

Uncaria tomentosa and acute ischemic kidney injury in rats

UNCÁRIA TOMENTOSA E A LESÃO RENAL AGUDA ISQUÊMICA EM RATOS

UNCÁRIA TOMENTOSA E A LESÃO RENAL AGUDA ISQUÊMICA EM RATOS

NMaria de Fátima Fernandes Vattimo¹, Natalia Oliveira da Silva²

ABSTRACT

The objective of this study was to evaluate the renoprotective effects of *Uncaria Tomentosa* (cat's claw) on ischemic acute kidney injury induced by renal clamping in rats. The hypoxia and hypoperfusion increase the production of reactive species already present in the inflammatory process. Results showed that the renal function evaluated by creatinine clearance, the urinary excretion of peroxides and malondialdehyde indexes demonstrated that UT induced renoprotection, probably related to its antioxidant activities.

KEY WORDS

Cat's claw.
Kidney.
Therapeutics.
Rats.

RESUMO

O objetivo deste estudo foi avaliar o efeito renoprotetor do fitoterápico *Uncaria tomentosa* sobre a lesão renal aguda isquêmica induzida pelo clampeamento dos pedículos renais de ratos. A hipóxia e a hipoperfusão geradas com a isquemia intensificam a produção de espécies reativas já presentes no processo inflamatório. Os resultados mostraram que a função renal avaliada pelo clearance de creatinina, a excreção de peróxidos urinários (FOX) e a excreção urinária de malondialdeído (TBARS) desses animais apresentou renoproteção induzida pela UT, provavelmente relacionada às suas atividades antioxidantes.

DESCRIPTORIOS

Unha-de-gato.
Rim.
Terapêutica.
Ratos.

RESUMEN

El objetivo de este estudio fue evaluar el efecto rinoprotector del fitoterápico *Uncaria tormentosa* sobre la lesión renal aguda isquémica inducida por el clampeo de los pedículos renales de ratones. La hipoxia y la hipoperfusión generadas con la isquemia intensifican la producción de especies reactivas ya presentes en el proceso inflamatorio. Los resultados mostraron que la función renal evaluada por el clearance de creatinina, la excreción de peróxidos urinarios (FOX) y la excreción urinaria de malondialdehído (TBARS) de esos animales demostró rinoprotección inducida por la UT, probablemente relacionada con sus propiedades antioxidantes.

DESCRIPTORIOS

Uña de gato.
Riñón.
Terapéutica.
Ratas.

¹PhD. Professor at School of Nursing, University of São Paulo. São Paulo, SP, Brazil. nephron@usp.br ²Undergraduate at School of Nursing, University of São Paulo. Holder of a FAPESP Scientific Initiation grant. São Paulo, SP, Brazil. nati_sioli@hotmail.com

INTRODUCTION

The kidney injury of ischemic origin corresponds, together with those related to the toxicity of drugs, to more than 70% of the categories of acute kidney injury, which persist with alarming mortality rates even when sophisticated techniques of function recovery are instituted⁽¹⁾. It is a condition in which the glomerular filtration rate is quickly reduced, causing sudden retention of endogenous metabolites⁽²⁾.

The acute kidney injury (AKI) takes place in a broad way in the population, with a sad prognosis, which may evolve to multiple failure of the organs and septicemia⁽³⁾. It is characterized by high mortality rates, around 50%, and in cases in which dialysis is needed, these rates may reach 88%⁽³⁾. It is a complication corresponding to approximately 5% of the hospitalizations and over 30% of ICU hospitalizations⁽⁴⁾. These significant data suggest that physiopathologic and clinical studies are fundamental for its effective control. Among these studies, notability is given to those aimed at the identification of agents that present a protective role in the AKI, especially experimental studies with animals like the one here presented.

In the ischemia, the reduction of the energetic source, the ATP, aggravates the production of reactive species of oxygen which, due to the excess, generates an unbalance between pro-oxidants and reservations of endogenous antioxidants⁽⁵⁻⁶⁾. The simple restoration of this flow is often not capable of preventing the maintenance stage of the AKI, in case there is tissue lesion, which is related to the time of exposure to the injury⁽⁷⁻⁸⁾.

In the ischemic AKI, the tubular lesion represents the final event, involving the cell death and/or dysfunction, which predisposes the cell to the tubular obstruction and consequent backscattering of the filtration, that is, the reflow of the ultrafiltration with reduction of the final urinary volume. This obstruction may lead to an increase of the intratubular pressures, also caused by the inflammatory process in which, despite of being a physiological process, release toxic metabolites and proteases through chemical mediators and favor the lesion of the tissue that has already been affected, causing vascular congestion, besides intensifying the redox unbalance. The failure and detachment of tubular epithelial cells favor the backscattering of the glomerular filtration to the peritubular capillaries. The obstruction and backscattering events contribute to the reduction of the glomerular filtration rhythm, besides causing edema of the tubular cells. The association of these lesions, in the kidney medulla region, supports the perpetuation of the kidney lesion, through positive feedback^(3,6).

Experimental studies in rats show the involvement of reactive oxygen species (ROS) in the ischemic lesion through the intensification of the redox unbalance in post-ischemia kidneys⁽⁹⁾.

The World Health Organization (WHO) recognizes the importance of the phytotherapy in the health care through a resolution (WHA 31.33, 1978), suggesting it is a viable alternative, particularly for populations of developing countries⁽¹⁰⁻¹¹⁾. In Brazil, parallelly, the phytotherapy is institutionalized through interministerial recommendation and resolution of the Health Ministry conditioning the use of medicinal plants in the Unique Health System to studies of characterization of active principles, action mechanisms of the drugs, among other scientific investigations⁽¹¹⁾.

Medicinal plants have been used for centuries in the Peruvian forest regions. Researchers have studied properties of plants and vegetal species of the region for years, making discoveries about the several ways of using this biodiversity of the flora, being the medicinal use the most common and important. In Lima, there are countless informal producers of phytotherapeutic products, as well as several pharmaceutical industries and laboratories obtaining extracts of medicinal plants through sophisticated methods. One of the applications of these plants is, also, as a type of dietary supplement, mainly for those with antioxidant properties, such as the cat's claw, or those with recovering properties⁽¹²⁾.

The use of
phytotherapeutic
products[...]may
bring relevant benefits
for the control of the
acute kidney injury
in its several
manifestations.

The *Uncaria Tomentosa* (UT or cat's claw) has been used for centuries by Indian tribes from the Amazon rainforest who inherited the knowledge of its therapeutic use from the old Incan people. It is a spiny climbing plant that can reach a total height of 30m, using its thorns (which resemble the claws of a cat, justifying its popular denomination) to hold and climb higher trees⁽¹³⁻¹⁴⁾. Among the characteristics attributed to it, it presents im-

mune-stimulating and antioxidant properties, probably related to the high concentration of flavonoids that act against reactive oxygen species, decreasing the oxidative stress in the inflammatory process⁽¹⁵⁾. Studies show that new quinovic acids and other effects of the plant may be related to treatments implemented against cancer, arthritis, gastritis and dermatological diseases⁽¹⁶⁾. Isolated extracts of its components, such as pentacyclic oxindole alkaloids, may be related to the stimulus of in vitro endothelial cells in the production of lymphocyte-proliferation-regulating factors⁽¹⁷⁾.

The clinical repercussion and the impact of the acute kidney injury in the public health have revealed to be resistant to traditional treatment strategies, like the methods of function substitution. The preventive approach of this disorder is in good time and may certainly result in more favorable advances. The use of phytotherapeutic products in this scenario, similarly to what has already happened in cardiovascular diseases with the use of wine due to the antioxidant properties derived from flavonoids⁽¹⁸⁾, may bring relevant benefits for the control of the acute kidney injury in its several manifestations.

Therefore, the main objective of this study was to evaluate the antioxidant and anti-inflammatory effects of the *Uncaria tomentosa* (UT) as a renoprotection strategy in animals preconditioned with this treatment and submitted to ischemic AKI, however, it did not stick to aspects like its toxicity and side effects as those were not the study focus. The phytotherapy analysis in the protection of the kidney function after ischemic dysfunction brings significant data for the real benefits of the clinical use of this agent. The scientific foundation for the clinical application of the UT, as well as for the advance of clinical studies about the respective thematic, may add unusual values in the prevention of the ischemic AKI, and may bring economical benefits for the health system/society through the discovery of a less complex and lower cost strategy when compared to hard technologies.

OBJECTIVE

The objective of this study was to evaluate the renoprotective effect of the *Uncaria tomentosa* on the renal function, the urinary excretion of peroxides and malonaldehyde in rats with ischemic AKI experimentally induced.

METHOD

This is an experimental quantitative study with animal model, approved by the Committee of Ethics in Animal Experiments of the Biological Sciences Institute of the University of São Paulo under the protocol no. 88/O7/CEEA and developed in the Laboratory of Experimental Research with Animal Models (LERAM) of the Nursing School of the University of São Paulo.

Dry extract of cat's claw 100mg (Herbarium Ltd) was used in a total number of approximately 35 Wistar adult male rats, distributed in the following groups:

Sham (control): rats submitted to laparotomy, without renal clamping.

Ischemia: rats submitted to renal ischemia of 45 minutes, through renal clamping.

Sham + *Uncaria tomentosa* (UT): rats treated with UT prior to the simulation of ischemia (20mg, VO, 1x day, 5 days).

Ischemia + *Uncaria tomentosa* (UT): rats treated with UT prior to the surgery day (20mg, VO, 1x day, 5 days).

The measurement of the urinary and plasma creatinine for the verification of the creatinine clearance, which was the renal function biomarker employed in this study, was developed through the Jaffe method⁽⁵⁾.

The xylenol orange method version 2 (FOX - 2) was used to measure the excretion of urinary peroxide⁽¹⁹⁾. The urinary excretion of malonaldehyde (MDA) was measured through the TBARs method⁽²¹⁾. Both, FOX-2 and MDA, were

used as oxidative parameters for the evaluation of the redox mechanism.

RESULTS

The analysis of the results was performed through the analysis of variance (ANOVA) followed by the test of Tuckey for multiple comparisons among the groups. The results are presented in tables containing the mean and standard deviation. The level of significance adopted was 0.05.

Renal Function

As described in Table 1, there were no significant variances of weight observed among the groups.

Table 1 - Results of body weight and global renal function of the groups: Sham, Ischemia, Sham +*Uncaria tomentosa*; Ischemia+*Uncaria tomentosa* - São Paulo - 2009

Groups(n)	Weight(g)	U (ml/min)	Clcr/100gr (ml/min)
Sham(7)	293±22	0.0062±0.004	0.54±0.09
Ischemia(7)	299±32	0.0176±0.004*	0.17±0.05*
Sham+UT (7)	278±11	0.0134±0.008*	0.54±0.08
Ischemia + UT(7)	287±11	0.0277±0.010**	0.54±0.07**

Notes: W = body weight, U = urinary flow and Clcr/100g = clearance of creatinine/100g of body weight. *p<0.05 versus Sham and ** versus Ischemia

Results show that the surgical procedure of the group SHAM (without renal clamping) did not determine any disadvantage of the renal function of these animals when compared to the control group of previous studies⁽⁵⁻⁶⁾, which confirms that the group SHAM may be used as control for this study.

Variations of urinary flow were observed in the animals from the groups that were submitted to the model of ischemia through clamping.

The ischemic animals presented reduction of the renal function when compared to those in the group Sham (0.17±0.05 vs. 0.54±0.09), confirming the episode of ischemic AKI with maintenance of the urinary flow.

The group that received pre-treatment for five days with *Uncaria tomentosa* (Ischemia+UT) presented attenuation in the reduction of creatinine clearance (0.54±0.07 vs. 0.17±0.05) in comparison to the group that was not treated. This result confirms a significant improvement of the renal function with the pre-treatment.

Urinary Peroxide

In Table 2, the group Sham presented results corresponding to the normality standard for this parameter. The group Ischemia, when compared to the group Sham, presented increase in the excretion of urinary peroxides (11.0±1.5 vs. 3.0±0.7), according to the standard expected for the implemented model of oxidation.

Table 2 - Results regarding the values of urinary peroxides of the groups: Sham, Ischemia, Sham+Uncaria tomentosa; Ischemia+Uncaria tomentosa - São Paulo - 2009

Groups	N	Urinary peroxides (nmol/g of creatinine)
Sham	7	3.0±0.7
Ischemia	7	11.0±1.5*
Sham+UT	5	3.8±1.3
Ischemia+UT	6	3.6±1.0**

*p< 0.05 versus Sham and ** versus Ischemia

In the comparison between the groups Ischemia+UT and Ischemia it was possible to observe a statistically significant decrease in the excretion of urinary peroxides in the group submitted to the pre-treatment (3.6 1.0 vs. 11.0±1.5).

Urinary TBARs (MDA)

Table 3 presents the values of urinary TBARs. The results of the group Sham corresponded to the standard described for urinary peroxides. The group Ischemia presented a statistically significant increase when compared to the control group (281 90 vs. 98±26).

Table 3 - Results regarding the values of urinary TBARs of the groups: Sham, Ischemia, Sham+Uncaria tomentosa; Ischemia+Uncaria tomentosa - São Paulo - 2009

Groups	N	Urinary TBARs (nmol/g of creatinine)
Sham	6	98±26
Ischemia	5	281±90 *
Sham+UT	7	108±35**
Ischemia+UT	5	151±33**

*p< 0.05 versus Sham and ** versus Ischemia

The values of lipid peroxidation, or redox mechanism, were reduced in the group treated with UT in comparison to the group Ischemia (151 33 vs. 281±90), remaining higher than in the control group, without statistical significance though.

DISCUSSION

Kidney injury is a clinical condition that affects several serious patients, mainly those in septicemia, as it currently consists in its main cause. The mortality due to AKI is high, at least 50%, and it stands out in cases in which it coexists with other morbidities, a scenario that is not rare in intensive care patients. The progresses in the care to subjects that experience the AKI are clear, but limited to the area of therapies of function substitution through dialytic methods. The persistence of mortality in over twenty years of studies presupposes that the mentioned advances have not resulted in any impact in the lesion epidemiology, revalidating the need to adopt measures of functional protection. Over the last years, antioxidant agents have stood out

in this field and, more recently, the use of phytotherapeutic products has been proving itself as an interesting therapeutic possibility, particularly in countries where the natural flora is so abundant as in Brazil. This study developed the acute kidney injury model in rats aimed at verifying the renoprotective effect of the UT.

The physiopathology of the AKI is complex and multifactorial. In it, the tissue hypoxia the hypoperfusion result in the decrease of the ATP levels, in the degradation of AMP to the accumulation of hypoxanthine. Simultaneously, there is an increase in the levels of Ca²⁺ and the activation of dependent proteases, such as the calmodulin that converts the xanthine dehydrogenase into xanthine oxidase⁽³⁾. On the other hand, it starts to catalyze the transference of electrons to the NAD and oxidates the xanthine and the hypoxanthine into uric acid, generating oxygen reactive species during the reperfusion⁽⁶⁾.

The ischemia-reperfusion guides the activation and expression of adhesion molecules in endothelial cells and leucocytes. Leucocytes activate oxidants such as superoxide, nitric oxide, hydroxyl radical, peroxynitrous acid and hypochlorous acid that destroy the endothelial and epithelial cells⁽⁷⁾. These oxygen reactive species compromise the cellular vitality, the action of growth factors and the cellular proliferation, the regeneration and restoration of tissue, of the inflammatory and immune processes, besides the regulation of the hemodynamic, hemostatic and vascular systems⁽⁵⁻⁶⁾. The indicators of oxidative stress include altered DNA bases, products of protein oxidation and products of lipid peroxidation⁽⁶⁾.

AUT is composed by oxindole and indole alkaloids that present immune-stimulating properties, including quinovic glycoside acids that present anti-inflammatory action and antioxidant compounds that demonstrate cellular repairing action⁽¹⁶⁻¹⁷⁾. Its antioxidant action seems to be the consequence of the high concentration of flavonoids that abduct oxygen reactive species, decreasing the oxidative stress in the inflammatory process^(8,15).

The administration of antioxidant elements to these animals, probably, inhibits the action of the oxidase xanthine and/or abducts the free radicals, decreasing the oxidative damage⁽⁶⁾. Active principles of this phytotherapeutic, like flavonoids, may act in the removal of oxygen reactive species and in the reduction of the lipid peroxidation, as observed in the study here presented and confirmed by the parameters of urinary excretion of peroxides and malonaldehyde.

The experimental model of the acute kidney injury reproduced in this study aimed, through renal clamping for 45 minutes, at simulating this process of hypoperfusion and ischemia-reperfusion, which was confirmed. The pre-treatment of the groups with Uncaria tomentosa induced improvement in the renal function with an increase of the values of creatinine clearance in animals with ischemic AKI, besides the reduction of urinary peroxides verified through the decrease of the values of cell lesion markers (FOX-2, TBARs), an improvement that is probably related to the antioxidant activities of the phytotherapeutic.

Therefore, the results presented emphasize the protective effect of the plant *Uncaria tomentosa* in the renal function of rats submitted to the model of kidney ischemia by clamping, with association to the reduction of urinary peroxides and malonaldehyde in these animals. The combination of improvement in the renal functional with reduction of the lipid peroxidation rates presupposes that the functional damage established by the ischemia has oxidative origin, as it has been already indicated in other lesion models. These data also reinforce that the phytotherapy may be considered as a therapeutic possibility, with anti-inflammatory and antioxidant action, in situations of risk of imminent kidney injury as well.

REFERENCES

- Amend WJC, Vincenti FG. Oligúria; Insuficiência renal aguda. In: Smith DR, editor. *Urologia geral*. Rio de Janeiro: Guanabara Koogan; 1985. p. 433-6.
- Robbins SL. *Patologia estrutural e funcional*. Rio de Janeiro: Interamericana; 1975. Rim; p. 977-1030.
- Brady HR, Clarkson MR, Lieberthal W. Acute renal failure. In: Brenner BM. *Brenner and Rector's the kidney*. Philadelphia: Saunders; 2007. p. 1201-62
- Schwilk B, Wiedeck H, Stein B, Reinelt H, Treiber H, Brothner U. Epidemiology of acute renal failure and outcome of haemodiafiltration in intensive care. *Intensive Care Med*. 1997;23(12):1204-11.
- Oliveira VCR, Tejos CR, Hosaka EM, Andrade SC, Araújo M, Vattimo MFF. Interferência do intervalo de administração da droga sobre a nefrotoxicidade da gentamicina em ratos. *Rev Esc Enferm USP*. 2001;35(2):200-4.
- Watanabe M, Neiva LBM, Santos CXC, Laurindo FRM, Vattimo MFF. Isoflavone and the oxygenase system in ischemic acute kidney injury in rats. *Food Chem Toxicol*. 2007;45(12):2366-71.
- Quintaes PSL, Noronha IL. Papel dos neutrófilos e moléculas de adesão na fisiopatologia da insuficiência renal aguda isquêmica. *J Bras Nefrol* [Internet]. 1998 [citado 2007 jul. 6];20(1). Disponível em: http://www.jbn.org.br/audiencia_pdf.asp?aid2=758&nomeArquivo=20-01-17.pdf
- Seguro AC, Yu L, Burdmann E, Helou CMB, Zatz R. Insuficiência renal aguda. In: Zatz R, editor. *Fisiopatologia renal*. São Paulo: Atheneu; 2000. p. 261-82.
- Paller MS, Hoidal JR, Ferris TF. Oxygen free radicals in ischemic acute renal failure in the rat. *J Clin Invest*. 1984;74(4):1156-64.
- Rezende AH, Cocco MIM. A utilização da fitoterapia no cotidiano de uma população rural. *Rev Esc Enferm USP*. 2002;36(3):282-8.
- Miguel MD, Miguel OG. Desenvolvimento de fitoterápicos. Ribeirão Preto: Tecmedd; 2004. p. 18-9.
- Technology Food Chain. The processing and sustainable management of amazonian medicinal plants [Internet]. 2006 [cited 2010 Apr 7]. Available from: http://practicalaction.org/energy/docs/agroprocessing/food_chain_26.pdf
- Unha-de-Gato (*Uncaria tomentosa*): descrição e indicações [Internet]. [citado 2010 abr. 7]. Disponível em: <http://www.fitoterapicos.info/unha-de-gato.php>
- Carvalho JCT. *Fitoterápicos antiinflamatórios: aspectos químicos, farmacológicos e aplicações terapêuticas*. Ribeirão Preto: Tecmedd; 2004. p. 423-28.
- Williams JE. Review of antiviral and immunomodulating properties of plants of the Peruvian rainforest with a particular emphasis on uña de gato and sangre de grado. *Altern Med Rev*. 2001;6(6):567-79.
- Cerri R, Aquino R, De Simone F, Pizza C. New quinovic acid glycosides from *uncaria tomentosa*. *J Nat Prod*. 1988;51(2): 257-61.
- Keplinger K, Laus G, Wurm M, Dierich M, Teppner H. *Uncaria tomentosa*: ethnomedical use and new pharmacological, toxicological and botanical results. *J Ethn*. 1998;64(1):23-4.
- Bezerra JS, Teixeira W, Vattimo MFF. Efeito protetor da vitis vinifera na lesão renal aguda isquêmica em ratos. *J Bras Nefrol*. 2008;30(2):99-104.
- Wolff SP. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods Enzymol*. 1994;233(1):182-9.
- Halliwel B, Long LH, Yee TP, Lim S, Kelly R. Establishing biomarkers of oxidative stress: the measurement of hydrogen peroxide in human urine. *Curr Med Chem*. 2004;11(9):1085-92.
- Lima ES, Abdalla DSP. Peroxidação lipídica: mecanismo de avaliação em amostras biológicas. *Braz J Pharm Sci*. 2001;37(3):293-303.

CONCLUSION

The generation of urinary peroxides confirms the oxidative lesion in the model of ischemic acute kidney injury instituted in this study.

The pre-treatment with *Uncaria tomentosa* promoted functional protection evaluated through the increase of the creatinine clearance, reduction of the peroxidation and urinary TBARs, which may be related to the antioxidant activities of the phytotherapeutic agent.