“Effect of renal ischemia on sub-chronically exposed rats to fluoride evaluated by the expression of hypoxia-inducible factor 1α (HIF-1α)”

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INTRODUCTION

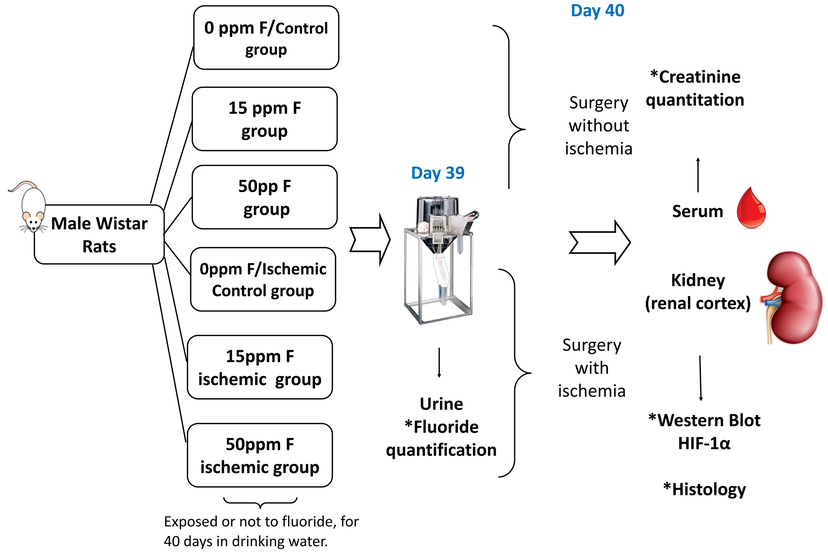
A new approach to study the causes of kidney diseases includes environmental xenobiotics. Fluoride (F) is known to cause deleterious effects in the kidney, however the precise mechanism is not fully understood. F can inhibit cellular respiration affecting intracellular oxygen. Low oxygen tensions alter the cell homeostasis and activates the hypoxia-inducible factor 1 (HIF-1). HIF-1 is an heterodimeric protein, constituted by HIF-1α and HIF-1β. Under hypoxic conditions, HIF-1α stabilizes, translocate to the nucleus and dimerizes with HIF-1β to induce the expression of genes involved in cellular adaptation and survival. Previous studies from our group showed a possible preconditioning effect by the exposure to F and a subsequent nephrotoxic stimulus (gentamicin), to rule out a possible interaction of pharmacokinetics, we used another way of inducing renal damage: ischemia (which stabilizes HIF-1a).

OBJETIVE

The aim of this study was to evaluate if previous exposure to F could affect the expression of HIF-1α induced by a subsequent renal ischemia.

METHODOLOGY

The care and experimental procedures were conducted after approval of the study by the Institutional (CINVESTAV-IPN) Animal Care and Use Committee (CICUAL) in accordance with their Guidelines for the are and Use of Laboratory Animals.



RESULTS

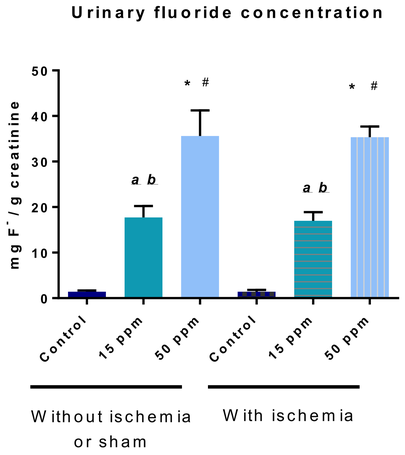


Fig.1 Urinary concentration of Fluoride. The data are presented as the mean ± standard error of the mean (SEM),*n* = 9. An [a] indicates *P*<0.05 vs sham control, [b] indicates *P*<0.05 vs ischemic control, * denotes P <0.001 vs sham control group and # denotes P <0.001 vs ischemic control group. (Kruskal-Wallis multiple comparison test + Dunn).

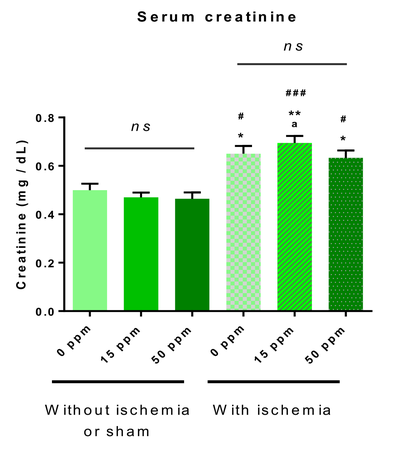


Fig.2 Serum creatinine (Scre). The data are represented as the mean ± SEM, *n* = 9. An [a] *P*=0.05 compared to 0 ppm sham group , an * *P*=0.05 or ** *P*=0.01 vs 15 ppm sham group, and a # *P*=0.05, ### *P*=0.001 vs 50 ppm sham group, ns = no statistically significant difference (Kruskal-Wallis multiple comparison test + Dunn).

Protein expression of HIF-1a

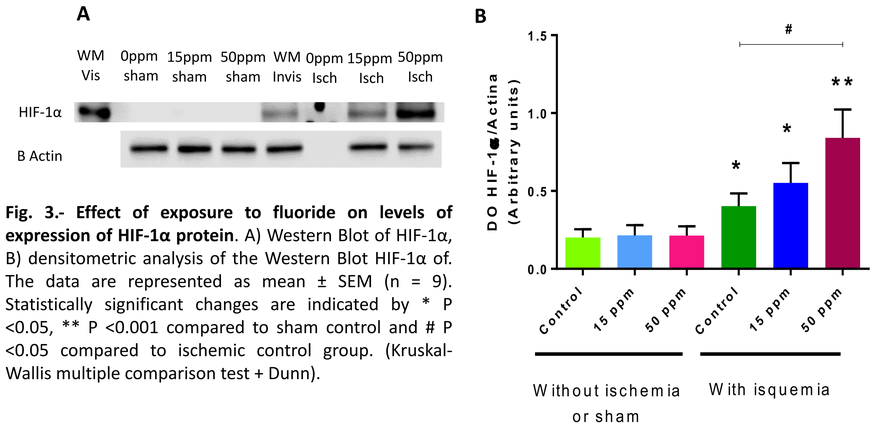


Fig. 3.- Effect of exposure to fluoride on levels of expression of HIF-1α protein. A) Western Blot of HIF-1α, B) densitometric analysis of the Western Blot HIF-1α of. The data are represented as mean ± SEM (n = 9). Statistically significant changes are indicated by * P <0.05, ** P <0.001 compared to sham control and # P <0.05 compared to ischemic control group. (Kruskal-Wallis multiple comparison test + Dunn).

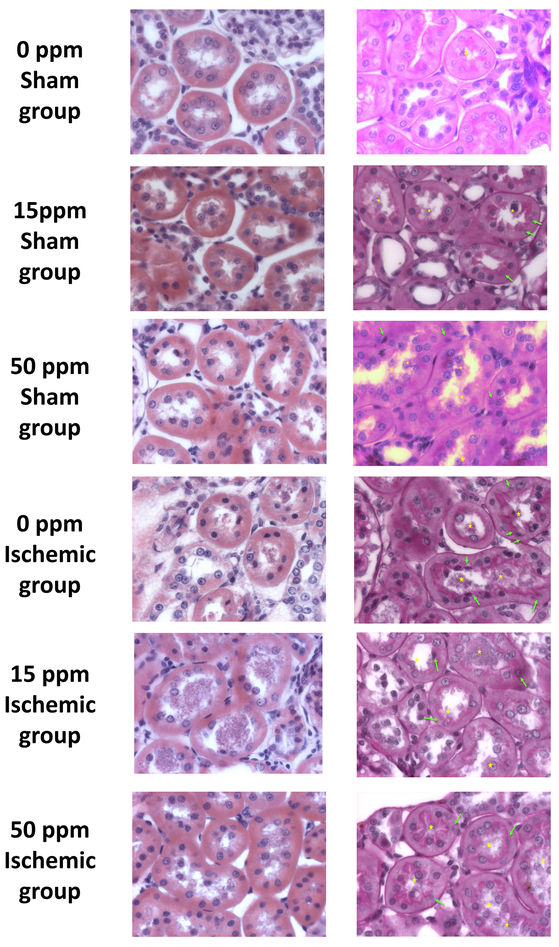
Histological analysis

C

	0 ppm F		15ppm F		50ppm F	
	A	N	A	N	A	N
Sham	12.28	13.52	31.5	18.55	28.48	15.97
Ischemia	34.8*	24.5*	26.3	31.19****	41.75***	26.03**

Fig.4 Histological analysis. C) Percentage of apoptosis (A) or necrosis (N) at the proximal tubule. D) Histology and renal cortex staining (H & E, PAS, AO) Apoptosis is indicated by a green arrow, yellow star indicates necrosis. Statistically significant changes are indicated by * *P*<0.05, ** *P*<0.05, *** *P*<0.001 compared to 0 ppm sham group (Kruskal-Wallis multiple comparison test + Dunn).

D



CONCLUSIONS

*Urinary excretion of fluoride depends on the concentration of exposure.
*The increase in serum creatinine is due to ischemia, not to exposure to fluoride.
*In normoxia, fluoride does not affect the expression of HIF-1α protein, however under hypoxia, groups exposed to fluoride increases its expression in a concentration dependent manner.
*Fluoride causes tubular damage evidenced by increased apoptosis and necrosis in proximal tubules.
*The decrease in apoptosis of ischemic group exposed to 15 ppm fluoride relative to 15 ppm sham group could be due to the expression of HIF-1 protein.

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