Reviewer's report

Title: Time course study of oxidative and nitrosative stress and antioxidant enzymes in K2Cr2O7-induced nephrotoxicity

Version: 2 Date: 31 December 2004

Reviewer: KAZIM HUSAIN

Reviewer's report:

General
This manuscript describes the time response of a single subcutaneous injection of potassium dichromate-induced nephrotoxicity and its relationship with renal antioxidant enzymes and oxidative/nitrosative stress in rats from day 1 till day 12. The chromium concentration in the serum and kidney but not in the urine was also measured. Nephrotoxicity (renal histology and physiological parameters), oxidative/nitrosative stress (protein carbonyls, 3-NT and NOx) and antioxidant enzymes were analyzed. The results show the accumulation of chromium in the kidney, which was persisted till last day of the study. Nephrotoxicity parameters such as decrease in creatinine clearance, increase in serum creatinine levels and urinary excretion of NAG were observed 1-4 days after potassium dichromate treatment. Other nephrotoxicity parameters such as BUN, urinary proteins and structural damage to the proximal tubules were noticed 1-6 days after potassium dichromate treatment. Renal oxidative/nitrosative stress was evident on days 1-8 after treatment with potassium dichromate. Renal antioxidant enzyme activities and immunohistochemical localization of these enzymes were not consistent following the potassium dichromate treatment. The conclusion of the study was that there is an association between oxidative/nitrosative stress and not the antioxidant enzymes with functional and structural renal damage after potassium dichromate treatment in rats.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
The conclusions are long and should be concise and may be modified as ‘The data show that association between oxidative/nitrosative stress and not the antioxidant enzymes with functional and structural renal damage induced by K2Cr2O7 in rats at different time points.’

Background: The authors are suggested to move the first three sentences from “Results and Discussion” section on page 10 of the manuscript to the beginning of the Background on page 4. The last three sentences in Background on page 5 should be removed. There are earlier reports pertaining to the dose and time response of K2Cr2O7 -induced nephrotoxicity (biochemical and histological changes) in rats (Gumbleton and Nicholls, Food Chem. Toxicol. 26 (1): 37-44, 1988; Nagaha E.O. Gen. Pharmacol. 12(6): 497-500, 1981). Authors are suggested to include these reports in Background and Discussion of the manuscript.

Results and Discussion: This portion of the manuscript reveals more results and less discussion reviewer suggests more discussion in light of the present data.
First three sentences should be moved to Background.
Page 12: last para, first sentence, to be modified as “Further we investigated the time response of the renal antioxidant enzymes in K2Cr2O7 - treated rats.”
Page 13: Para 1, last sentence, Explain why these enzymes remained low in spite of abolished oxidative/nitrosative stress.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Abstract: Authors stated that “serum and kidney chromium content increased reaching the highest
value on day 1” but what was the value from 0-20 hrs after K2Cr2O7 administration?
“Activity of GR decreased on days 2-10” but in the background it is stated that “renal activity GR remained unchanged at 24 and 48 h”

Methods: Why the authors did not use monoclonal instead of polyclonal antibodies for specificity?
Did the authors find any cross reactivity using polyclonal antibodies?
The age of the animals and total number of rats used in each group is missing and exactly how many rats from each group were sacrificed at 8 different time points?
From which tissues/organs the blood was collected i.e. from heart, orbital plexus or jugular vein?
In “western blot” w should be capitalized.
How many animals in each group used for metal analysis, histology, immunohistochemistry, Western blot and antioxidant enzyme assays?
For chromium analysis whole kidney or corticomedullary region was used.
The spelling of “hematoxilin” needs to be checked.
For antioxidant enzyme activity assay how the tissue homogenate was prepared?
What was the rationale for only CAT protein analysis by Western blot?
The spelling of “polyacrilamide” needs to be checked on page 9.

Discretionary Revisions (which the author can choose to ignore)

Page 10: Para 1, last sentence “In both cases------on day 6.” The pharmacokinetics/pharmacodynamics of chromium needs to be discussed in light of the published reports.
Page 11: Para 1, last sentence, explanation is needed to improve the statement.

What next?: Accept after minor essential revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests