In vivo comparison between two nephrotoxic agents, sodium fluoride and uranyl nitrate: phenotypic aspects and molecular mechanisms involved

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1 Background

Humans can be exposed at low concentrations to toxic agents with anthropogenic and natural origins such as uranium and fluoride. Because of its functions of filtration, transport and reabsorption the kidney is a primary target organ of toxicity to foreign compounds. Uranium and fluoride are both known to be nephrotoxic, nevertheless there is a lack of knowledge of their mechanisms of nephrotoxicity and of the underlying molecular pathways involved [1, 2]. This study aims to compare these agents to identify the cellular and molecular pathways of nephrotoxicity in mouse.

2 Methods and results

C57Bl6 mice are exposed to uranyl nitrate (UN) (0, 2, 4, 5 mg/kg) or sodium fluoride (NaF) (0, 2, 5, 7.5, 10 mg/kg) by intraperitoneal injection and put into metabolism cages for 24 hours before and directly after injection. Collected urines are used to quantify uranium or fluoride, to measure specific and sensitive biomarkers of nephrotoxicity levels and to assess clinical bioassays. Finally, animals are euthanized 48h and 72h after exposure, chosen as the peak of nephrotoxicity is observed after intraperitoneal injection [3]. The protocol is shown in figure 1.

Renal phenotypic aspects and biological mechanisms are evaluated by urinary biochemistry, gene and protein expressions, enzyme activity, and histological analyses. Exposure to UN and NaF induces nephrotoxicity in a dose-dependent manner. A 5 mg/kg injection of UN induces mild histopathological alterations and respectively 44 and 6-fold increase in gene expressions of nephrotoxicity markers KIM1 and osteopontin. In comparison, 10 mg/kg of NaF induces high nephrotoxicity with histopathological alterations scored as severe and late appearing parameters of toxicity whereas 7.5 mg/kg

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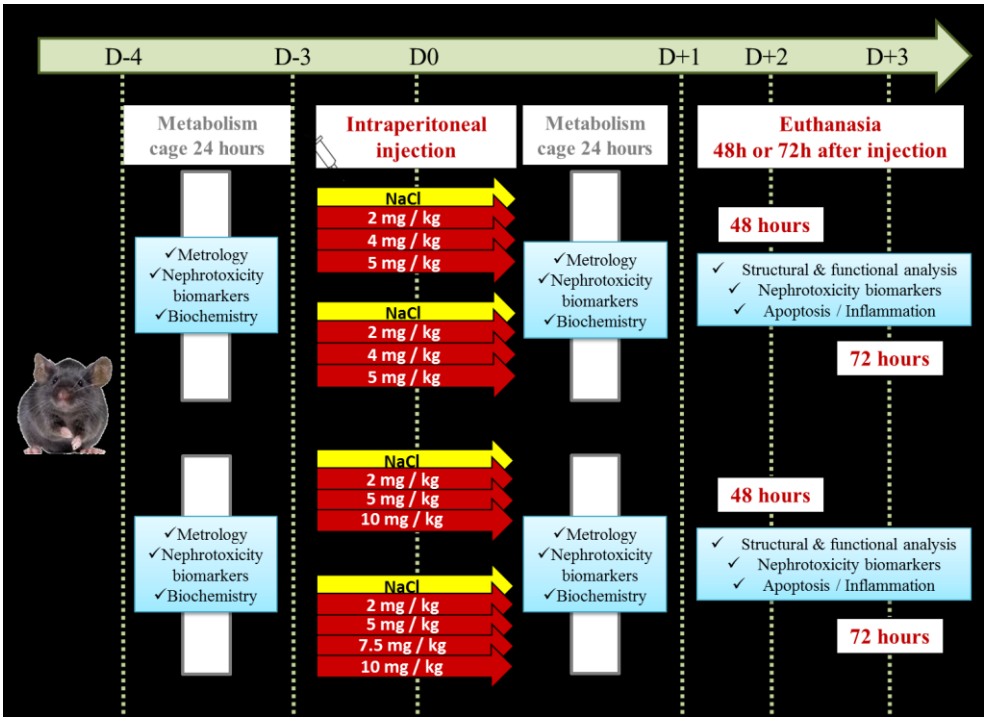

Fig. 1. Protocol of internal exposure of uranium and fluoride in mice C57Bl6. Scale of time is given at the top of the figure (in days). Day 4 before injection mice are put 24 hours in metabolism cages to collect urines, at day 0 mice are injected intraperitoneally with either uranium or fluoride and directly put in metabolism cages again. Day 2 or day 3 after injection mice are euthanized to extract kidneys.

induces mild histopathological scoring and gene expressions of KIM1 and clusterin enhanced respectively by 70 and 4-fold compared to control. No signs of nephrotoxicity are observed below 5 mg/kg of NaF. Clusterin is respectively increased by 2.4 and 4.4-fold in urines after 7.5 and 10 mg/kg injection of NaF, whereas 4 and 5 mg/kg of UN induce respectively a 2.2 and 2.7-fold increase of clusterin in urines. Apoptosis is evaluated through caspases 3/7 activity which is increased by 210% after UN treatment (5mg/kg) whereas NaF does not induce apoptosis significantly. Inflammation is implied in UN and NaF acute nephrotoxicity as shown by gene and *in situ* overexpressions of ICAM and VCAM measurement by immunohistochemical staining.

3 Conclusions

UN and NaF acute exposures resulted in dose and time-dependent nephrotoxicity with a higher nephrotoxicity after 72h. Inflammation and apoptosis are both involved in UN or NaF toxicity. These observations allow us to identify the mechanisms that will be studied in a low-dose exposure protocol after a chronic exposure.

References

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