ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effects of Atorvastatin against Cisplatin Induced Nephrotoxicity in Rabbits

Ali H. Abady[1*], Ahmed R. Abu-Raghif[1], Ban J Qasim[2]

1.Department of Pharmacology, College of Medicine, AL-Nahrain University, Baghdad, Iraq.
2.Department of Pathology, College of Medicine, AL-Nahrain University, Baghdad, Iraq.

Manuscript Info

Manuscript History:

Received: 15 August 2015
Final Accepted: 22 September 2015
Published Online: October 2015

Key words:

Atorvastatin, Cisplatin, Induced Nephrotoxicity in Rabbits

Corresponding Author

Ali H. Abady

Abstract

Nephroprotective effect of atorvastatin was investigated in rabbits with acute renal injury induced by a single i.p. injection of cisplatin (6.5 mg/kg). Atorvastatin treatment (10 mg/kg/day, orally) was applied for 7 consecutive days, starting 4 days before cisplatin administration. Atorvastatin significantly reduced serum creatinine levels and increased serum albumin levels which were altered by cisplatin. Atorvastatin significantly compensated deficits in kidney tissue glutathione level,suppressed lipid peroxidation, and decreased the elevations of serum tumor necrosis factor-alpha resulted from cisplatin administration. Also, histopathological renal tissue damage mediated by cisplatin was mildly ameliorated by atorvastatin treatment. Atorvastatin showed no significant effect on serum urea, or inflammatory cell infiltration. It was concluded that atorvastatin represents a potential therapeutic option to protect against acute cisplatin nephrotoxicity commonly encountered in clinical practice.

INTRODUCTION

Cisplatin has been widely used for chemotherapy. It is potent, demonstrating one of the highest cure rates, for example, over 90% in testicular cancers. Cisplatin and related platinum-based therapeutics are now being used for the treatment of testicular, head and neck, ovarian, cervical, non-small cell lung carcinoma, and many other types of cancer (Sidik, 2003; Wang *et al*, 2005). Its use is mainly limited by two factors: acquired resistance to cisplatin and severe side effects in normal tissues especially renal tissues.

Cisplatin-induced nephrotoxicity is a major complication in the cancer therapy and had a dose limiting toxicity (Saleh *et al*, 2014). Several interrelated mechanisms of actions have been hypothesized to induce the nephrotoxicity. These include apoptosis and inflammatory mechanism, hypoxia, production of tumor necrosis factor (TNF)-α by renal parenchymal cells, and oxidative stress (Tanaka *et al*, 2005).

Atorvastatin is an HMG-CoA reductase inhibitor that exerts lipid independent benefits against renal injury in experimental states of chronic or acute renal function impairment as well as anti-inflammatory and antioxidant effects.

MATERIALS AND METHODS

Animals

Male New Zealand white rabbits, weighing 1.0 to 1.5 kg were obtained from local market in Baghdad. The animals were housed in the animal house of Al-Nahrain University at a maximum of three per cage on wood shavings with free access to food and water. The animals were fed on standard rodent pellet diet and water ad libitum. Before starting the study, the animals were left for 48 hours to acclimatize to the animal room conditions which were maintained on an environment of controlled temperature, a 12 hours light-dark cycle, and proper ventilation. The study protocol was approved by the ethical committee of College of medicine, Al-Nahrain University.

Drugs
Cisplatin solution was obtained from ebewe pharma, Austria. Atorvastatin powder was obtained from Julphar pharma, UAE. The required doses were taken and reconstituted in 5 ml of distilled water just before oral administration.

Experimental Design
The rabbits were randomly divided into three equal groups (n= 8, each). The first group received a single i.p. injection of normal saline (vehicle of cisplatin) and served as negative control. Nephrotoxicity was induced in animals of the second and third groups by a single i.p.
injection of cisplatin at a dose of 6.5 mg/kg. The second group received distilled water orally for 7 days and served as positive control. The third group was treated with treated with atorvastatin 10mg/ kg/ day for 7 consecutive days starting 4 day before cisplatin administration.

Sample preparation and biochemical analysis
The animals were sacrificed 72 hours following cisplatin administration. The blood aspirated from rabbits' heart was immediately transferred into plastic test tubes without anticoagulant and allowed to clot overnight at 4°C before centrifugation for 15 minutes at 1000 rpm.
The supernatant (serum) was carefully aspirated and transferred into another clean plastic test tube, then stored in refrigerator at -20°C for subsequent measurement of serum urea, serum creatinine, and serum albumin using colorimetric assay kits according to the instructions of the manufacturer (BioMérieux, France). Serum TNF-α levels was measured using ELISA kit according to the instructions of the manufacturer (Cusabio, China). Kidneys were extracted free of their peritoneal attachments. Right kidneys were placed in 10%
neutral buffered formalin for histopathological examination while left kidneys were homogenized in Phosphated buffered saline (PBS) and stored overnight in the refrigerator at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the
homogenates were centrifuged for 5 minutes at 5000 rpm at 2 - 8°C. The supernatant was collected and stored at -20°C to be used for the estimation of tissue glutathione (GSH) and tissue malondialdehyde (MDA) by ELISA according to the instructions of the kit's manufacturer (Cusabio, China).

Histopathological examination of renal tissue
The right kidneys were fixed in10% formalin solution and then dehydrated in ascending grades of alcohol and embedded in paraffin. Sections at 5µm-thickness were taken, stained with hematoxylin and eosin (H&E) and examined under light microscope by a pathologist
unaware of the treatment conditions.
Scores were assigned according to the percentage of cortical tubules having epithelial necrosis Scores were assigned according to the percentage of cortical tubules having epithelial necrosis by examining 10 high power fields per kidney under higher power magnification (x200) (Table 1).

Table 1: Cortical tubular necrosis scores

Score	Score Percentage of tubules with necrosis
0	0%
1	<10%
2	10%-25%
3	26%-75%
4	>75%

The number of neutrophils per high-power field (x400) was counted by investigator blinded to treatment conditions and the average number in 10 fields of each sample was recorded16.

Statistical analysis

Statistical analysis was performed using IBM SPSS version 21 statistical software and Microsoft Excel 2010. Descriptive statistics for the numerical data were formulated as mean and standard error. Parametric independent samples t-tests were carried out for comparison between two groups whenever data were normally distributed, while non-parametric Mann-
Whitney U tests were carried out whenever the data were not normally distributed. The significant difference level (p-value) is below 0.05.

RESULTS

Effects of atorvastatin on renal function and TNF-α
Cisplatin administration (6.5 mg/ kg I.P.) resulted in significant increase in serum urea, serum creatinine, serum TNF-α and decrease in serum albumin levels compared to the negative control. Treatment with atorvastatin resulted in a significant reduction in serum creatinine, serum TNF-α and elevating serum albumin levels but not to the normal ranges. Atorvastatin had no significant effect on serum urea level (Tables 2, 3).

Effects of atorvastatin on oxidative stress parameters
Treatment with atorvastatin significantly ameliorated the depletion of the antioxidant defense mechanisms (GSH level) and suppressed lipid peroxidation (MDA level) in renal tissue resulted from cisplatin administration (Tables 2, 3).

Effects of atorvastatin on renal histopathology
Kidney sections of the positive control group showed renal damage mostly in form of glomerular and tubular injury. The tubular injury is seen mostly in the proximal convoluted tubules. Some of the renal tubules are distended and dilated with proteinaceous casts especially in severe nephrotoxicity. Glomerular damage including glomerular capillary necrosis with obliteration of capillary lumens and increase of cellularity as a result of acute
inflammatory cells infiltration (neutrophils) and mesangial cells hyperplasia resulting in the reduction of the glomerular filteration rate and protein urea with cast formation in renal tubules. Section of kidney tissue of Atorvastatin group showed histological picture of acute renal injury similar to that of cisplatin group, with presence of tubule dilatation and proteinaceous casts, but less extensive; 79% of cortical tubules were found to be necrotic in cisplatin group while it was 36% in atorvastatin group (Figure 1).

Atorvastatin seems to have mild effect on tubular necrosis score in this study. Lower score was measured in atorvastatin group compared to the positive control group and the difference was statistically significant.

The study revealed no statistical significant difference between the positive control group and the atorvastatin group in neutrophil count (Tables 2, 3) (Figure 2).

Table 2: Comparison between negative control group (normal group) and positive control group (cisplatin group)

Groups Parameters	Negative control group (normal group)	Positive control group (cisplatin group)
Serum urea	Mean: (35.34±1.40)	Mean: (100.23±0.86)*
Serum creatinine	Mean: (0.42±0.09)	Mean: (4.84±0.08)*
Serum albumin	Mean: (2.92±0.03)	Mean: (0.92±0.02)*
Serum TNF-α	Mean: (42.91±1.84)	Mean: (322.55±5.33)*
Tissue MDA	Mean: (180.50±0.64)	Mean: (505.39±1.70)*
Tissue GSH	Mean: (62.41±0.60)	Mean: (11.40±0.19)*

Tubular necrosis score	Mean: (00.00±0.00)	Mean: (3.63±0.18)*
Neutrophil count	Mean: (00.00±0.00)	Mean: (5.03±0.92)*

*Statistically significant difference ($p \leq 0.05$).

Table 3: Comparison between positive control group (cisplatin group) and treatment group (atorvastatin group).

Groups Parameters	Positive control group (cisplatin group)	atorvastatin group
Serum urea	Mean: (100.23±0.86)	Mean: (99.48±0.84)
Serum creatinine	Mean: (4.84±0.08)	Mean: (2.92±0.08)*
Serum albumin	Mean: (0.92±0.02)	Mean: (1.30±0.05)*
Serum TNF-α	Mean: (322.55±5.33)	Mean: (224.82±7.37)*
Tissue MDA	Mean: (505.39±1.70)	Mean: (488.55±1.38)*
Tissue GSH	Mean: (11.40±0.19)	Mean: (22.15±0.71)*
Tubular necrosis score	Mean: (3.63±0.18)	Mean: (2.75±0.16)*
Neutrophil count	Mean: (5.03±0.92)	Mean: (3.32±0.57)

*Statistically significant difference ($p \leq 0.05$).

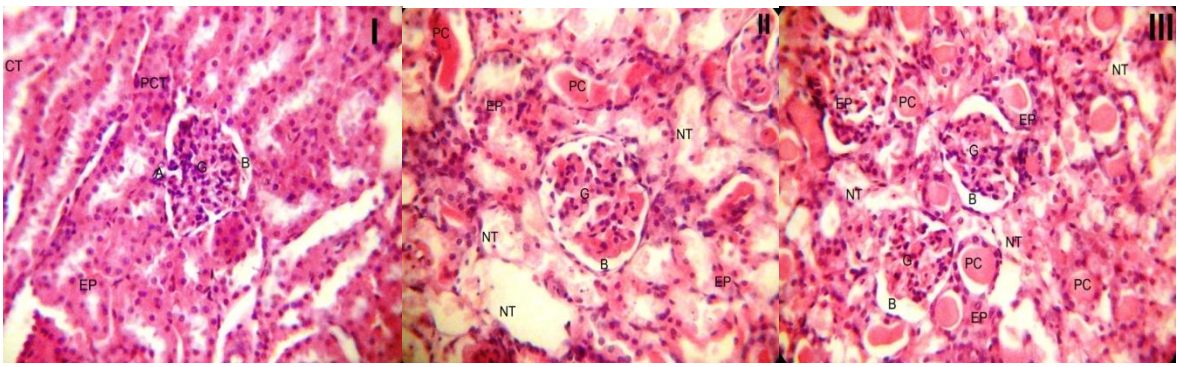
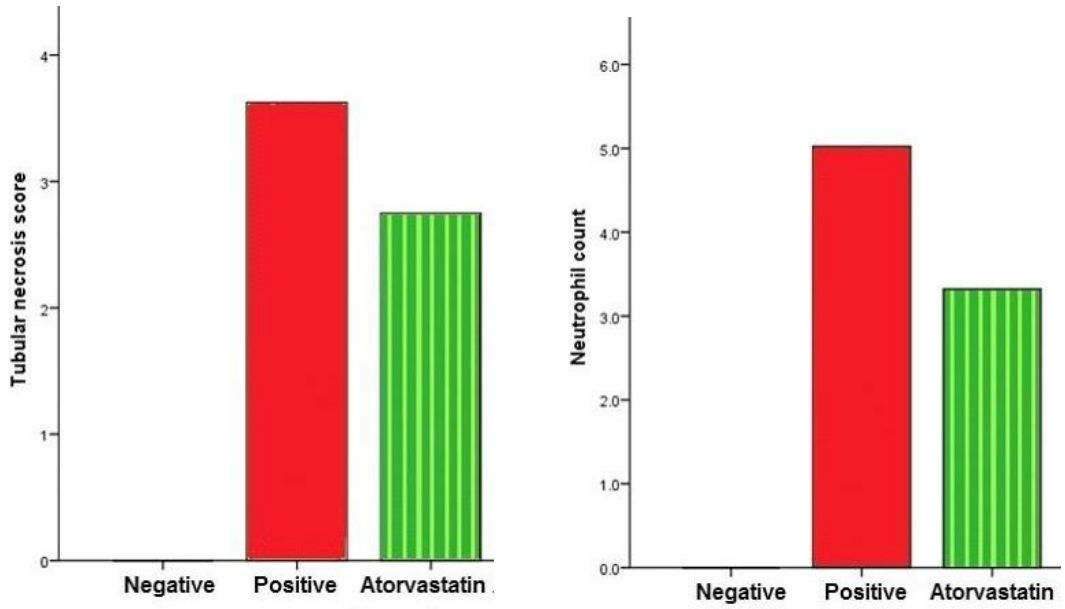


Figure 1: Section of right kidneys of a White rabbits of study groups(I: Normal group, II: Cisplatin group, III: Atorvastatin group) on day 8 of experiment 72 hours after administration of 6.5mg/ kg cisplatin I.P. 40X, H&E.A: Afferent glomerular arteriole, B: Bowman's capsule, CT: Collecting tubule, EP: Epithaelial cells, G: Glomerulus, NT: Necrotic tubule, PC: proteinaceous cast, PCT: Proximal convoluted tubule.



A: Tubular necrosis scores B: Neutrophil count

Figure 2: Histopathological parameters of study groups on day 8 of experiment (72 hours after nephrotoxicity induction by cisplatin).

DISCUSSION

Cisplatin is one of the potent anticancer agents that is not only cause nephrotoxicity but also induces vascular endothelial dysfunction mediated by inflammation and oxidative stress. Cisplatin-induced nephrotoxicity is a major complication in the cancer therapy and had a dose limiting toxicity (Saleh *et al*, 2014). In the present study, using an experimental model of cisplatin induced nephrotoxicity in rabbits (single dose of 6.5 mg/ kg I.P.) characterized by alterations in renal function as a significant increase in serum creatinine, urea, albumin, and TNF-α as well as increased MDA and decreased GSH levels compared to normal group and this results are compatible with those observed by many other studies (Gamal el-Din *et al*, 2006; Kim *et al*, 2010; Saleh *et al*, 2014). It has been reported that cisplatin induced nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney manifested by increased MDA as well as a decrease in anti-oxidant activity with depletion of GSH which is in agreement with the results obtained in the present study (Joy *et al*, 2008).

The present study along with other studies have shown increased tissue content of inflammatory mediators together with inflammatory cell infiltration, suggesting that inflammation plays an important role in cisplatin induced renal injury (Fouad *et al*, 2010; Miyawaki *et al*, 2012; Domitrovic *et al*, 2013).

Although the precise inflammatory mechanisms are unknown, marked attenuation of cisplatin induced renal damage by inhibition of tumor necrosis factor alpha (TNF-α) indicates that TNF-α has a central role of mediation cisplatin induced renal injury (Kim *et al*, 2010). Histopathological results of this study showed that cisplatin had induced severe kidney damage characterized by severe necrosis of tubular cells and inflammatory cell infiltration that could be correlated with the harmful effects of cisplatin parallel to high MDA and low GSH levels. These results are in agreement with the results in other studies (Gamal el-Din *et al*, 2006; Fouad *et al*, 2010; Kim *et al*, 2010; Miyawaki *et al*, 2012; Saleh *et al*, 2014).

The increase in thickness of the glomeruli basement membrane could be a result of membrane disturbance due to cisplatin administration. Pirincci mentioned that lipid peroxidation mediated by oxygen free radicals causes destruction and damage to cell membranes resulting in necrosis (Pirincci *et al*, 2009).

Results of the present study showed that pretreatment with atorvastatin (10mg /kg /day) for 4 days before cisplatin administration followed by 3 more days had mild effects against cisplatin induced nephrotoxicity. Atorvastatin has no significant effect on serum urea in this study. Although it reduced serum creatinine levels and increased serum albumin levels in a statistically significant difference, the results still beyond the normal levels. Atorvastatin alleviates inflammation as it was shown by the results of the present study as atorvastatin significantly reduced serum TNF-α level. Previous studies had indicated that atorvastatin as well as other statins exert anti inflammatory effect through stimulation of down regulation of proinflammatory cytokine (IL- 6) and chemokine

(IL- 8) expression also reduction in C-reactive protein, and TNF-α and have direct effects on the gene expression and function of cells of both the innate and adaptive immune systems, including endothelial cells, macrophages, dendritic cells and T cells (Sever *et al*, 2003; Ozbek *et al*, 2009; Panonnummal *et al*, 2011; Mehany *et al*, 2013). Atorvastatin reduced tissue MDA and increased tissue GSH levels in statistically significant difference but not to the normal levels.

Histopathological examination of kidney tissues in the present study showed that atorvastatin had mild effect. Although atorvastatin had decreased cortical tubular necrosis scores in a statistically significant difference, Degenerative histopathological changes still present characterized by necrosis, glomerular atrophy, dilated tubules, and presence of proteinacious casts but to a lesser extent. Atorvastatin has no significant effect on inflammatory cells infiltration in the present study.

This study is not in agreement with the results of the previous studies which stated that atorvastatin had greatly maintained normal kidney functions, significantly compensated deficits in kidney tissue glutathione level, suppressed lipid peroxidation, and greatly ameliorated the histopathological changes, examination showed mild inflammatory cells infiltration, mild congestion, no atrophy, and tubules almost back to normal (Ozbek *et al*, 2009; Panonnummal *et al*, 2011; Mehany *et al*, 2013). The difference in the results may be due to the difference in the duration of the experiments; atorvastatin was administered for 17 days in experiments of Ozbek et al., 2009 and Panonnummal et al., 2011 and for 16 days in experiment of Mehany et al., 2013 which may allowed more time for atorvastatin to exert its nephroprotective effect. Also, it may be due to the difference in the nephrotoxic agents used in the experiments; Potassium Dichromate, Vancomycin and Gentamicin were used in the previous studies.

CONCLUSION

Inflammation and oxidative stress were found to be major factors in the nephrotoxicity of cisplatin. Atorvastatin had some protective effects against cisplatin induced nephrotoxicity in rabbits. The treatments were found to induce significant effects in alleviating inflammation & oxidative stress. Further experimental study is necessary to view the nephroprotective effect of atorvastatin, a longer treatment period is recommended.

REFERENCES

Ali, B. H., Mansour Al-Moundhri, M. Tag Eldin, Abderrahim Nemmar, Sultan Al-Siyabi, and Kanthi Annamalai (2008): Amelioration of cisplatin-induced nephrotoxicity in rats by tetramethyl pyrazine, a major constituent of the Chinese herb Ligusticumwallichi. Experimental biology and medicine. 233(7): 891-896.

Domitrović, Robert, Olga Cvijanović, Ester Pernjak-Pugel, Marko Škoda, Lorena Mikelić, and ŽeljkaCrnčević-Orlić. (2013) :Berberine exerts nephroprotective effect against cisplatin induced kidney damage through inhibition of oxidative/nitrosative stress, inflammation, autophagy and apoptosis. Food and chemical toxicology. 62: 397- 406.

Fouad, Amr A., Ali Ibrahim Al-Sultan, Shereen M. Refaie, and Mohamed T. Yacoubi. (2010): Coenzyme Q10 treatment ameliorates acute cisplatin nephrotoxicity in mice. Toxicology. 274(1): 49-56.

Gamal el-Din, Ayman M., and Abdullah M. Al-Bekairi (2006): Carvedilol, a beta adrenoceptor blocker with antioxidative potential, attenuates cisplatin-induced nephrotoxicity in rats. *J Applied Sci Res* 2: 331-355.

Joy, Jisha, and Cherupally Krishnan Nair (2008): Amelioration of cisplatin induced nephrotoxicity in Swiss albino mice by Rubiacordifolia extract." Journal of cancer research and therapeutics. 4(3): 111.

Kim, Myung-Gyu, Ha Na Yang, Hye-Won Kim, Sang-Kyung Jo, Won Yong Cho, and Hyoung-Kyu Kim (2010): IL-10 mediates rosiglitazone-induced kidney protection in cisplatin nephrotoxicity." Journal of Korean medical science. 25(4): 557-563.

Mehany, H. A., Abo-youssef, A. M., Ahmed, L. A., Arafa, E. S. A., & Abd El-Latif, H. A. (2013). Protective effect of vitamin E and atorvastatin against potassium dichromate-induced nephrotoxicity in rats. Beni-Suef University Journal of Basic and Applied Sciences, 2(2), 96-102.

Miyawaki, Yuki, Masaaki Ueki, Masaki Ueno, Takehiko Asaga, Masaaki Tokuda, and Gotaro Shirakami (2012): D-allose ameliorates cisplatin-induced nephrotoxicity in mice." The Tohoku journal of experimental medicine. 228(3): 215-221.

Ozbek, E., Cekmen, M., Ilbey, Y. O., Simsek, A., Polat, E. C., & Somay, A. (2009). Atorvastatin prevents gentamicin-induced renal damage in rats through the inhibition of p38-MAPK and NF-kB pathways. Renal failure, 31(5), 382-392.

Panonnummal, R., Varkey, J., & Dinoop, D. R. (2011). Protective effect of atorvastatin against vancomycin induced nephrotoxicity in albino rats.Pharmacie Globale, 2(8), 1-6.

Pirincci, P. A., & Bolkent, S. (2009): The role of GLP-2 on oxidant-antioxidant system at an in vivo mouse model of intestinal injury induced by TNF-alpha/Act D. *FEBS JOURNAL* 276: 186-18.

Saleh, Rasha M., Walaa F. Awadin, Yousef Y. Elseady, and Faheim E. Waheish (2014): Renal and cardiovascular damage induced by cisplatin in rats." Life Sci J. 11(2): 191-203.

Sever, Peter S., et al. (2003): Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. *The Lancet* 361.9364: 1149-1158.

Siddik, Zahid H.(2003): Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene. 22(47): 7265-7279.
Tanaka, Tetsuhiro, Ichiro Kojima, Takamoto Ohse, Reiko Inagi, Toshio Miyata, Julie R. Ingelfinger (2005): Hypoxia-inducible factor modulates tubular cell survival in cisplatin nephrotoxicity. American Journal of Physiology-Renal Physiology. 289(5): F1123-F1133.

Wang, Dong, and Stephen J. Lippard (2005): Cellular processing of platinum anticancer drugs. Nature reviews Drug discovery. 4(4): 307-320.