Antioxidant activity of simvastatin prevents ifosfamide-induced nephrotoxicity

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Abstract: Ifosfamide is an anticancer agent used largely in treatment of solid tumors. The mainstay dose-limiting toxicity of ifosfamide is nephrotoxicity. This is largely believde to be a result of ifosfamide-induced oxidative stress. In this study, we investigated the antioxidant activity of simvastatin and the possible protective role of simvastatin against ifosfamide induced nephrotoxicity. Thirty Sprague-Dawely rats were divided into five groups and given orally different drug combinations. Group I and II were regarded as control groups and received 0.1% DMSO and normal saline, respectively. Group III received ifosfamide at 50mg/kg, group IV received simvastatin at 0.3mg/kg and group V received both ifosfamide and simvastatin. All animals were decapitated 2 days after the last ifosfamide administration. Findings revealed that ifosfamide induced nephrotoxicity as indicated by a significant increase in plasma creatinine and lipid per oxidation. This increase was significantly inhibited in animals pretreated with simvastatin. Histopathological observations were in correlation with the biochemical parameters in that simvastatin minimized ifosfamide-induced renal tubular damage. The above results promote a future use of simvastatin in combination with ifosfamide in treatment of cancer patients to indicated that simvastatin protectics against ifosfamide-induced nephrotoxicity in terms of oxidative stress and might be given in combination.

Keywords: Ifosfamide, simvastatin, nephrotoxicity, histopathology, antioxidants enzymes.

INTRODUCTION

Ifosfamide is an isomer of cyclo-phosphamide belonging to Oxazaphosphorinane drug family (Dechant *et al.*, 1991). It is widely used in treatment of many cancers such as Wilms tumor, rhabdo- myosarcoma, Ewing's sarcoma, testicular germ cell tumors, gynecologic, head and neck cancers, lymphomas, and osteogenic sarcoma (Straka *et al.*, 2003).

The efficacy of ifosfamide is limited by its dose-limiting nephrotoxicity (Skinner *et al.*, 1990). Ifosfamide-induced nephrotoxicity is particularly caused by its metabolite chloroacetalaldehyde (Elias *et al.*, 1990). It is closely associated with increased levels of lipid per oxidation in kidney tissues, formation of reactive oxygen species, and inhibition of antioxidant enzymes activity (Chen *et al.*, 2007).

Statins are clinically used as lipid lowering agents. This is primarily by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Tobert, 2003). Beyond this effect, several studies have shown that statins possess antioxidant properties. This might occur by increasing the nitric oxide (NO) bioavailability, improvement in plasma antioxidants levels, and reducing lipid peroxidation and reactive oxygen species (ROS) production (Tobert, 2003; Scalia *et al.*, 2001; Stoll *et al.*, 2004; Wilson *et al.*, 2001; Wassmann *et al.*; 2001). Recently and due to their antioxidant properties, it has been shown that statins might suppress the growth of different cancers such as prostate cancer (Sekine *et al.*, 2008).

In the present study, we evaluated the potential of ifosfamide to induce nephrotoxicity in Sprague-Dawely rats and the protective effects of simvastatin against ifoswfamide-induced oxidative stress.

MATERIALS AND METHODS

Male Sprague Dawley rats, weighing 160-210g, aged 8-12 weeks were used in this study. Drugs were administered orally using a ball tipped stainless steel gavage attached to a syringe. Simvastatin was dissolved in dimethyl sulfoxide (DMSO) and ifosfamide in normal saline. Five experimental groups were established: Group I: 0.1% DMSO, Group II: saline, Group III: ifosfamide (50mg/kg), Group IV: 0.5ml simvastatin (0.3mg/kg), and Group V: ifosfamide (50mg/kg) and simvastatin (0.3mg/kg). Drugs were administered for five days in all groups except Group V in which simvastatin was administered one day before the start of overall treatment. Approval of the study was conducted by Institutional Animal Care Committee at Jordan University of Science and Technology (Irbid, Jordan).

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Animals in all groups were scarified in an ether chamber after 48 h from the last application of the treatment drug. Blood samples were taken by intracardiac puncture and collected into heparinized tubes. Samples were centrifuged at 3000rpm for 10min; plasma was separated and stored at -20°C for determination of urea and creatinine. Kidneys were removed from each rat. One kidney was placed in 10% formaldehyde solution for histopathology examination by light microscopy as described previously (Chen *et al.*, 2007). The other one was homogenized with phosphate buffered solution (pH 7.2) to obtain a 1:5 (w/v) homogenate. The homgenate was stored at -80°C, later thawed, and glutathione S-transferase activity (GST), catalase (CAT) activity, and lipid per oxidation levels were determined.

GST activity was measured by monitoring the rate of 1-chloro-2,4-dinitrobenzene conjugation with reduced glutathione at 340nm (Habig *et al.*, 1974). CAT activity was measured by monitoring the rate of hydrogen peroxide decomposition at 240 nm (Aebi, 1984). Level of lipid per oxidation was determined at 532 nm by a method described earlier (Ohkawa *et al.*, 1979). GST, CAT and lipid per oxidation levels were expressed in terms of protein content. Total protein concentration (mg/ml) was estimated according to Bradford method using bovine serum albumin standard (Bradford, 1976). Urea and creatinine blood levels were measured using two commercial kits according to the manufacturer's instructions (Biorex, United Kingdom).

Histopathological examination was performed in that the rats were euthanized by ether. Kidneys were preserved in formalin 10%. Fixed materials were washed in 70% ethanol repeatedly. Dehydration was performed by passing the materials in upgraded ethanol concentrations as follows: 80%, 90%, 95% and absolute (two hours in each change), and then cleared in xylene for 20min. Infiltration was carried out using paraffin wax (melting point 49°C), embedding of the samples with pure melted paraffin wax was achieved by pouring it in cassettes in order to make blocks. Blocks were then trimmed and they were serially sectioned using a wild microtome at 5µm thickness. Glass slides were used to collect the serial sections floating in distilled water and placed on a hot plate for stretching.

Serial sections were stained by using Ehrlich hematoxylin and Eosin stains (H&E). The stained sections were mounted with Canada balsam and covered with cover slip, examined on CX31 microscope. Images were captured using DP20 camera set from Olympus™. DP20 is a 2 Megapixel color CCD digital microscope camera, with a resolution of 1600 x 1200 pixels and pixel size of 4.2µm x 4.2µm.

Data were analyzed using the SPSS package version 17 (SPSS Inc, Chicago, IL, USA) for windows. Data were expressed as a mean ± standard error (SE). The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-test for pair wise comparisons. Discrete variables were expressed as counts and were compared using Chi square test. A P-value of less than 0.05 was considered statistically significant.

RESULTS

No mortality was observed during the study period. The activities of two different antioxidant enzymes, lipid per oxidation levels (MDA), and creatinine and urea are shown in fig. 1. According to ANOVA test results, the mean value of GST specific activity in ifosfamide group was significantly lower than controls ($P \leq 0.05$). Results also indicated no significant differences ($P > 0.05$) in the mean values of CAT enzyme activity in ifosfamide and simvastatin / ifosfamide compared to controls (fig. 2).

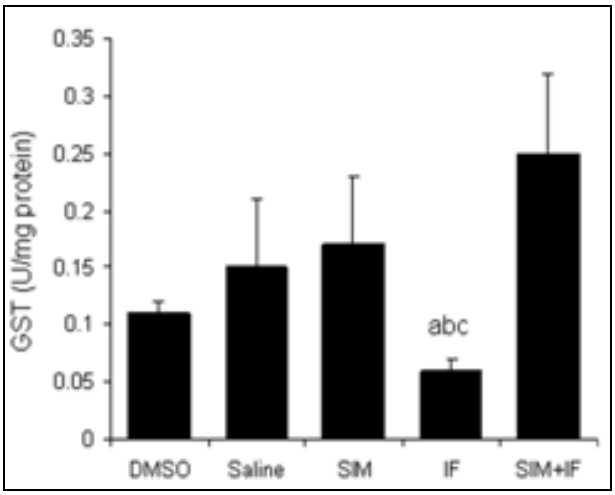

Fig. 1: The effect of ifosfamide (IF) and/or simvatatin (SIM) on GST activity in the rat kidney, n=4. Values are means ± SE. Analysis of variance (ANOVA), $^{a}P<0.05$ when compared with saline; $^{b}P<0.05$ when compared with combined group (IF+SIM). $^{c}P< 0.05$ when compared with DMSO and SIM groups.

Results shown in fig. 3 revealed that lipid per oxidation levels in the ifosfamide treated animals were significantly higher than those in the control group. Co-administration of simvastatin has significantly reduced the increase in lipid per oxidation levels induced by ifosfamide. Plasma creatinine was significantly higher in plasma of ifosfamide treated group compared with other groups (fig 4). However, administration of simvastatin significantly reduced the increase in creatinine levels. In contrast to creatinine, the average mean values of urea were not significantly different among the other experimental groups (fig. 5).

Haematoxylin and eosin staining (H & E) of kidney showed regular morphology of the tubules and glomeruli in normal saline (fig. 6a) and 0.1% DMSO (fig. 6b) groups. Ifosfamide at 50mg/kg induced a significant morphological changes compared to the control group (fig. 6d). These include severe wide ischemia of PCT and missing in Bowman's space. There was also a significant amount of cell death, hemorrhage, edema and congestion. Animal group received simvastatin (0.3mg/kg) showed normal renal tubules and glomeruli (fig. 6c). Pretreatment with simvastatin protected kidney from histological damage induced by ifosfamide and pathological scores were significantly decreased (fig. 6e).

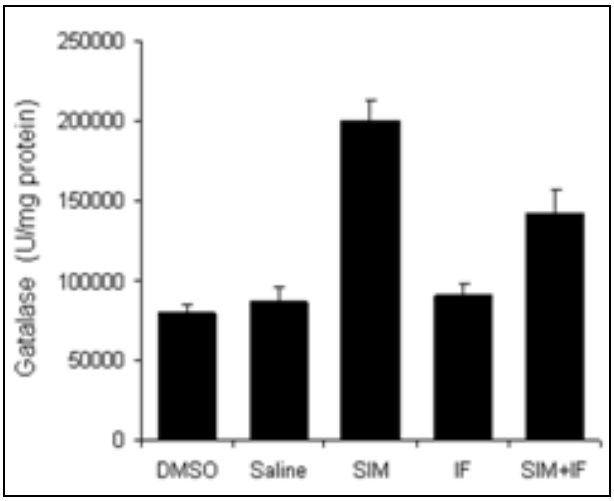

Fig. 2: The effect of ifosfamide (IF) and/or simvatatin (SIM) on catalase activity in the rat kidney, n=4.

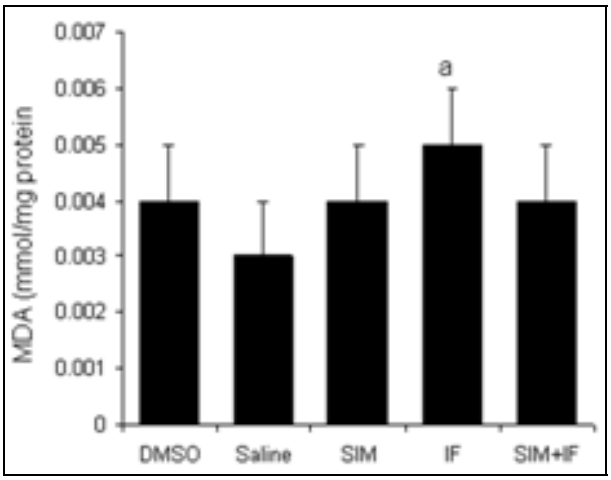

Fig. 3: The effect of ifosfamide (IF) and/or simvastatin (SIM) on lipid peroxidation in the rat kidney after treatment with ifosfamide 50mg/kg for 5 days. n=4. [a]*P*< 0.1 when compared with control groups of saline and DMSO.

DISCUSSION

In the present study, results indicated that administration of ifosfamide in rats resulted in perturbation of renal function as indicated by a significant increase in plasma levels of creatinine. This result is consistent with the previous studies performed on ifosfamide induced nephrotoxicity in both experimental animals and human being (Springate and Van Liew, 1995; Springate, 1997; Badary, 1998; Sener *et al.*, 2004; Nissim *et al.*, 2006; Skinner *et al.*, 1990; Skinner *et al.*, 1993). Pretreatment of animals with simvastatin significantly reduced the increase in creatinine levels. These findings revealed a valuable protective effect of against ifosfamide-induced nephrotoxicity.

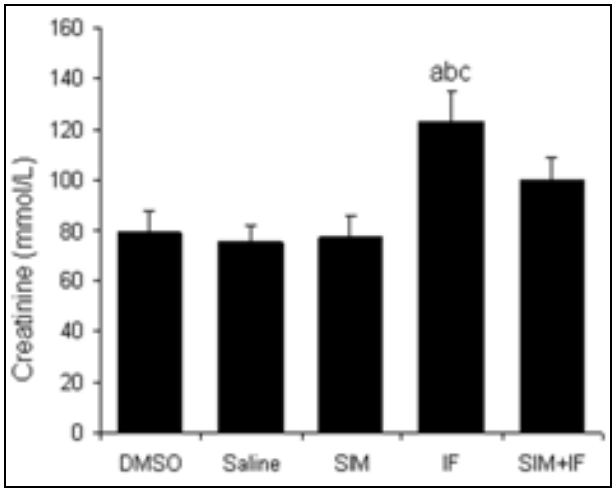

Fig. 4: Ifosfamide at 50 mg/kg/day significantly increased plasma creatinine levels when compared with saline [a]*P*< 0.05. Pretreatment with simvastatin (SIM) significantly reduced the elevation of plasma creatinine levels. [b]*P*< 0.05 when compared with combined group (IF+SM). [c]*P*< 0.05 when compared with DMSO and SIM groups.

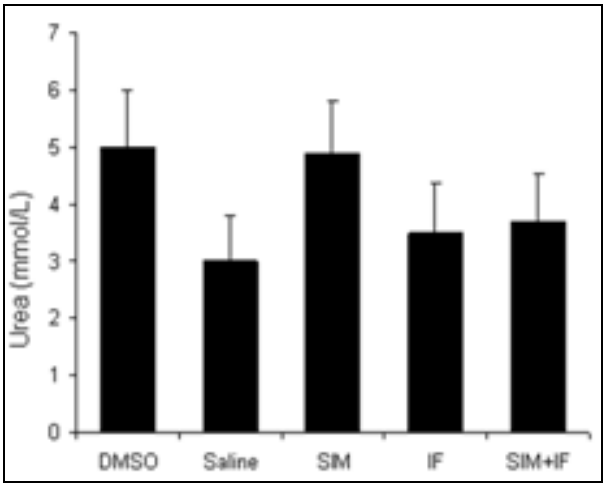

Fig. 5: The effect of ifosfamide (IF) and/or simvatatin (SIM) on plasma urea levels.

It has been shown that chemotherapy-induced nephrotoxicity is largely mediated by lipid per oxidation. The later was found to be well correlated with depletion of GSH and impaired antioxidant defense modalities (Mansour *et al.*, 1999). Measurement of MDA has been

utilized as an indicator of lipid per oxidation (Esterbauer *et al.*, 1991; Srour *et al.*, 2000). In here, levels of MDA were significantly increased in ifosfamide-treated group compared to those of saline group. In parallel, GSH was critically depleted in ifosfamide-treated group. This oxidative stress induced by ifosfamide might be due to decreased cellular Co-A, acetyl-CoA content and cellular ATP levels (Jez and Cahoon, 2004), or due to increased lactate dehydrogenase (Dubourg *et al.*, 2001).

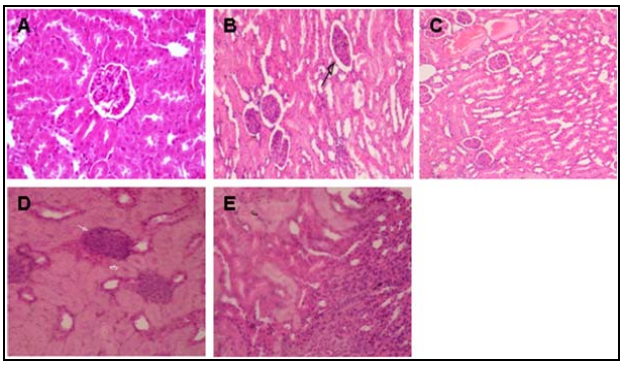

Fig. 6: a. Normal kidney morphology of DMSO group. Normal glomerulus (arrow). X: 100. b. Normal kidney morphology of normal saline group. X: 400. c. Ischemic changes (thin arrow) of rats administrated IF (100X). d. Microphotograph shows increasing of mesangial cells (black thin arrow) and ischemic changes of the tubules and interstitium (white arrow) of rats administrated IF+SIM (100X). e. photomicrograph of kidney section treated with SIM showing normal morphology (100X).

Previous studies indicated an important role of ROS in the development of nephrotoxicity by ifosfamide leading to oxidative renal damage (Arouma *et al.*, 1989). Results in the present study indicated that simvastatin significantly reduced MDA concentration in kidney tissue. This is probably due to free radicals scavenging and antioxidant properties of simvastatin (Stoll *et al.*, 2004).

Glutathione S-transferase is largely believed to facilitate conjugation of GSH with free radicals leading to formation of thioether bond masking their reactivity. It functions as a scavenger of ROS, including hydroxyl radicals, single oxygen, nitric oxide and peroxynitrite (Gutteridge *et al.*, 1989). Data of our study indicate that GSH increased when animals pretreated with simvastatin. More importantly, simvastain has not only prevented the decrease but also increased the GST activity when co-administered with ifosfamide. This might explore the mechanism by which simvastatin prevent nephrotoxicity-induced by ifosfamide. Results of antioxidant effects of simvastatin against chemotherapy-induced nephrotoxicity are in agreement with other studies (Abd Elbaky *et al.*, 2006).

Furthermore, results indicated that levels of catalase enzyme were highly induced by simvastatin but were not significantly reversed to normal levels when co-administered with ifosfamide. In addition, ifosfamide did not cause a dramatic change in the enzyme levels. This might be due to resistance of catalase toward ifosfamide particularly after either a long-term exposure or at high chemotherapy dose. In addition, results showed that ifosfamide induced serum creatinine, which is regarded as a sign of early glomurlus dysfunction (Levey *et al.*, 1988). Pretreatment with simvastatin has significantly inhibited this increase adding another evidence of simvastatin protective effect against ifosfamide-induced renal damage.

In summary, biochemical and histopathological findings indicate that administration of simvastatin inhibited the nephrotoxic effects of ifosfamide. Consequently, simvastatin should be considered as an important potential candidate in clinical trials designed to minimize ifosfamide-induced nephrotoxicity, a serious obstacle in treatment of cancer.

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