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No Significant Paraquat-Induced Oxidative DNA Damage in Rats

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The metabolism of paraquat generates oxygen radicals. Paraquat has thus been suggested as a model compound to induce oxidative damage to DNA, lipids and proteins in different cells and tissues, although experimental data are inconsistent. In order to explore the possibilities for an animal model of oxidative DNA damage *in vivo*, rats were treated with 20 mg/kg paraquat or vehicle i.p. One and five days later we measured DNA oxidation in terms of 7-hydro-8-oxo-2′-deoxyguanosine (8-oxodG) in the liver and lung as well as the urinary excretion of 8-oxodG. No significant effects on the level of 8-oxodG in the liver, the lung or the urinary excretion, could be distinguished following paraquat treatment. We found, however, a significant correlation ($r = 0.69$; $p < 0.0002$) between the 8-oxodG level in the lung and the urinary excretion, but no significant correlation between the level in the liver and the urinary excretion or between the levels in the liver and the lung. During the experiment the rats were clearly affected by the paraquat as they were very lethargic compared to the controls. Accordingly, even at toxic doses, paraquat did not cause detectable oxidative damage to DNA. The data do not support the use of paraquat as a model compound in experiments investigating effects or prevention of oxidative damage to DNA.

Keywords: Paraquat, oxidative DNA damage, 8-oxodG

Abbreviations: ROS, reactive oxygen species; 8-oxodG, 7-hydro-8-oxo-2′-deoxyguanosine; •OH, hydroxyl radical; FPG, formanidopyrimidine glycosylase

INTRODUCTION

Oxidative damage to DNA is thought to be involved in various diseases including cancer and degenerative disorders.[1,2] Such DNA damage is frequently assessed by measuring the level of the oxidatively modified nucleoside 7-hydro-8-oxo-2′-deoxyguanosine (8-oxodG) in tissue DNA and/or its urinary excretion.[3,4] A large number of xenobiotics have been shown to induce oxidative DNA damage in animal experiments[5,6] and some of these have been used as model compounds to demonstrate effects of various antioxidants and other preventive substances.[7,8] The use of these xenobiotics as model compounds, however, all involves different drawbacks, particular with respect to the generation of non-oxidative DNA damage.[9]

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Paraquat is a toxic xenobiotic, which generates reactive oxygen species (ROS) through redox cycling.[10] Paraquat undergoes a one-electron reduction catalysed by the cellular enzymes xanthine oxidase and NADPH-cytochrome P450 reductase. This reduction leads to the formation of a paraquat radical which will react rapidly with molecular oxygen to produce superoxide anion radicals with subsequent regeneration of paraquat. Superoxide possess limited reactivity,[11] but via hydrogen peroxide and transition metal reduction it may be an important source of hydroxyl radical (•OH) formation.[10]

Paraquat is used as a herbicide but is also toxic to humans and animals with frequent fatal consequences. The lung is the critical organ with pathological changes usually occurring in two phases. In the first phase massive destruction of the alveolar epithelium is followed by pulmonary oedema. In the second phase, an extensive alveolar fibrosis leads to death from anoxia.[12,13]

Several studies have investigated the effects of paraquat on DNA oxidation both *in vitro* and *in vivo* with somewhat conflicting results. *In vitro* paraquat has been found to both increase[14,15] and have no effect[16] on DNA oxidation. *In vivo* experiments also show conflicting results on DNA oxidation.[17,18]

In the pursuit of a suitable animal model for investigating consequences of oxidative stress, we tested paraquats ability to induce DNA oxidation in terms of 8-oxodG in the liver and lung as well as the urinary excretion of 8-oxodG in rats.

MATERIALS AND METHODS

Chemicals

Paraquat, trizma, triton X-100, $MgCl_2$, 8-oxodG, dG, EDTA, glycerol, nuclease P1 and sucrose were all obtained from Sigma (St. Louis, USA); NaCl, HCl, mannitol, H_3PO_4 85% and $NaClO_4$ were from Riedel-deHäen (Seelze, Germany); NaOH, NaAc (sodium acetate), $ZnCl_2$, SDS and $C_5H_{12}O$ (isoamylalcohol) were from Merck (Darmstadt, Germany); alkaline phosphatase and HEPES were from Boehringer Mannheim (Mannheim, Germany); and chloroform and acetonitrile were from Romil (Cambridge, UK).

Animals and Treatment

Male Wistar rats, approximately six weeks old and of 180–250 g body weight were used. The animals received standard laboratory diet and tap water *ad libitum*. They were housed four to a cage with aspen wood bedding (Finntapwei, Finland) in an environmentally controlled animal facility operating on a 12 h dark/light cycle and 55% humidity. Thirty-two rats were randomly allocated in four treatment groups with six rats in the two control groups and eight rats in the groups receiving paraquat. For 24-h urine collection immediately after and four days after paraquat treatment, the rats were placed in individual metabolic cages. Paraquat was dissolved in saline and 20 mg/kg body weight was injected i.p. Control rats received vehicle i.p. On day one and day five after treatment, two groups (one control group and one treatment group) were sacrificed by decapitation. The liver and lungs were dissected and immediately frozen in liquid nitrogen and stored at −80°C until use.

8-OxodG Analysis

Livers and lungs (600 mg) were homogenised in 30 ml HEPES buffer (250 mM mannitol, 70 mM sucrose, 5 mM HEPES, pH 7.4). The homogenates were then centrifuged at 1000× *g* in 10 min. The pellet was resuspended in 600 μl saline. All operations were carried out at 4°C. The DNA was extracted, precipitated and digested as previously described.[8] The 8-oxodG to dG ratio was measured using a HPLC system with electrochemical and UV detection as previously described.[19] The urine concentration of 8-oxodG was measured by HPLC with electrochemical detection as previously described.[19]

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Statistics

The four groups were compared by means of analysis of variance (ANOVA), followed if significant by a least significant difference test (LSD-test) for identification of differences between the groups. Some variables were log transformed in order to obtain a normal distribution before analysis.

In bivariate analysis Pearson's product–moment correlation coefficients were calculated whereas predictors of urinary 8-oxodG excretion were analysed by stepwise multiple linear regression. Probability values less than 0.05 were considered statistically significant.

RESULTS

Table I summarises the effects of paraquat treatment in the liver and the lung. There were no significant changes in the 8-oxodG levels in the liver or the lung although the actual levels were around 10% higher in the treatment groups. Similarly, there was no significant effect of paraquat treatment on the urinary excretion of 8-oxodG.

All the animals that received paraquat treatment showed signs of poisoning, as they were very inactive and lethargic compared to the control. In the group to be sacrificed on day five after the paraquat administration one rat died.

A significant correlation was found between the 8-oxodG level in the lung and the urinary excretion ($p < 0.0002$, $r = 0.69$) (Figure 1). There was no significant correlation between the 8-oxodG level in the liver and the urinary excretion ($r = 0.25$) or between the levels in the liver and the lung ($r = 0.28$).

DISCUSSION

In the present study no significant effect of paraquat on oxidative DNA damage was found, although paraquat is thought to be a powerful generator of superoxide and an overt toxic dose was used.

Treatment with paraquat did not result in any significant changes in DNA oxidation in the liver or lung, as measured by 8-oxodG, neither one nor five days after administration, although slight nominal increases were seen in both organs (Table I). In contrast, Tokunaga and co-workers found significant increases ($p < 0.01$) in 8-oxodG levels in the liver (2-fold), kidney (2-fold), brain (4-fold) and lung (4-fold), five days after administration of paraquat 20 mg/kg, i.v. to male Wistar rats.[17] Accordingly, the same dose and rat strain were used and it seems unlikely that a difference in route of administration could account for the large differences in the results obtained in the two experiments. As i.p. injection was used in the present study, paraquat would at least have been expected to have an effect on the liver. The age of the rats used in the experiments could also affect the results, as it has been found that young rats are more resistant to the toxic effects of paraquat than old rats.[20] However, the age of the rats used in the experiment of Tokunaga and co-workers[17] are assumed to be similar to the present, as the weight of the rats was the same.

TABLE I Effects of paraquat treatment on oxidative DNA damage in rat

	Day 1		Day 5	
	Control	Paraquat	Control	Paraquat
DNA oxidation				
8-OxodG, liver (per 10^5 dG)	0.75 ± 0.09	0.81 ± 0.15	0.81 ± 0.12	0.88 ± 0.30
8-OxodG, lung (per 10^5 dG)	0.90 ± 0.54	1.07 ± 0.56	0.87 ± 0.51	0.97 ± 0.59
8-OxodG, urine (pmol/24 h)	89.6 ± 50.2	85.6 ± 39.0	76.3 ± 14.2	89.6 ± 37.2

8-OxodG; 7-hydro-8-oxo-2′-deoxyguanosine; data are mean ± sd.

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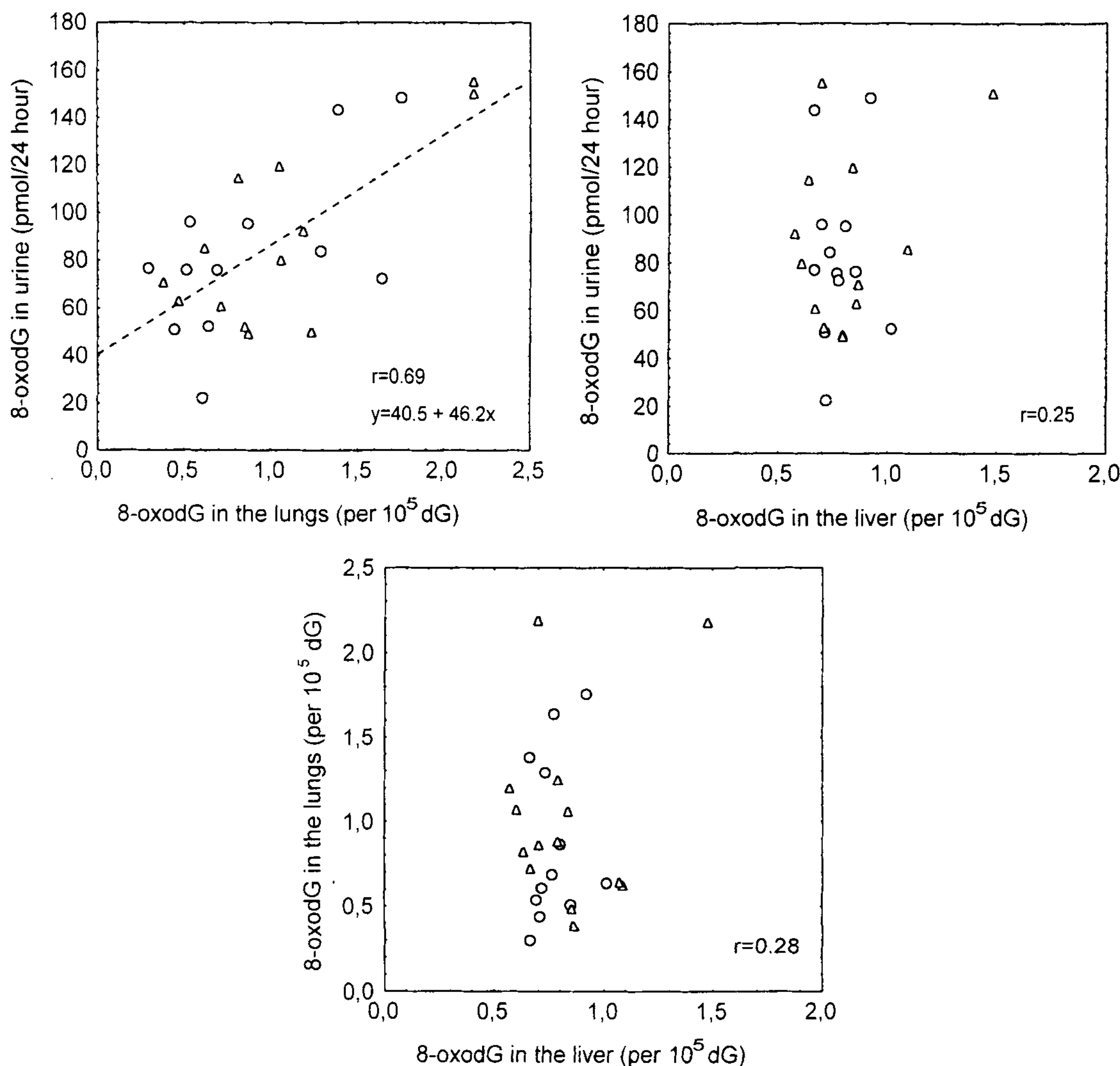

FIGURE 1 Relationships between 8-oxodG levels in the lung, the liver and the urinary excretion in rats one or five days after treatment with paraquat (△) or vehicle (○). The line of regression for all groups ($n = 25$) is shown for the levels in lung and urinary excretion. No significant correlation was found for the other relationships.

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Alternative explanations for differing results could be related to artefactual oxidation of DNA with 8-oxodG formation, which is known to occur during sample handling and analysis.[19,21,22] However, in the study by Tokunaga and co-workers[17] the 8-oxodG level was 1.3 and 1.7 per 10^5 dG in the liver and the lung of the control group, i.e. almost twice as high as the background levels in the present study. Accordingly, it appears unlikely that artefactual 8-oxodG formation in the present study could have obscured a true effect corresponding to the earlier study.[17] In support of the present data, another *in vivo* study reported unaffected 8-oxodG levels in the liver of gilthead seabream 8, 24, 48 and 72 h following paraquat administration i.p.[18]

Three *in vitro* studies have with conflicting results investigated the effect of paraquat on

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DNA damage by means of the comet assay with the use of formanidopyrimidine glycosylase (FPG) and endonuclease III for introducing strand breaks at oxidised bases.[14–16] In one study paraquat in concentrations of approximately 1 μM increased strand breaks and FPG sensitive sites in alveolar macrophages and epithelial type II cells.[14] A second study found that paraquat in cytotoxic concentrations of 50–300 μM induced strand breaks and FPG sensitive sites in two human transformed cell lines (HeLa and Hep G2) as well as in freshly isolated human lymphocytes, although the concentration response curve appeared bell shaped.[15] In contrast to this no changes in strand breaks and FPG sensitive sites were found in V79 Chinese hamster cells despite cytotoxicity, at paraquat concentrations of 0–10 mM.[16] The differences observed both *in vivo* and *in vitro* call the role of oxidative DNA damage in paraquat toxicity in question. Indeed, paraquat generates superoxide which *per se* do not induce 8-oxodG formation in isolated DNA.[23] Whether the excess superoxide generated by paraquat results in generation of $^{\bullet}OH$ *in vivo* is controversial, as it depends on the presence of free transition metals.[24,25] Under normal conditions free transition metals are not present *in vivo*, but can be released by the extensive cellular damage seen in the lungs during paraquat poisoning.[26] In the present study paraquat may have been completely metabolised before any possible release of metal ions.

In the present study there was no significant effect of paraquat on the urinary excretion of 8-oxodG. This variable reflects the overall rate of oxidative damage to DNA and the nucleotide pool in the animal. Repair of oxidised guanine DNA can result in excision of either the nucleotide resulting in 8-oxodG or the base 7-hydro-8-oxoguanine (8-oxoGua),[27,28] which both are water-soluble and excreted in the urine without further metabolism.[29] Sanitation of the nucleotide pool by specific enzymes removing 8-oxodGTP and possible oxidised DNA from turnover of cells and mitochondria are other potential sources of 8-oxodG. In contrast to the present results the injection of paraquat 11.5 mg/kg has been reported to cause a significant increase of 116% in the urinary concentration of 8-oxoGua related to creatinine on the following day.[30] However, the excretion of 8-oxoGua is mainly dependent on the amount of oxidised guanine in the diet and such data are thus difficult to interpret without a purine free diet.[31]

Previously it has been demonstrated that administration of inducers of oxidative DNA damage (e.g. 2-nitropropane) cause excess 8-oxodG levels in the target organs (liver, kidney, bone marrow etc.) that corresponded reasonably to the increase in the urinary excretion.[8] Interestingly, this experiment found a correlation between the levels in the lung and the urinary excretion independent of treatment, whereas there was no correlation between the levels of 8-oxodG in the liver and the urinary excretion. This may suggest that oxidation of guanine in DNA in the lungs may be an important contributor to the urinary excretion of 8-oxodG.

In conclusion, we found that paraquat did not induce significant oxidative damage to DNA in rats at a clearly toxic dose. The data therefore do not support paraquat as a model compound in experiments investigating effects or prevention of oxidative DNA damage.

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