Reviewer's report

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

Version: 2 Date: 7 January 2005

Reviewer: Leonard Rybak

Reviewer's report:

General
This is an interesting study that investigates the time course of structural and functional damage to the kidney, oxidative and nitrosative stress, and the behavior of the antioxidant enzymes, Cu/Zn SOD, Mn-SOD, glutathione reductase and peroxidase, and catalase in rats treated with nephrotoxic doses of K2Cr2O7. Previous studies have suggested that reactive oxygen species are involved in chromium (VI)-induced cell injury, and that antioxidant compound protect against renal damage caused by this agent. The questions addressing the time course and biochemical changes in relation to renal structure and function appear appropriate and original.

There are some questions and concerns about the methods. Was the dose of K2Cr2O7 selected on the basis of previous studies indicating that this is an appropriate nephrotoxic dose of chromium to employ? What was the total number of rats used in the study, and how were the numbers per group decided upon? What is the sensitivity of the atomic absorption spectrophotometric method for the detection of chromium? Presumably, it does not distinguish among various metabolites and reduction intermediates of the parent compound. This makes it difficult to make firm conclusions about the association between chromium concentrations in tissues and the toxic effects measured. For example, the authors state on p. 11, “Interestingly, the renal damage disappeared in spite of the kidney still had high levels of chromium on days 8-12.” They do not discuss why this is the case. They should consider that chromium at these time points is present in the kidney tissues in some bound form that is nontoxic, that it has been converted into a nontoxic metabolite, that chromium itself induced the formation of heme-oxidase-1, that the level, although significantly elevated above control is below the threshold for nephrotoxicity, or other possible mechanisms to explain this finding.

The method to quantitate the percentage of tubules with histopathological alterations is unclear. Did the investigators measure damaged tubules per high power field to generate a percentage of tubular damage, or how did they obtain a percent value? Were the evaluators of the tubular damage blinded to the treatment?

The data in Figure 4 are not presented in a consistent manner. Some of the micrographs are reported in the figure legend as being 400X (A-C). However, it is obvious that other micrographs shown in this figure vary in magnification. Is there some justification for this variation? The actual magnification for each panel should be specified. In 4H, the authors state that there was “strong” immunostaining for DNP. However, the micrograph presented appears to show moderate staining at best, and the cells appear to be just membrane ghosts in H and I. The micrographs in Figure 7 also vary in magnification. The authors in the figure legend for 7C appropriately state that strong CAT immunoreactivity was observed in the renal tubular epithelium 12 days after K2Cr2O7 administration. Actually, it appears that the immunostaining is more intense in these tubules than that observed in controls. Did the authors notice some sort of “rebound” phenomenon whereby the CAT immunoreactivity in the kidneys of rats recovering from chromium-induced acute tubular necrosis was greater than it was in untreated controls? In the immunostained micrographs for Cu/Zn SOD and Mn-SOD, it appears that there are differences in the intensity of immunostaining that is not described by the authors. It looks like the intensity of immunostaining for Cu/Zn-SOD was greatest 2 days following chromium treatment (E), of
intermediate intensity 12 days after treatment (F), and of lowest intensity in controls (D). The intensity for immunostaining for Mn-SOD seems greatest in controls (G), of intermediate intensity in kidney from rats 2 days after treatment (H) and lowest in rat kidneys 12 days after treatment (I). Other than the concerns mentioned above, the conclusions appear to be based on solid data. The last sentence of the body of the manuscript on p.13 under conclusions does not make sense, however. This sentence needs to be restated for clarity. I think that the authors are trying to state that the decrease in the urinary excretion of NO metabolites was related to protein nitration that would result in a reduced elimination of nitrite and nitrate in the urine. There are numerous other sentences that lack clarity and need to be edited. General examples are the statements like “along the study” (p.13 and in other parts of the manuscript), “Regarding to the SOD enzymes” (p. 12) and in the figure legends and text stating “after 12 days of treatment.” The latter implies that the rats were treated for 12 days with chromium, whereas they only received a single dose on day 1. The two lines preceding the Conclusions section (p.13) do not form a complete sentence.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)