L-ARGININE SUPPLEMENTATION REDUCES FIBROSIS IN MOUSE MODEL OF CHRONIC ARISTOLOCHIC ACID-INDUCED NEPHROPATHY

Inès Jadot [1], Vanessa Colombaro [1], Blanche Martin [1], Isabelle Habsch [1], Olivia Botton [1], Joëlle Nortier [2], Anne-Émilie Declèves [3], Nathalie Caron [1]

[1]Molecular Physiology Research Unit - URPhyM - University of Namur (UNamur), 61, rue de Bruxelles, B-5000 Namur, Belgium.
[2]Laboratory of Experimental Nephrology, Faculty of Medicine, Université Libre de Bruxelles (ULB), B-1070 Brussels, Belgium.
[3]Laboratory of Molecular Biology, Faculty of Medicine and Pharmacy, Research Institute for Health Sciences and Technology, University of Mons (UMons), B-7000 Mons, Belgium.
Corresponding autor: Inès Jadot, PhD Student, University of Namur, ines.jadot@unamur.be

BACKGROUND

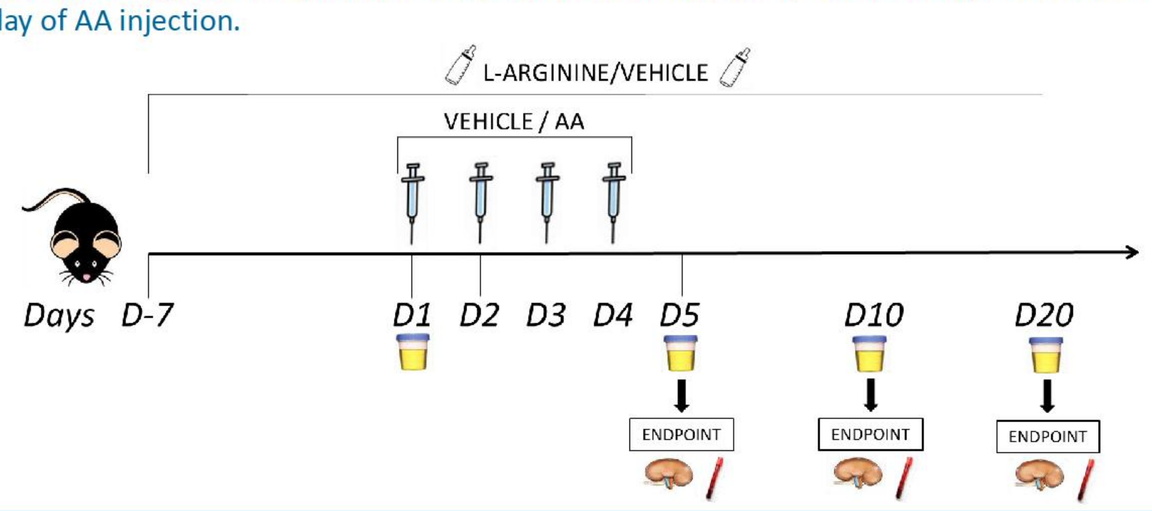
Aristolochic acid nephropathy (AAN), a progressive tubulointerstitial injury of toxic origin, was originally described in early 1990's in Belgian women after ingestion of root extracts of *Aristolochia fangchi* for slimming purposes. AAN is characterized by a biphasic evolution with an early and transient phase of acute tubular necrosis, followed by a chronic phase of fibrosis and tubular atrophy leading to end stage renal disease. Nowadays, AAN is considered as a worldwide health problem with a highly underestimated incidence. Indeed, aristolochic acids (AA) are still widely used in traditional medicines in Asian countries and they have also been identified as the causative agent of Balkan-endemic nephropathy.

A **reduced nitric oxide (NO) production** in AAN has been demonstrated, which might lead to renal dysfunction. Indeed, we have previously demonstrated that **L-Arginine (L-ARG)** supplementation restored NO bioavailability in acute AAN mouse model thereby improving the outcome of AA-induced acute kidney injury (AKI) in association with a morpho-functional protection.

Since the severity of the AKI phase is a strong predictor of chronic kidney disease, we aimed to evaluate the impact of L-ARG supplementation on the AKI-to-CKD transition. Indeed, decreased NO might act as a key factor on both loss of renal function and fibrosis development.

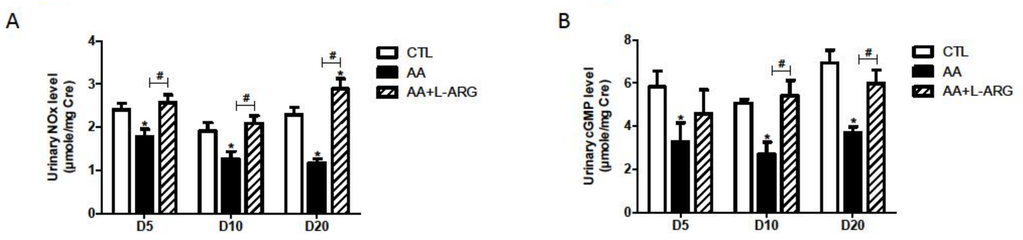
METHODS

C57BL/6J male mice (n=72) were subjected to daily i.p. injection of AA (3,5 mg/kg) for 4 days. L-ARG was supplemented in drinking water (5%) for 7 days before AA treatment, as well as throughout the protocol. Mice were euthanized at 5, 10 or 20 days after the first day of AA injection.



RESULTS

1 > L-Arginine increases NO bioavailability

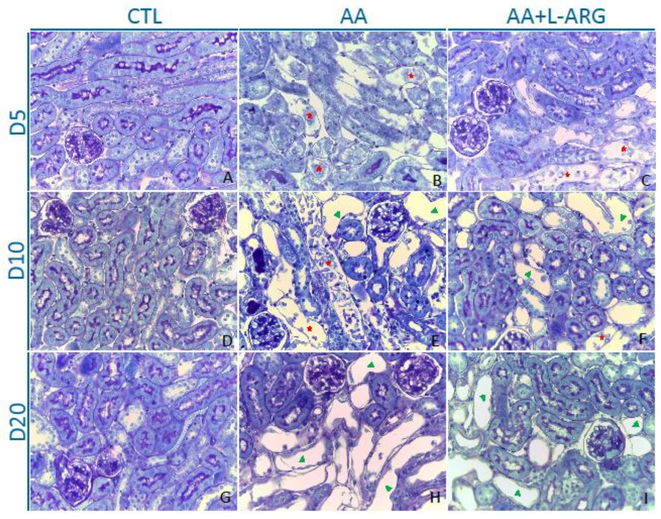


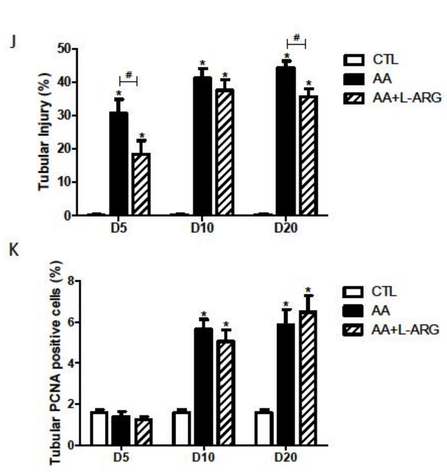
	eNOS	*iNOS*
Day 5		
CTL	1.00 ± 0.32	1.00 ± 0.20
AA	1.52 ± 0.45	1.29 ± 0.13
AA + L-ARG	1.73 ± 0.39	1.34 ± 0.24
Day 10		
CTL	1.17 ± 0.34	1.03 ± 0.26
AA	1.11 ± 0.33	1.65 ± 0.34
AA + L-ARG	1.14 ± 0.28	1.56 ± 0.34
Day 20		
CTL	1.02 ± 0.41	0.94 ± 0.25
AA	1.09 ± 0.26	2.42 ± 0.26 *
AA + L-ARG	1.07 ± 0.25	2.54 ± 0.61 *

Quantitative urinary nitrite/nitrate (NOx) level **(A)** and urinary cGMP level **(B)** in CTL, AA and AA+L-ARG mice, 5, 10 and 20 days after first AA injection. Relative kidney expression of endothelial nitric oxide synthase (*eNOS*) and inducible nitric oxide synthase (*iNOS*) mRNA ($2^{-\Delta\Delta CT}$) in CTL, AA and AA+L-ARG mice 5, 10 and 20 days after first AA injection **(Table)**. Values are means ± SEM. N=8 in each group. *p< 0,05 *vs* CTL; #p< 0,05 *vs* AA. Statistical analysis were performed by two-way ANOVA followed by Newman-Keuls test.

2 > L-Arginine improves renal function and reduces tubular injury

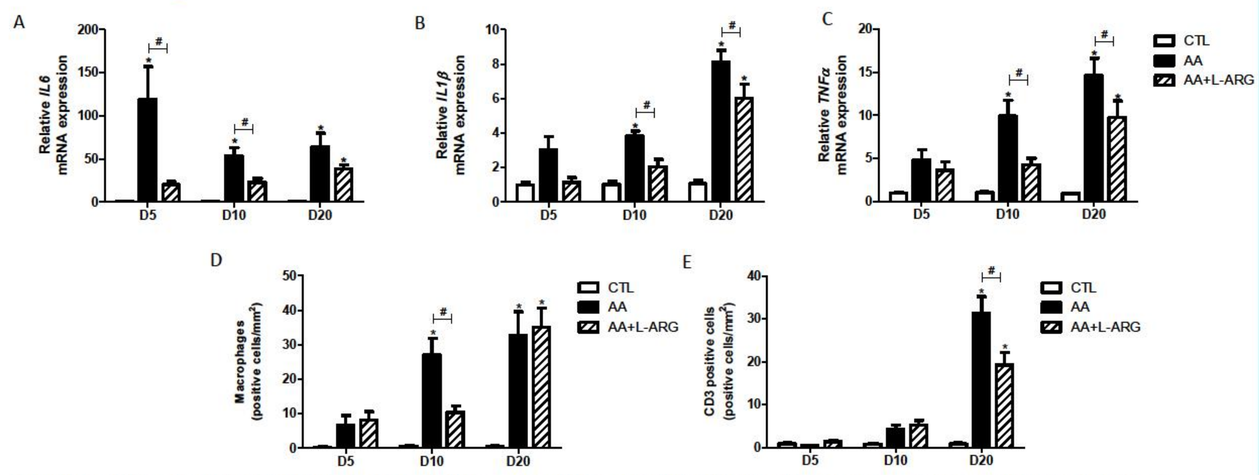
	Diuresis – ml/24h	Osmolarity – mOsm/kg	Creatinine clearance – ml/min	Plasma creatinine level – µmol/l	Blood urea nitrogen – µmol/l	Proteinuria – mg/mg Cre
Day 5						
CTL	0.43 ± 0.10	5034 ± 95	1.29 ± 0.10	3.51 ± 0.27	11.31 ± 1.15	5.31 ± 0.18
AA	0.45 ± 0.21	2597 ± 245 *	0.15 ± 0.02 *	12.01 ± 2.28	9.16 ± 1.44	16.09 ± 3.71 *
AA + L-ARG	0.45 ± 0.12	6400 ± 723 #	1.12 ± 0.35 #	5.95 ± 1.31	7.55 ± 1.90	10.41 ± 2.51
Day 10						
CTL	0.59 ± 0.13	4329 ± 433	1.02 ± 0.16	4.02 ± 0.63	11.07 ± 1.46	5.45 ± 1.02
AA	1.35 ± 0.17 *	2009 ± 219 *	0.29 ± 0.09 *	27.29 ± 5.25 *	183.10 ± 41.72 *	14.28 ± 2.67 *
AA + L-ARG	0.67 ± 0.13 #	5202 ± 899 #	1.41 ± 0.43 #	3.72 ± 1.01 #	100.80 ± 30.81 *#	12.08 ± 1.34 *
Day 20						
CTL	0.79 ± 0.20	4491 ± 418	1.11 ± 0.19	3.91 ± 0.63	11.65 ± 1.42	6.92 ± 0.37
AA	2.02 ± 0.28 *	2096 ± 325 *	0.57 ± 0.11	17.06 ± 3.11 *	100.80 ± 18.60	11.01 ± 0.96
AA + L-ARG	1.43 ± 0.19 *#	3626 ± 363 #	0.47 ± 0.04	12.52 ± 1.56	111.30 ± 17.10	11.87 ± 0.51





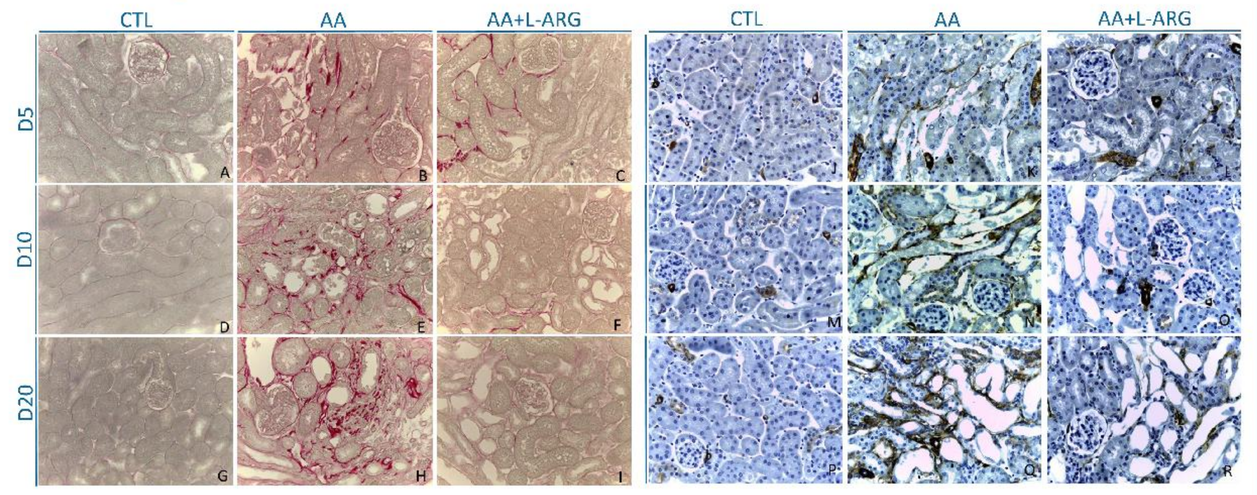
Effects of L-Arginine supplementation on renal function in CTL, AA and AA+L-ARG mice, 5, 10 and 20 days after first AA injection **(Table)**. Effects of L-Arginine supplementation on tubular injury in CTL, AA and AA+L-ARG-treated mice. Representative hemalun, Luxol fast blue and Periodic Acid Schiff stained kidney sections (x400) from CTL **(A,D,G)**, AA **(B,E,H)** and AA+L-ARG **(C,F,I)** mice, 5, 10 and 20 days after first AA injection. Necrotic tubules **(*)** with cell debris in tubular lumens are visible in AA and AA+L-ARG treated mice, 5 and 10 days after first AA injection and cystic tubules **(▲)** are visible in AA and AA+L-ARG treated mice, 10 and 20 days after first AA injection. Quantitative analysis of tubular injury in CTL, AA and AA+L-ARG mice, 5, 10 and 20 days after first AA injection **(J)**. Percentage of PCNA positive cells in tubules of CTL, AA and AA+L-ARG mice, 5, 10 and 20 days after first AA injection **(K)**. Values are means ± SEM. N=8 in each group. *p< 0,05 *vs* CTL; #p< 0,05 *vs* AA. Statistical analysis were performed by two-way ANOVA followed by Newman-Keuls test.

3 > L-Arginine reduces AA-induced inflammation

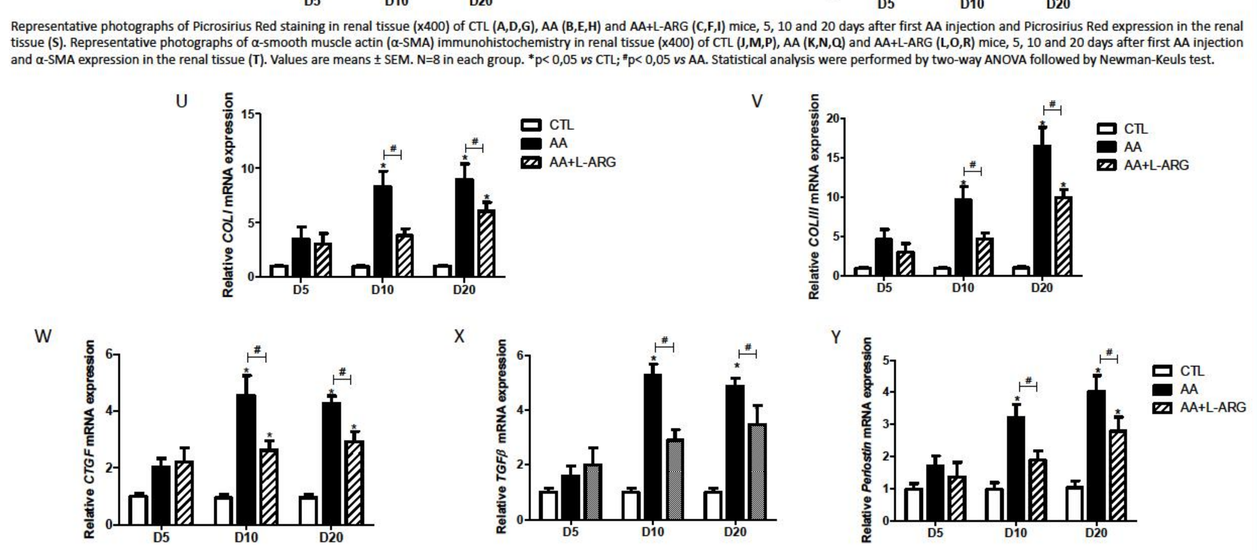


Relative kidney expression of interleukin 6 (*IL6*; **A**), interleukin 1β (*IL1β*; **B**), tumor necrosis factor α (*TNFα*; **C**) mRNA ($2^{-\Delta\Delta CT}$) in CTL, AA and AA+L-ARG mice, 5, 10 and 20 days after first AA injection. Macrophages **(D)** and lymphocytes T **(E)** infiltration within the interstitium of the kidney of CTL, AA and AA+L-ARG mice, 5, 10 and 20 days after first AA injection. Values are means ± SEM. N=8 in each group. *p< 0,05 *vs* CTL; #p< 0,05 *vs* AA. Statistical analysis were performed by two-way ANOVA followed by Newman-Keuls test.

4 > L-Arginine reduces renal fibrosis



Representative photographs of Picrosirius Red staining in renal tissue (x400) of CTL **(A,D,G)**, AA **(B,E,H)** and AA+L-ARG **(C,F,I)** mice, 5, 10 and 20 days after first AA injection and Picrosirius Red expression in the renal tissue **(S)**. Representative photographs of α-smooth muscle actin (α-SMA) immunohistochemistry in renal tissue (x400) of CTL **(J,M,P)**, AA **(K,N,Q)** and AA+L-ARG **(L,O,R)** mice, 5, 10 and 20 days after first AA injection and α-SMA expression in the renal tissue **(T)**. Values are means ± SEM. N=8 in each group. *p< 0,05 *vs* CTL; #p< 0,05 *vs* AA. Statistical analysis were performed by two-way ANOVA followed by Newman-Keuls test.



Relative kidney expression of collagen 1 (*COLI*, **U**) and collagen 3 (*COLIII*, **V**), connective tissue growth factor (*CTGF*, **W**), transforming growth factor β (*TGFβ*, **X**) and *Periostin* **(Y)** mRNA ($2^{-\Delta\Delta CT}$) in CTL, AA and AA+L-ARG mice, 5, 10 and 20 days after first AA injection. Values are means ± SEM. N=8 in each group. *p< 0,05 *vs* CTL; #p< 0,05 *vs* AA. Statistical analysis were performed by two-way ANOVA followed by Newman-Keuls test.

SUMMARY / CONCLUSION

- ✓ L-Arg supplementation restores renal NO bioavailability in AAN.
- ✓ L-Arg supplementation improves renal function, decreases tubular injury and inflammation and prevents progression of fibrosis in AAN.
- ✓ Our results demonstrate a key role of NO in the AKI-to-CKD transition in AAN.

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