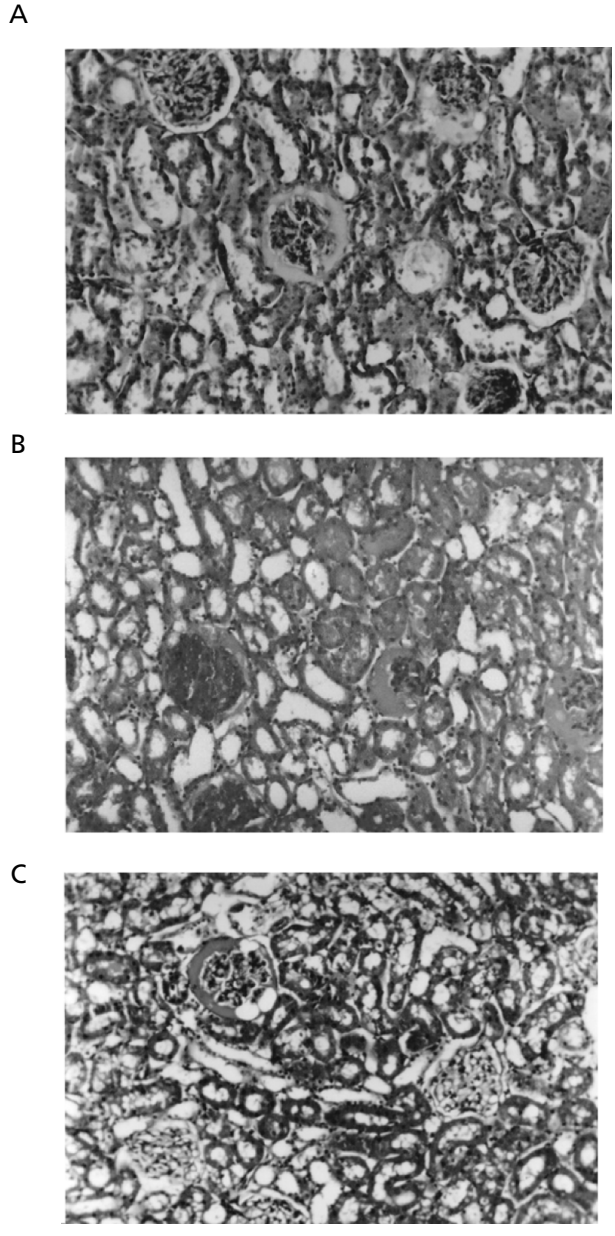

Figure 3 Histological evaluation of control kidney (A), kidney treated with *Vatairea macrocarpa* lectin in the absence of galactose (B) and kidney treated with *V. macrocarpa* lectin in the presence of galactose (C).

Physicochemical features such as specificity to the carbohydrate complex, the dimer/tetramer balance related to pH, and relative orientation of the binding site of carbohydrates may interfere with the amino acid position in the primary structure of protein and, consequently, in the biological activity of the lectin (Cavada et al 2001). It has been shown that the specificity toward carbohydrate is directly reflected in the intensity of the biological effect and not its primary structure, as the lectin with 98% structural homology caused effects of different intensity in the renal physiology (Havt et al 2001).

We conclude that lectin from the seeds of *V. macrocarpa* has important effects on the carbohydrate-binding sites of the renal system, given that it showed a reversal of renal effects when the specific inhibitor was used. To our knowledge, this is the first demonstration of the biological effects of *V. macrocarpa* lectin in isolated rat kidney.

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