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Article

The effect of *Cratylia floribunda* lectin on renal hemodynamics and ion transport

Alexandre Havt[1], Ana Maria Sampaio Assreuy[2], Nilberto Robson Falcão do Nascimento[2], Manassés Claudino Fonteles[2], Lívia de Paula Pereira[2], Sandra Maria Nunes Monteiro[1], Paulo Sergio Ferreira Barbosa[1], Kyria Santiago do Nascimento[3], Benildo Sousa Cavada[4], Alice Maria Costa Martins[4], Helena Serra Azul Monteiro[1]

[1]Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, UFC, Fortaleza, Ceará, Brasil, [2]Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, UECE, Fortaleza, CE, Brazil, [3]BioMol-Lab, Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, UFC, Fortaleza, CE, Brazil, [4]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Odontologia e Enfermagem, Universidade Federal do Ceará, Fortaleza, CE, Brazil

Lectins have been described as glycoproteins that reversibly and specifically bind to carbohydrates. Legume lectins isolated from the subtribe Diocleinae (*Canavalia*, *Dioclea* and *Cratylia*) are structurally homologous with respect to their primary structures. The Diocleinae lectins of *Canavalia brasiliensis*, *Dioclea guianensis* and *Canavalia ensiformis* have been shown to distinctly alter physiological parameters in isolated rat kidneys. Thus, the aim of this study was to investigate the effect of Cratylia floribunda lectin (CFL) on renal hemodynamics and ion transport in rats. In isolated perfused kidneys, CFL (10 µg/mL, n=5) increased RPP, RVR and decreased %TK+, but did not change urinary flow, glomerular filtration rate, sodium or chloride tubular transport. In isolated perfused mesenteric bed, CFL (3 and 10 µg/mL/min; n=4) did not alter tissue basal tonus or tissue contraction by phenylephrine (1 µM/mL/min). In conclusion, the seed lectin of *Cratylia floribunda* increased renal hemodynamic parameters showing a kaliuretic effect. This effect could be of tubular origin, rather than a result from haemodynamic alterations.

Uniterms: Plant lectin. Amiloride. *Cratylia floribunda*/lectina/renal hemodynamics. Renal hemodynamics. Ion-transport.

As lectinas são descritas como (glico)proteínas que se ligam, especificamente e reversivelmente, a carboidratos. Lectinas de leguminosas isoladas da subtribo Diocleinae (*Canavalia*, *Dioclea* e *Cratylia*) são estruturalmente homólogas em relação às suas estruturas primárias. Demonstrou-se que as lectinas de Diocleinae *Canavalia brasiliensis, Dioclea guianensis* e *Canavalia ensiformis* alteram diferentemente parâmetros fisiológicos em rins isolados de ratos. Dessa maneira, o objetivo deste estudo foi investigar o papel da lectina de *Cratylia floribunda* (CFL) na hemodinâmica renal e no transporte de íons em ratos. Em rins isolados perfundidos, CFL (10 µg/mL, n=5) aumentou a pressão de perfusão renal, a resistência vascular renal e reduziu o percentual do transporte tubular de K^+, mas não alterou o fluxo urinário, a taxa de filtração glomerular e o percentual de transporte tubular dos íons sódio e cloreto. No leito mesentérico isolado perfundido, CFL (3 e 10 µg/mL/min, n=4) não alterou o tônus basal ou a contração do tecido induzida por fenilefrina (1 µM/mL/min). Em conclusão, a lectina de sementes de *Cratylia floribunda* altera parâmetros hemodinâmicos renais, provavelmente de origem tubular, e não por alterações hemodinâmicas.

Unitemos: Lectina vegetal. Amilorida. *Cratylia floribunda*/lectina/hemodinâmica renal. Hemodinâmica renal. Transporte de íons.

***Correspondence:** A. M. C. Martins. Departamento de Análises Clínicas e Toxicológicas. Faculdade de Farmácia, Odontologia e Enfermagem. Universidade Federal do Ceará. Rua Capitão Francisco Pedro, 1210 - 60430-370 - Fortaleza - CE, Brasil. E-mail: martinsalice@gmail.com

INTRODUCTION

Lectins have been described as carbohydrate-binding proteins widely distributed in animals, plants and microorganisms (Yamazaki *et al*., 2000). Plant lectins isolated from seeds of the Diocleinae subtribe present high structural homology (80-90%) and are the most investigated, especially those of the genera *Canavalia*, *Dioclea* and *Cratylia* (Ingham, 1990). The lectin studied here is a metalloprotein, isolated by affinity chromatography from seeds of *Cratylia floribunda* (CFL) (family *Leguminosae*, Tribe *Phaseoleae*, Subtribe *Diocleinae*), that requires divalent ions (Ca^{2+}/Mn^{2+}) for full biological activity (Oliveira, Cavada, Moreira, 1991). CFL presents glucose/mannose binding specificity for conserved binding sites of asparagine-linked oligosaccharides or deoxy analogs (Dam *et al*., 1998). In addition, CFL has a multimeric structure composed of identical monomers of 25.5 KDa and exhibits distinct pH-dependent dimer-tetramer equilibrium (Calvete *et al*., 1999; Del Sol, Cavada, Calvete, 2007).

Despite the structural similarities, Diocleinae lectins exhibit distinct biological activities, varying in potency and efficacy in comparison with CFL: lower mitogenic activity of *Canavalia ensiformis* lectin (ConA) in human blood mononuclear cells (Barral-Netto *et al*., 1992); higher histamine secretory activity of many Diocleinae lectins in rat peritoneal mast cells (Gomes *et al*., 1994). In addition, the higher affinity of CFL for glucose-mannose binding sites on the eye and mouth mucosal surfaces in relation with other glucose-mannose lectins (Banchonglikitkul *et al.,* 2000) and its intermediate inhibitory activity in relation to the lectins of *Dioclea violacea* and *Dioclea guianensis* (DguiL) on the rat peritonitis and paw edema (Assreuy *et al*., 1997).

In respect to renal effects of Diocleinae lectins, our group demonstrated that the lectin of *Canavalia brasiliensis* (ConBr) (Teixeira *et al*., 2001), DguiL and ConA (Havt *et al*., 2003) distinctly alter physiological parameters in *ex vivo* rat kidneys. Considering that kidneys are responsible for the maintenance and excretion of electrolytes in the human body and the structure-activity relationship among Diocleinae lectins, the direct renal effects promoted by the seed lectin of *Cratylia floribunda* in isolated perfused kidneys and mesenteric bed of rats were investigated.

MATERIAL AND METHODS

Wistar rats

Animals, maintained with free access to water and fasted for 24 h before experiments, were anesthetized with sodium pentobarbital (50 mg/kg body weight; i.p.). Experimental protocols were approved by the Animal Care and Use Committee of the Federal University of Ceara (UFC n° 107/07), according to international guidelines (NIH publication n°85-23, 1985). ‘’Guide to the Care and Use of Experimental Animal Care” (Canadian Council on Animal Care guidelines, 1984).

Rat kidney perfusion

The right kidney (n=5) of rats (260-320g) was carefully dissected and the renal artery was cannulated via mesenteric artery (Bowman, 1970; Fonteles et al., 1983) and perfused with modified Krebs-Henseleit solution (MKHS; mmol/L): 114.00 NaCl, 4.96 KCl, 1.24 KH_2PO_4, 0.5 $MgSO_4 \cdot 7H_2O$, 2.10 $CaCl_2$ and 24.99 $NaHCO_3$. Bovine serum albumin-fraction V (BSA; 6 g), urea (0.075 g), inulin (0.075 g) and glucose (0.15 g) were added to 100 mL of MKHS and the pH was adjusted to 7.4. In the experiments, 100 mL of MKHS were perfused for 120 min as control.

Renal perfusion pressure (RPP) was measured at the tip of the stainless steel cannula in the renal artery. Renal vascular resistance (RVR) was calculated by dividing the RPP by the flow, measured in a flowmeter. Urinary flow (UF) was measured directly and glomerular filtration rate (GFR) by the clearance of inulin (Walser, Davidson, Orloff, 1955, Fonteles *et al.* 1983). Osmolality was evaluated by means of a vapor pressure osmometer (Wescor 5100C, USA). Samples of urine and perfusate were collected at 10min intervals. Electrolytes (Na^+, K^+ and Cl^-) were measured using ion-selective electrodes (Rapid Chem 744®, Bayer Diagnostic, UK) and sodium (%TNa^+), potassium (%TK^+) and chloride (%TCl) tubular transport were calculated (Martinez-Maldonado, Opava-Stitzer, 1978). CFL (10 µg/mL) was added to the organ bath 30 min after the perfusion was initiated. In another set of experiments, amiloride (10^{-4} M), a distal tubular sodium channel blocker, was added at 0 time (immediately after a 20-min equilibration period), 30 min before the CFL challenge. All experiments lasted 120 min and results were compared to the internal control groups (inside the group) or to time-matched controls (between groups).

Mesenteric bed perfusion

The abdomen of rats (280-350 g) was opened for tightening of pancreatic-duodenal, ileum-colic and colic branches of the superior mesenteric artery. The superior mesenteric artery was cleaned of surrounding tissue

and cannulated with a polyethylene tube (PE20). The mesenteric bed (n=4) was perfused with MKHS with addition of 3.60 mM glucose at 37°C at a flow rate of 4 mL/min (MCGREGOR 1965). Direct vascular effects of CFL (3 and 10 µg/mL/min) or phenylephrine (1 µM/mL/min) after 10 min of infusion were compared to the vehicle. Endothelium integrity was evaluated by addition of acetylcholine (1 µM) in the plateau-phase of phenylephrine-induced contraction. Perfusion pressure was measured using a pressure transducer (Statham P23, Gould, Oxnard, CA, USA) and recorded on a four-channel physiograph (Narco BioSystems, Houston, TX, USA).

Statistical analysis

Results were presented as mean±S.E.M. and compared by the Student test. Statistical differences were adopted for p values < 0.05.

RESULTS AND DISCUSSION

CFL increased RPP and RVR at 90 min ($\%_{RPP}$ = 11.2; $\%_{RVR}$ = 8.7) and 120 min ($\%_{RPP}$ = 13.9; $\%_{RVR}$ = 18.6), whereas GFR and UF were unchanged (Table I). Amiloride decreased RPP and RVR at 60 min ($\%_{RPP}$ = 15.1; $\%_{RVR}$ = 16.0), 90 min ($\%_{RPP}$ = 21.4; $\%_{RVR}$ = 25.3) and 120 min ($\%_{RPP}$ = 25.1; $\%_{RVR}$ = 24.2). However, the addition of amiloride before CFL potentiated the CFL increased effect on RPP and RVR at 90 min ($\%_{RPP}$ = 12.5; $\%_{RVR}$ = 8.9) and 120 min ($\%_{RPP}$=34.6; $\%_{RVR}$ = 39.2). Moreover, CFL plus amiloride increased GFR and UF at 60 min ($\%_{GFR}$ = 90.6; $\%_{UF}$ = 52.6), 90 min ($\%_{GFR}$ = 175; $\%_{UF}$ = 78.9) and 120 min ($\%_{GFR}$ = 178; $\%_{UF}$ = 126.3) (Table I). In the mesenteric bed, CFL did not alter either the tissue basal tonus nor decreased the high tonus induced by phenylephrine (Figure 1).

These results are suggestive of an indirect effect of CFL on renal hemodynamics. It is known that the increase in renal vascular resistance can lead to the increase in glomerular filtration and urinary flow. However, the present results showed that these effects could follow independent mechanisms (Koeppen, Stanton, 1997). Previous studies had demonstrated important vasodilator effects of lectins belonging to the genus *Canavalia*, that

TABLE I - Renal effects of the lectin from *Cratylia floribunda* seeds

Renal parameters	30 min	60 min	90 min	120 min
RPP (mmHg)				
Control	110.11 ± 3.68	108.27 ± 4.88	108.69 ± 5.09	110.28 ± 3.69
Amiloride	111.10 ± 1.30	91.90 ± 5.70*	85.40 ± 2.30*	82.60 ± 1.50*
CFL	107.70 ± 2.30	116.80 ± 2.40	120.90 ± 4.00*	125.60 ± 4.20*
CFL + Amiloride	107.80 ± 1.70	106.90 ± 3.90	122.30 ± 5.20*	148.40 ± 5.20*
RVR (mmHg/mL.g^{-1}.min^{-1})				
Control	5.39 ± 0.10	5.57 ± 0.02	5.76 ± 0.05	5.49 ± 0.08
Amiloride	5.60 ± 0.20	4.68 ± 0.37*	4.30 ± 0.18*	4.16 ± 0.14*
CFL	5.58 ± 0.23	6.00 ± 0.16	6.26 ± 0.30*	6.51 ± 0.32*
CFL + Amiloride	5.54 ± 0.17	5.48 ± 0.23	6.27 ± 0.27*	7.64 ± 0.34*
GFR (mL.g^{-1}.min^{-1})				
Control	0.77 ± 0.05	0.74 ± 0.02	0.70 ± 0.03	0.73 ± 0.03
Amiloride	0.74 ± 0.08	0.67 ± 0.11	0.58 ± 0.09	0.60 ± 0.07
CFL	0.76 ± 0.04	0.85 ± 0.07	0.87 ± 0.14	0.81 ± 0.11
CFL + Amiloride	0.90 ± 0.09	1.41 ± 0.21*	1.93 ± 0.32*	2.03 ± 0.29*
UF (mL.g^{-1}.min^{-1})				
Control	0.17 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Amiloride	0.16 ± 0.01	0.15 ± 0.02	0.14 ± 0.01	0.14 ± 0.01
CFL	0.18 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.18 ± 0.02
CFL + Amiloride	0.19 ± 0.02	0.29 ± 0.04*	0.34 ± 0.05*	0.43 ± 0.05*

RPP: Renal perfusion pressure; RVR: renal vascular resistance; UF: urinary flow; GFR: glomerular filtration rate. CFL (10 µg/mL), amiloride (10^{-4} M) or CFL + amiloride. Mean ± S.E.M. (n=5) and compared by Student test. *p< 0.05 compared to control (Krebs-Henseleit).

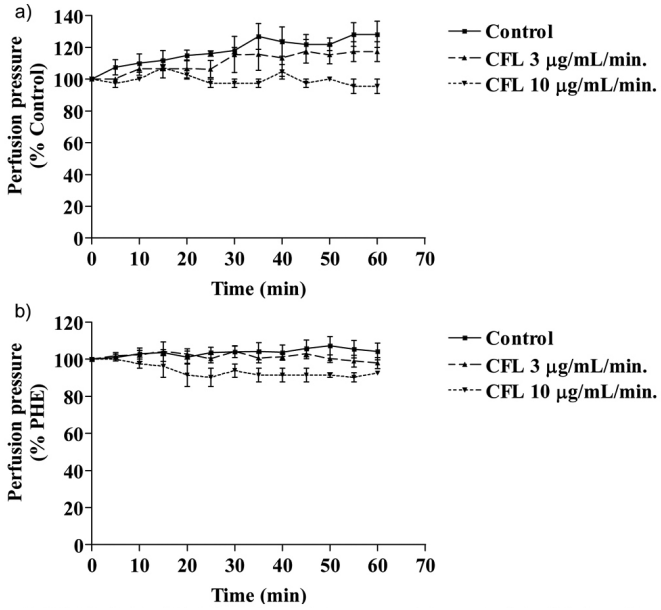

FIGURE 1 - Effect of *Cratylia floribunda* lectin (CFL; 3 or 10 µg/mL/min) on mesenteric vascular bed. Panel (A) shows its effect in the basal mesenteric vascular tonus and (B) shows the effects of CFL in vascular bed contracted with phenylephrine (PHE; 5 µM). Mesenteric bed was perfused with Krebs Henseleit solution at 37 °C. Data were expressed as mean ± S.E.M. (n=4) and compared by using Student t test. *p< 0.05 compared to control (Krebs-Henseleit).

varied in efficacy and mechanisms either *in vivo* (paw edema) or *in vitro* (isolated aorta), and involved

the nitric oxide pathway (Assreuy *et al*., 2009). Thus, changes in renal hemodynamics and tubular physiology may be a consequence of the release of inflammatory mediators from kidney cells (Koeppen; Stanton, 1997). Moreover, the increased release of renin elicited by amiloride can interfer in its mild natriuretic and diuretic effects (Endemann *et al*. 2002). In fact, amiloride intensified the vascular effects (RPP and RVR) elicited by CFL.

CFL did not alter the tubular transport of sodium or chloride, but reduced the tubular transport of potassium at 60 min ($\%_{TK}^{+}$ = 7.19), 90 min ($\%_{TK}^{+}$ = 20.79) and 120 min ($\%_{TK}^{+}$ = 22.53). As expected, amiloride reduced the transport of sodium at 60 min ($\%_{TNa}^{+}$ = 8.16), 90 min ($\%_{TNa}^{+}$ = 10.14) and 120 min ($\%_{TNa}^{+}$ = 7.25), but not that of chloride or potassium, whereas its addition to the perfusion solution before CFL did not alter none of these ion transports (Table II). In respect to renal tubular transport, CFL decreased $\%TK^{+}$, but did not alter $\%TNa^{+}$ or $\%TCl^{-}$. Since amiloride, recognized as a potent potassium sparing drug, increases sodium levels in the distal segments, its effect opposes potassium reabsorption by CFL in this renal segment. The tubular alteration in

TABLE II - Effects of the lectin from *Cratylia floribunda* seeds on ions tubular transport

Ions transport	30 min	60 min	90 min	120 min
$\%TNa^{+}$				
Control	80.86 ± 0.16	81.11 ± 0.26	80.66 ± 0.31	81.28 ± 0.26
Amiloride	79.66 ± 1.68	74.49 ± 2.79*	72.48 ± 2.72*	75.38 ± 1.45*
CFL	79.42 ± 1.04	79.15 ± 0.89	78.58 ± 1.28	78.88 ± 1.80
CFL + Amiloride	80.06 ± 1.11	80.18 ± 1.77	81.59 ± 1.60	78.17 ± 2.02
$\%TK^{+}$				
Control	69.13 ± 0.52	69.40 ± 0.60	71.84 ± 1.18	69.94 ± 0.88
Amiloride	69.57 ± 1.67	67.41 ± 2.94	65.20 ± 3.12	67.21 ± 1.75
CFL	70.52 ± 1.87	64.41 ± 3.89*	56.90 ± 5.49*	54.18 ± 7.63*
CFL + Amiloride	71.94 ± 1.52	72.62 ± 1.76	73.37 ± 1.55	69.48 ± 2.10
$\%TCl^{-}$				
Control	80.90 ± 0.34	82.25 ± 1.08	78.32 ± 0.90	79.53 ± 0.81
Amiloride	77.29 ± 2.69	73.03 ± 5.25	70.93 ± 4.78	72.26 ± 2.52
CFL	77.09 ± 1.75	76.17 ± 2.18	75.05 ± 2.78	75.78 ± 3.34
CFL + Amiloride	77.17 ± 2.58	75.41 ± 4.93	76.99 ± 4.44	73.00 ± 4.18

$\%TNa^{+}$: sodium tubular transport percentage; $\%TK^{+}$: potassium tubular transport percentage; $\%TCl^{-}$: chloride tubular transport percentage. CFL (10 µg/mL), amiloride (10^{-4} M) or CFL + amiloride. Mean ± S.E.M. (n=5) and compared by Student test. *p < 0.05 compared to control (Krebs-Henseleit).

$\%TK^+$ elicited by CFL could be a consequence of an indirect mechanism.

Renal effects elicited by different Diocleinae lectins are quite diverse. For instance, CFL and ConBr greatly increased RPP and RVR in the perfused rat kidney, but the increase caused by CFL was twice longer than that induced by ConBr (Teixeira *et al*., 2001. On the other hand, ConA and DguiL showed minor alterations in RPP and RVR (Havt *et al*., 2003). ConA increased these parameters at 60 min, whereas DguiL increased from 90 to 120 min. In addition, only ConBr, but not DguiL, ConA (Teixeira *et al.,* 2001, Havt *et al*., 2003) or CFL increased UF and GFR. With respect to tubular ion transport, ConBr reduced all transports (potassium, sodium and chloride) (Teixeira *et al*., 2001), while ConA only potassium (Havt *et al*., 2003). However, DguiL did not influence any of these parameters (Havt *et al*., 2003). Remarkably, CFL decreased potassium but not sodium or chloride transport.

Diocleinae lectins display high homology in their primary structures and share biochemical and structural features such as the conserved amino acid residues present in the carbohydrate binding sites (Tyr12, Asn14, Leu99, Tyr100, Asp208 and Arg228), metal binding sites (Glu8, Asp10, Tyr12, Asn14, Asp19, His24, Val32, Ser34, Asp208 and Arg228) and in hydrophobic cavities (Tyr54, Leu81, Leu85, Val89, Val91, Phe111, Ser113, Val179, Ile181, Phe191, Phe212 and Ile214) (Cavada *et al*. 2001). Despite this structural homology, the observed antinociceptive and anti-inflamatory responses of *Diocleinae lectins* were quite different: the contortions induced by acetic acid in mice were inhibited by 55% by DguiL and by 46% by CFL (Holanda *et al*., 2009); in the formalin test, ConBr inhibited the neurogenic phase by 26% (De Freitas *et al*., 2013), while DguiL did not express such behaviour (Holanda *et al.,* 2009). With respect to the anti-inflammatory activity, the rat peritonitis induced by carrageenan was inhibited by 63% by DguiL and by 62% by CFL (Assreuy *et al.,* 1997; Alencar *et al*., 1999; Figueiredo *et al*., 2009); the rat paw-edema induced by carrageenan was reduced by 17% by DguiL and by 30% by CFL, but not by ConBr (Assreuy *et al*., 1997).

The presence of specific amino acids in the structure of Diocleinae lectins (Del Sol *et al*., 2007) may explain such differences in pharmacological activities. The substitution of the amino acids Gln155 and Glu155, present in all Diocleinae by His155 solely on ConBr (Calvete *et al*., 1999) could also explain the better efficacy of ConBr, either in its antinociceptive property as well as in its renal hemodynamic changes compared to other lectins. Furthermore, the different activities and potencies observed between CFL and ConBr could be explained by the presence of specific amino acids in CFL, which are not present in ConBr due to the evolutionary processes in ancestors (Del Sol *et al*., 2007).

The knowledge of structural aspects and phylogenetic proximity among Diocleinae lectins is essential for the understanding of its relation structure-activity. Besides, this study opens avenues for the use of CFL as an important pharmacological tool to elucidate mechanisms implied in cardiovascular disorders.

CONCLUSION

The seed lectin of *Cratylia floribunda* alters renal hemodynamic parameters showing a kaliuretic effect, that could be of tubular origin, rather than a result from haemodynamic alterations.

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