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Original Paper

Novel Superoxide Dismutase Mimetics for Protection against Paraquat-induced Acute Renal Injury

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ABSTRACT

Paraquat-induced nephrotoxicity involves severe renal damage caused by reactive oxygen species (ROS), specifically by increasing superoxide ($O_2^{\bullet-}$) generation in the kidney. While proven to be of benefit in animal models of organ injury involving $O_2^{\bullet-}$, superoxide dismutase (SOD) and superoxide dismutase mimetics (SODm) can suffer problems regarding their bioavailability and toxicity. Since ROS has been incriminated in the pathogenesis of several disease conditions including acute kidney injury, the search for ideal SODm therefore continues unabated. Thus, the current study aims at investigating the therapeutic potential of Manganese (II) complexes of ethylenebis (oxyethylenenitrilo) tetraacetic acid (EGTA) and ethylenebis hydroxyphenylglycine (EHPG), novel SODm, against paraquat-induced nephrotoxicity using *an in vivo* rodent model. Administration of a single intraperitoneal dose of 10-50 or 100 mg/kg paraquat to male Wistar rats (200-250g) produced acute kidney injury within 48 and 24 hours respectively; as evidenced by a significant increase in serum creatinine, fractional excretion of sodium and a reduction in creatinine clearance. Unlike Mn (II)-EGTA (2mg/kg), Mn (II)-EHPG (4mg/kg) was able to significantly attenuate the acute kidney injury induced by 10-50 mg/kg paraquat. These complexes were not toxic at the doses examined unlike SOD or conventional SODm which can display pro-oxidant actions at higher concentrations. Since the clinical toxicity profiles of EGTA and EHPG are already known, these novel SODm particularly Mn (II)-EHPG could be beneficial in attenuating disease conditions involving ROS generation.

Keywords: Acute kidney injury, Oxidative stress, Paraquat, Superoxide anion, Superoxide dismutase mimetics

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INTRODUCTION

Paraquat (1,1'-dimethyl-4,4'-bipyridium dichloride, also known as methyl viologen), is a widely used broad-spectrum, fast acting herbicide; which is extremely toxic, causing fatalities due to accidental or intentional poisoning prevalently in developing countries (Gunnell and Eddleston, 2003; Eddleston and Phillips, 2004). Paraquat poisoning causes severe multiple organ failure, with the degree of poisoning dependant on the route of administration, the amount administered and duration of exposure. It is rapidly distributed within the body with highest concentrations accumulating within the kidneys where it produces early and severe nephrotoxicity (Rose and Smith, 1977). Additionally, as it is primarily excreted unchanged via the kidneys, the consequent reduction in renal function increases plasma concentrations by up to 5-fold which contributes to paraquat toxicity in other organs; especially the lungs (Hawksworth *et al*., 1981; Smith, 1987). Ultimately, respiratory failure, in the presence of nephrotoxic acute renal failure (ARF), is responsible for most deaths caused by paraquat (Smith, 1987; Haley, 1979; Nagata *et al*., 1992).

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Therefore, maintaining renal function in patients suffering from paraquat poisoning remains a therapeutically important treatment strategy. The role of superoxide anions ($O_2^{\bullet-}$) in the pathogenesis of paraquat toxicity has prompted much interest in the development of safe and effective antioxidants to negate its injurious effects (Suntres, 2002). Unfortunately, native superoxide dismutase (SOD) cannot be used effectively due to recognised problems with its short half-life, poor bioavailability and immunogenicity (Freeman *et al*., 1985; Muzykantov, 2001; Muscoli *et al*., 2003). Furthermore, SOD can have pro-oxidant activities at higher concentrations, e.g. Cu/Zn-SOD can promote the generation of hydroxyl radicals (Mao *et al*., 1993; Jewett *et al*., 1999) and inhibit the ability of superoxide to regulate lipid peroxidation (McCord and Edeas, 2005; Nelson *et al*., 1994). This has generated much interest in SOD mimetics, leading to the development of agents including macrocyclics, manganese salens, nitroxides, porphyrins and many other catalytic antioxidants (Muscoli *et al*., 2003; Salvemini *et al*., 2002; Mitchell *et al*., 1990; Cuzzocrea *et al*., 2001; Spasojevic *et al*., 2001; Batinic-Haberle, 2002; Sharpe *et al*., 2002; Fisher *et al*., 2003). However, many of these have also suffered problems ranging from cytotoxicity to poor stability (Liu *et al*., 1994; Collman *et al*., 2004). Furthermore, in common with native SOD, higher concentrations of some of these SOD mimetics can have pro-oxidant actions (Paller and Eaton, 1995; Czapski *et al*., 2002; McCord and Edeas, 2005).

Recently, the observation that complexes formed between metal ions and the common chelator sodium edentate or ethylenediaminetetraacetic acid (EDTA) possess significant SOD and catalase mimetic properties (Fisher *et al*., 2004a) prompted investigation of the SOD and catalase activities of other commonly used chelators and their metal complexes. Low concentrations of the calcium ion chelator ethylenebis (oxyethylenenitrilo) tetraacetic acid (EGTA) have been shown to solubilise amyloid plaques in post-mortem brain samples from patients suffering from Alzheimer's disease and to reduce nitric oxide (NO)-induced calcium influx into endothelial cells and prevent consequent cell death (Cherny *et al*., 1999; David-Dufilho *et al*., 2001). Some of these beneficial effects of EGTA have been credited to its ability to bind the excess copper (II) (Cu^{2+}) ions, which contribute, to disease pathology; however, it is now also clear that complexing EGTA with Cu (II) or Mn (II) promotes significant SOD activity (Fisher *et al*., 2004b). In addition, the related chelating agent ethylenebis hydroxyphenylglycine (EHPG), which is used as a contrast agent for magnetic resonance imaging and as a transferring mimic in the study of manganese transport (Richardson *et al*., 1999; Bihari *et al*., 2002), has also been shown to exhibit significant SOD activity when complexed with Mn (II) or Cu (II) (Fisher *et al*., 2004b).

We have previously demonstrated that Mn (II) complexes of EGTA and EHPG are beneficial in reducing paraquat-mediated cellular injury and death of confluent NRK-52E cells (renal cell line) in culture by attenuating paraquat-mediated increase in superoxide anion and hydroxyl radical generation (Samai *et al*., 2008). The current study was therefore conducted with the aim of investigating whether these complexes could produce similar beneficial effect in an *in vivo* rodent model of paraquat nephrotoxicity.

MATERIALS AND METHODS

Chemicals

Unless otherwise stated, all compounds used in this study were purchased from Sigma-Aldrich Company Ltd. (Poole, Dorset, UK). Mn (II) complexes of EGTA and EHPG were kindly synthesized as described previously (Fisher *et al*., 2004b) and provided by Dr Theresa Hague and Professor Declan Naughton, Biomedical & Pharmaceutical Sciences Research Group, School of Life Sciences, Kingston University, Surrey, UK.

Animals

A total of one hundred and eight (108) male wistar rats (weighing 200-250 g) purchased from Charles River laboratories (Margate, UK) were used. These animals were allowed free access to a certified rodent diet (expanded pellets R & M no. 3) from special diet services and water throughout the experimental period. They were all housed in a room kept at a temperature of 19 ± 2 ^{0}c and relative humidity of 46–55 % and were maintained on a light/dark cycle of 12 hours/12 hours and were recruited into the study a week after delivery. The animals were handled and treated in accordance with the British Home Office Animals (Scientific Procedures) Act 1986 and the University of Brighton Local Ethical and Health Safety Regulations.

The experimental protocol was approved by the University of Brighton Ethics Committee and was performed under British Home Office Project License 70/6553.

Characterization of Rodent Model of Paraquat-induced Acute Kidney Injury (AKI)

The rats were randomly allocated into two groups for the characterization of paraquat-mediated acute kidney injury (AKI). In the first group (the paraquat treated group), paraquat dissolved in normal saline was administered intraperitoneally as a single dose to five subgroups at a dose of 2.5, 5, 10, 50 and 100 mg/kg respectively. An equal volume of the vehicle (normal saline) was administered intraperitoneally to the second group to serve as control. The doses of paraquat were based on previously published work in which the effects of paraquat on gene expression in the kidney was investigated in male Wistar rats (Tomita *et al*., 2006).

Following the injections, the rats were housed separately in metabolism cages for the daily collection of urine. During the treatment period the rats were observed for features of paraquat toxicity such as bleeding and respiratory distress. At the end of the experimental period, usually 48 hours, except for rats treated with 100 mg/kg paraquat, which was 24 hours, the rats were terminally anaesthetized with intraperitoneal pentobarbital (50 mg/kg) and placed on a temperature-regulated table to maintain their body temperature (37 ^{0}c). The abdominal cavity was exposed via a high mid-line incision and a total bilateral nephrectomy was performed. The kidney capsule was removed and the kidneys were kept in formaldehyde for histopathological examination.

In addition, blood samples were obtained by cardiac puncture and the rats were subsequently sacrificed. Serum was collected following centrifugation of the blood samples at 13000 rpm for 5 minutes. The serum and urine samples were subsequently used for biochemical analysis for markers of AKI (specifically creatinine). In addition, the urine samples were qualitatively analysed for protein, blood and glucose using urine dipsticks. Furthermore, serum and urinary sodium concentrations were evaluated via flame photometry with the subsequent calculation of fractional excretion of sodium (FE_{Na}) and creatinine clearance.

Intervention against Paraquat-induced AKI

We have previously shown that Mn (II) complexes of EHPG and EGTA are efficacious in protecting against paraquat-mediated renal cytotoxicity of confluent NRK-52E cells *in vitro* (Samai *et al*., 2008). Thus, the hypothesis that these novel SOD mimetics could also protect against paraquat - induced nephrotoxicity (specifically AKI) in an *in vivo* rodent model was tested. For this model, the rats were randomly allocated into six initial groups. The first, second, third and fourth group of rats were exclusively treated with a single intraperitoneal dose of normal saline, paraquat only (10 mg/kg), Mn (II)-EGTA only (2 mg/kg), and Mn (II)-EHPG only (4 mg/kg) respectively. A single dose of paraquat (10 mg/kg) was also co-administered with either Mn (II)-EHPG (4mg/kg) or Mn (II)-EGTA (2mg/kg) intraperitoneally into fifth and sixth group of rats respectively. The results of the initial intervention study indicated that Mn (II)-EHPG at the dose examined completely reversed the nephrotoxic effect produced by 10 mg/kg paraquat whilst Mn (II)-EGTA did not offer any protection. Thus, the ability of Mn (II)-EHPG (4mg/kg) to protect against the nephrotoxic effect of 50 mg/kg paraquat was further investigated. For this experiment the rats were randomly allocated into four groups comprising of control (normal saline only), Mn (II)-EHPG only (4 mg/kg), paraquat only (50 mg/kg), and paraquat (50 mg/kg) plus Mn (II)-EHPG (4 mg/kg) treated groups. At the end of the experimental period (48 hours), the animals were treated as described above. Creatinine and sodium concentrations were subsequently determined in the serum and urine samples; with urinalysis performed for proteins, blood and glucose. Finally, creatinine clearance and FE_{Na} were evaluated.

Measurement of Serum and Urinary Creatinine

Serum and urinary creatinine levels were evaluated using the recently characterized method of Piero *et al*. (1988), which is a colorimetric assay based on the measurement of hydrogen peroxide.

Measurement of Creatinine Clearance and Fractional Excretion of Sodium

All the urine passed in a 24-hour period was collected and one sample of blood was taken during the day of collection.

The creatinine clearance was computed in mL/min from the product of the total urinary creatinine (µmol/L) and the urine flow (mL/min) divided by the serum creatinine (µmol/L). FE_{Na} is not a test, but rather a calculation based on the concentrations of sodium and creatinine in the blood and urine (as shown below). Serum and urinary sodium concentrations were measured by a flame photometer using the standard technique.

$$FE_{Na} = \frac{U_{Na} \times P_{Cr}}{P_{Na} \times U_{Cr}} \times 100\%$$

Where U_{Na} and P_{Na} represent urinary and serum sodium and P_{Cr} and U_{Cr} serum and urinary creatinine respectively.

Statistical Analysis

Results are expressed as mean ± SEM. Means were obtained from multiple experiments performed in triplicate. Data were analyzed with the commercially available statistical software (Graphpad Prism, version 3.0, Graphpad software, San Diego, CA, USA). Differences between mean values within the groups were determined using Student's t-test and one way analysis of variance (ANOVA) followed by a Dunnett's test for comparison of multiple means. A *P* value of less than 0.05 was taken to indicate significance.

RESULTS

Administration of 100 mg/kg dose of paraquat intraperitoneally to a subgroup of rats resulted in severe intercoastal recession, nasal flaring and respiratory distress, including bleeding from the nostrils, eyes, and mouth, with spastic paralysis of the hind limbs, polyuria, haematuria, glycosuria and proteinuria within the first 24 hours. These rats were euthanized after 24 hours to reduce pain and distress. The subgroup of rats which received 50 mg/kg paraquat also manifested polyuria within the first 24 hours. However, proteinuria, haematuria, glycosuria and a significant reduction in urine production with moderate to severe respiratory distress, were only noted in this subgroup of rats by the end of 48 hours as compared to the control group; with some manifesting anuria. Thus, the 48 hour time point was used as the hallmark for the onset of the paraquat -induced AKI. In contrast, there were no apparent differences between the physical features and the urinalysis profile of the control group and the 2.5-10 mg/kg paraquat subgroups (Table 1).

Table 1: Some Physical Features and Urinary Profile of the Paraquat Treated Subgroup of Rats.

Treatment group	Respiratory distress	Spastic paralysis of hind limbs	Proteinuria	Haematuria	Glycosuria
Control (0 mg/kg paraquat)	-	-	-	-	-
2.5 mg/kg paraquat	-	-	-	-	-
5.0 mg/kg paraquat	-	-	-	-	-
10 mg/kg paraquat	-	-	-	-	-
50 mg/kg paraquat	+++	+++	++	++	++
100 mg/kg paraquat	++++++	++++++	++++	++++	++++

***: "+"** indicates the presence of a symptom and the number of pluses indicates the intensity of the symptom and **"–"**indicates the absence of the symptom.*

Significant elevation of serum creatinine levels from baseline control values was used as a biochemical marker of AKI. AKI was noted in rats within 24 and 48 hours following single intraperitoneal administration of 100 and 10-50 mg/kg dose of paraquat respectively. A significant increase in the concentrations of serum creatinine by 2.7, 3.6 and 4.4 fold and FE_{Na} by 8, 13, and 29 fold was noted in rats treated with 10, 50, and 100 mg/kg paraquat respectively as compared to the control group ($P< 0.05$). This was concurrently accompanied by a significant reduction in creatinine clearance by 95% in all three paraquat treated subgroups ($P< 0.05$; Figure1 (A-C).

In the absence of either Mn (II)-EGTA or Mn (II)-EHPG, the 10 mg/kg dose of paraquat significantly increased the serum creatinine levels and FE_{Na} by 2 and 11 fold respectively with a significant reduction of 84 % in creatinine clearance when compared to the control group ($P< 0.05$).

In contrast, there were no significant differences in the serum creatinine levels, FE_{Na}, and creatinine clearance in rats treated exclusively with either Mn (II)-EGTA (2 mg/kg) or Mn (II)-EHPG (4 mg/kg) as compared to the control group. Co-administration of paraquat (10 mg/kg) and Mn (II)-EGTA (2 mg/kg) did not protect the rats against paraquat-induced AKI; as the increase in serum creatinine levels and FE_{Na}, and the reduction in creatinine clearance seen in these rats were statistically similar to the paraquat only treated group (P>0.05). However, Mn (II)-EHPG (4 mg/kg) reduced the increases in serum creatinine levels and FE_{Na}, and increased the reduction in creatinine clearance caused by 10 mg/kg paraquat to almost baseline control levels (P<0.05) (Figure 2 (A-C).

As Mn (II)-EHPG (4 mg/kg) completely protected the rats against the nephrotoxic effect of 10 mg/kg paraquat, its ability to provide similar protection against the nephrotoxic effect of 50 mg/kg paraquat was further investigated. Unlike the control and Mn (II)-EHPG only treated groups; proteinuria, haematuria, glycosuria, and spastic paralysis of the hind limbs with moderate-severe respiratory distress were noted in rats treated exclusively with 50 mg/kg paraquat. These physical manifestations and urinalysis profile were also observed though to a lesser degree, in the paraquat (50 mg/kg) plus Mn (II)-EHPG (4 mg/kg) treated group. In addition, administration of 50 mg/kg dose of paraquat significantly increased the serum creatinine levels and FE_{Na} by 3 and 12 fold respectively with a significant reduction in creatinine clearance by 98 % when compared to the control group (P<0.05; Figure 3 (A-C). In the presence of Mn (II)-EHPG (4 mg/kg) the paraquat -mediated increase serum creatinine and FE_{Na} were significantly reduced by 64 and 49 % respectively (P<0.05; Figures 3A and B). However, this was not accompanied by any significant increase in creatinine clearance (P>0.05; Figure 3C). As speculated, Mn (II)-EHPG (4 mg/kg) on its own did not alter the serum creatinine levels, FE_{Na}, and creatinine clearance to any significant extent when compared to the control group (P>0.05; Figure 3 (A-C)).

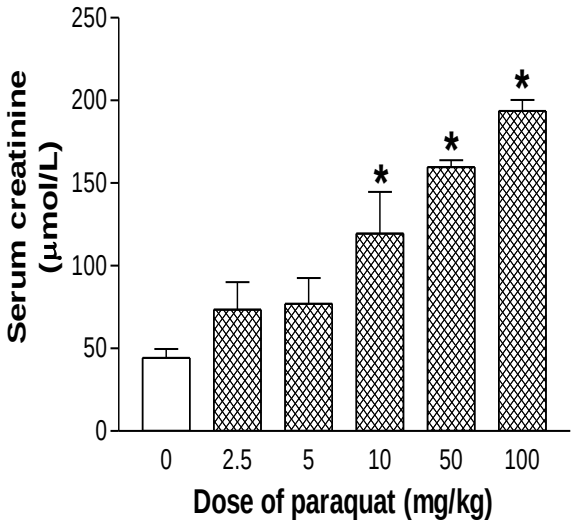

Figure 1A: Serum Creatinine Levels

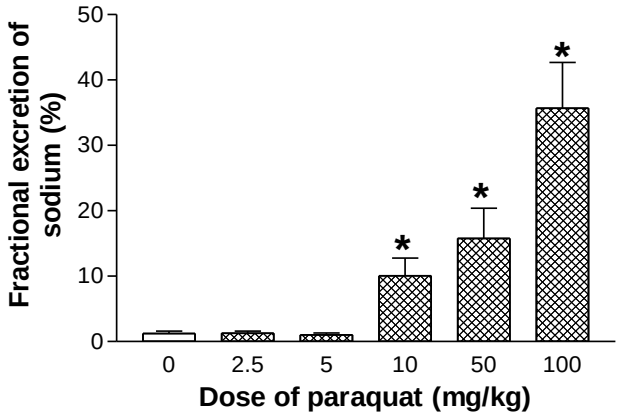

Figure 1B: Fractional Excretion of Sodium

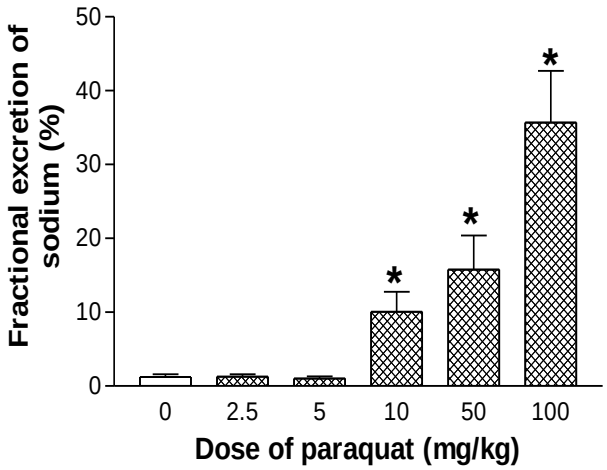

Figure 1C: Creatinine Clearance

Figure 1(A-C): Effects of paraquat on **(A)** Serum creatinine levels, **(B)** Fractional excretion of sodium and **(C)** Creatinine clearance. Rats were intraperitoneally treated with normal saline only or paraquat dissolved in normal saline (2.5-100 mg/kg) N=8 for each subgroup). *****P < 0.05 vs. control group.

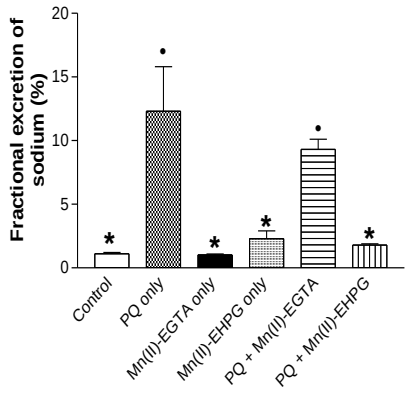

Figure 2A: Serum Creatinine Levels

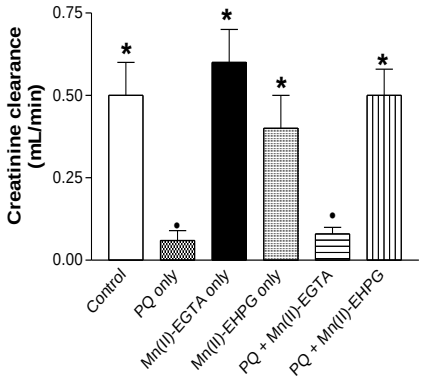

Figure 2B: Fraction Excretion of Sodium

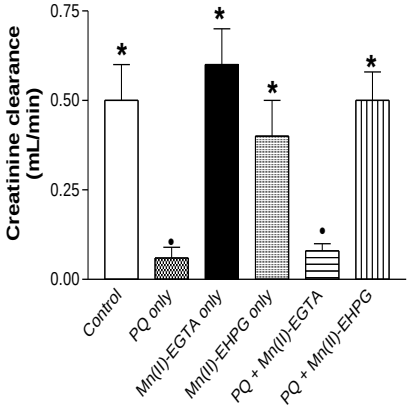

Figure 2C: Creatinine Clearance

Figure 2(A-C): Effects of Mn (II)-EGTA and Mn (II)-EHPG on paraquat-induced increase in serum creatinine levels **(A)**, and fractional excretion of sodium **(B)** and paraquat-mediated reduction in creatinine clearance **(C)**. Rats were treated intraperitoneally with normal saline only, Mn (II)-EGTA only (2 mg/kg), Mn (II)-EHPG only (4 mg/kg) or paraquat dissolved in normal saline (10 mg/kg) in the presence or absence Mn (II)-EGTA or Mn (II)-EHPG (N=6 for each subgroup). *****P< 0.05 vs. paraquat only treated group and ●P< 0.05 vs. control group (rats treated with normal saline only).

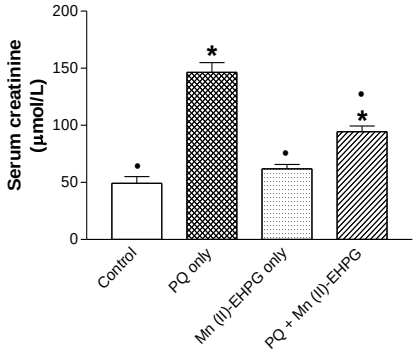

Figure 3A: Serum Creatinine Levels

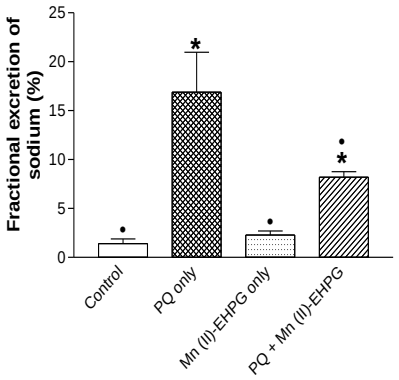

Figure 3B: Fractional Excretion of Sodium

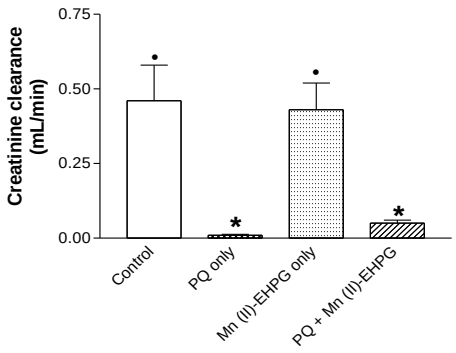

Figure 3C: Creatinine Clearance

Figure 3(A-C): Effects of Mn (II)-EHPG on paraquat-induced increase in serum creatinine levels **(A)**, and fractional excretion of sodium **(B)** and paraquat-mediated reduction in creatinine clearance **(C)**. Rats were treated intraperitoneally with normal saline only, Mn (II)-EHPG only (4 mg/kg) or paraquat dissolved in normal saline (50 mg/kg) in the presence or absence Mn (II)-EHPG (N=6 for each subgroup). *****P< 0.05 vs. control group (rats treated with normal saline only) and ●P < 0.05 vs. paraquat only treated group.

DISCUSSION

The results of the *in vivo* studies confirm the nephrotoxic effect of paraquat in addition to its cytotoxic action on other tissues particularly the pulmonary and neuromuscular tissues. As with the *in vitro* findings the nephrotoxic effects of paraquat was noted to be both dose and time dependent (Samai *et al*., 2007; 2008). Specifically, paraquat produces a dose dependent AKI as evidenced by a progressive increase in serum creatinine levels with increase in dose. Paraquat -mediated AKI has been shown to be due to a combination of pre-renal factors such as hypovolaemia and hypotension and/or renal factors such as acute tubular necrosis (ATN) (Beebeejaun *et al*., 1971; Harsanyi *et al*., 1987).

Severe dehydration secondary to polyuria/diuresis and coupled with the inability of the rats to drink due too ill health as seen in the current study, may have led to hypovolaemia and severe hypotension with a subsequent reduction in renal perfusion; thereby causing renal ischaemia. Renal ischaemia increases serum renin levels and hyperreninaemia has been demonstrated in dogs after toxic administration of paraquat (25 mg/kg) (Giri *et al*., 1982). Reduction in renal blood flow also reduces glomerular filtration rate (GFR); and a reduction in GFR by paraquat has been previously documented by Vaziri *et al*., (1979) and Prashad *et al*., (1981). This has been confirmed in the current study as evidenced by a significant reduction in creatinine clearance, which was used as an indicator of GFR.

In addition, ATN particularly of the proximal tubule (PT) has been showed on histopathological examinations of human kidneys taken from paraquat-poisoned patients (Beebeeja *et al*., 1971; Onyeama and Oehme, 1984). Although histopathological examination of the paraquat poisoned kidneys were not performed in the current study for physical evidence of ATN, intrinsic renal failure was confirmed biochemically by the measurement of the FE_{Na}. Fractional excretion of sodium between 1-3 % is usually normal and values less than 1 % or greater 3 % is suggestive of pre- or intrinsic- renal failure respectively. Intrinsic renal failure, possibly ATN has been confirmed biochemically in the current study as the values for the FE_{Na} were noted to be greater than 3 %.

Moreover, several PT dysfunctional parameters including diuresis, proteinuria, and glycosuria, with a concurrent increase in renal leakage of sodium, potassium and chloride ions have been shown in rats (Lock and Ishmeal, 1979), dogs (Giri *et al*., 1982; Nagata *et al*., 1992) and monkeys (Purser and Rose, 1979) following paraquat treatment. Additionally, other PT dysfunctional parameters, such as impaired phosphate and uric acid transport (Vaziri *et al*., 1979), lactic aciduria, 3-D-hydroxybutyric aciduria and a reduction in the excretion of citrate and succinate (Bairaktari *et al*., 1998) have been noted in paraquat poisoned patients. Furthermore, abnormal morphological findings have been demonstrated in the PT of rats (Lock and Ishmeal, 1979), rabbits (Yonemitsu, 1986), and dogs (Giri *et al*., 1982; Nagata *et al*., 1992) exposed to paraquat. Proximal tubule damage has been confirmed biochemically in the current study as evidenced by polyuria/diuresis, proteinuria, glycosuria and increase FE_{Na}.

Apart from its nephrotoxic effect, the highest dose of paraquat (100 mg/kg) examined *in vivo* also produced severe respiratory distress as evidenced by intercoastal recession, nasal flaring with a very rapid and shallow respiration; and spastic paralysis of the hind limbs within 24 hours. These manifestations were noted, though to a limited extent, in rats treated with 50 mg/kg dose of paraquat after 48 hours of treatment; but were not apparent in the 2.5-10 mg/kg paraquat treated rats. The spastic paralysis of the hind limbs observed in these rats could be either locally or centrally mediated. It is possible that paraquat may have produced succinylcholine-like action on the neuromuscular endplate. However, in view of reports that paraquat induces Parkinson's disease (Melchiorri *et al*., 1998; Vogt *et al*., 1998; Chun *et al*., 2001); it is more likely that paraquat may have increased the peripheral cholinergic action.

Although none of the rats in the current study died during the experimental period, pulmonary toxicity such as pulmonary fibrosis (Uhal *et al*., 1995) and pneumomediastinum (Sittipunt, 2005) which has a poor prognosis with a mortality rate of almost 100 % (Im *et al.,* 1991); has been widely documented to be responsible for most of the deaths associated with paraquat poisoning. In the presence of ARF, plasma and tissue paraquat levels including the lung tissues increase by 5-fold (Hawksworth, *et al*., 1981). Acute renal failure is a life threatening illness with a high mortality despite advances in supportive treatment.

In general, uraemia secondary to ARF has been documented to be associated with increased oxidative stress (Ichikawa *et al*., 1994; Witko-Sarsat *et al*., 1996). In addition, reduced antioxidant levels have been demonstrated in uraemic patients on haemodialysis or peritoneal dialysis (Epperlein *et al*., 1998). Moreover several experimental animal models and *in vitro* studies have established a role for reactive oxygen species and the therapeutic potential for free radical scavengers in renal dysfunction.

The safe profile of Mn (II) and Cu (II) complexes of EGTA and EHPG will allow investigations of these complexes *in vivo* to assess their efficacy against many pathological conditions involving ROS generation and oxidative stress (Samai *et al.*, 2008). However, some care may need to be taken when Cu (II) complexes are utilised in view of a report that Cu (II) derived from Cu/Zn-SOD may facilitate oxidative stress in the presence of glutathione (Paller and Eaton, 1995). Both EGTA and EHPG have the advantage that they have already been used in biological systems for their respective chelation and imaging properties and therefore their clinical toxicity profiles are already known. In addition, their long-term effects and stability in biological systems have been demonstrated (Fisher, *et al*., 2004b).

Furthermore, SOD and catalase activities of Mn (II), Cu (II) and Fe (III) complexes of another chelating agent, EDTA, have previously been reported (Fisher *et al*., 2004a) and subsequently, EDTA itself has been reported to protect against ischaemic renal injury via modulation of endothelial nitric oxide synthase (eNOS) and NO production (Foglieni *et al*., 2006). Since Mn (II) complexes of EGTA and EHPG have been previously demonstrated to be reno-protective and safe *in vitro* (Samai *et al*., 2008), it was anticipated that these complexes may provide similar benefits *in vivo*, a hypothesis which was further tested in a rodent model of paraquat -induced AKI.

In the rodent model of paraquat -induced AKI characterised in the current study, Mn (II)-EGTA at the dose examined (2 mg/kg) was found to be ineffective in protecting the subgroup of rats exposed to 10 mg/kg paraquat against the paraquat -mediated AKI. On the other hand, Mn (II)-EHPG (4 mg/kg) provided absolute protection against the paraquat-induced AKI in the subgroup of rats exposed to 10 mg/kg paraquat; as evidenced by a complete reversal of the serum creatinine, FE_{Na} and creatinine clearance to the baseline control values. This illustrates the reversible nature of paraquat-induced AKI; as paraquat -induced ARF is reversible if patients ingest less than 40 mg/kg paraquat (Vale *et al*., 1987). Although similar dose of Mn (II)-EHPG (4 mg/kg) significantly reduced the paraquat -induced AKI in the subgroup of rats treated with 50 mg/kg dose of paraquat, it did not provide absolute protection.

The inability of Mn (II)-EGTA to provide any protection against paraquat-induced AKI in the 10 mg/kg paraquat treated subgroup of rats, could be possibly related, in part, to the dose employed. Although doses greater than 2 mg/kg Mn (II)-EGTA were not examined as 2 mg/kg was the highest achievable dose; it is however anticipated that doses greater than or equal to 4 mg/kg could provide similar protection as that seen with 4 mg/kg Mn (II)-EHPG. As with the *in vitro* findings (Samai *et al*., 2008), Mn (II) complexes of EGTA and EHPG at the doses examined were not biochemically toxic to the renal system and particularly the kidney cells *in vivo.*

CONCLUSION

The novel SODm Mn (II)-EHPG is beneficial in the reduction of paraquat -mediated AKI in a rodent model. Since the clinical toxicity profiles of EHPG are already known, this novel SODm could be beneficial in attenuating disease conditions involving ROS generation.

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