Journal of Animal Research: v.5 n.4, p. 789-793. December 2015

DOI: 10.5958/2277-940X.2015.00131.X

Effect of Aminoguanidine-Hemisulphate on Amikacin Induced Hematological Alterations in Wistar Rats

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Received: 27 June, 2015 **Accepted:** 10 October, 2015

ABSTRACT

In the present study, haematological alterations induced by intraperitoneal administration of amikacin and the effect of aminoguanidine-hemisulphate alone and their combination was studied in wistar rats of either sex.Twenty-four healthy wistar rats divided into 4 groups (I, II, IIIand IV) were taken for the study. The intra-peritoneal administration of amikacinat at a dose rate of 15 mg/kg body weight for 28 days (Group-II) caused a significant decrease in haematological parameters like Hb, PCV, TEC and TLC as compared to control-group. Although a significant increase in parameters was found in aminoguanidine treated-rats on day 15th and 29th as compared to day zero within same group. However after the co-administration of amikacin and aminoguanidine, a non-significant change was found in same parameters (Hb, TEC, PCV and TLC) as compared to control.

Keywords: Amikicin, aminoguanidine-hemisulphate, haematological alterations, wistar rats

Amikacin is a semi-synthetic aminoglycoside derived from *Streptomyces kanamyceticus* in 1972 (Gilbert *et al.* 1995). It is primarily used against gram-negative-aerobic-organisms (Edson and Terrell, 1999) and also against gram-positive-pathogens (Isaksson *et al.* 1991). Amikacin can also be used in combination with beta-lactam-antibiotics to produce synergistic effect and also broaden the activity against both gram positive and gram negative bacteria (Sandhu *et al.* 2007). Most often it is used to treat severe hospital-acquired infections of gram-negative bacteria with multidrug resistance and also as a second-line drug for anti-tuberculosis drugs (Edson and Terrell, 1999). The pharmacokinetic behavior of the drug is known to be influenced by pathophysiological conditions (Zaske *et al.* 1992).During severe sepsis and septic shock, amikacin disposition is altered by an increased volume of distribution and a reduced total body clearance, decreased protein binding, and organ failure (Roberts and Lipman, 2006).Its nephrotoxicity and ototoxicity have widely guided attempts to rationalize the drug dosage strategy (Barclay and Begg, 1994). It produces free-radicals/reactive oxygen species (ROS) which participate in the patho-physiology of amikacin-induced-nephrotoxicity (Parlakpinar *et al.* 2004). Amikacin administration also led to granulovacular tubular degeneration in light microscopic examination and myeloid bodies, mitochondrial electron dense material deposition and mitochondrial swelling in the proximal tubule epithelium in the electron microscopic evaluation (Kubra

et al. 2009). Aminoguanidine-hemisulfate is an effective antioxidant (Ihm *et al.* 1999) and a free radical scavenger (Szabo *et al*. 1997) which is well-known to protectnephrotoxicity (Parlakpinar*et al.* 2004). It inhibits inducible nitric oxide synthase (iNOS) in a selective and competitive manner, leading to decreased generation of nitric-oxide (Misko *et al*. 1993) and free-radicals. The protective effects of AG have been previously addressed in other models of cell damage induced by drugs (Aoki *et al.* 1997; Gardner *et al.* 1998). In addition, the beneficial effects of AG in various experimental models of inflammation have also been reported (Shiomi *et al.* 1998). Recently, Al-Shabanah *et al.* (2000) showed that AG protects mice against hepatotoxicity induced by carbon tetrachloride.

MATERIALS AND METHODS

Chemicals

Chemical	Source/Company Name
Amikacin-sulphate	MACLEODS Maharashtra
Aminoguanidine-hemisulfate	Sigma chemicals

Animals and Experimental Protocol

In the present investigation, twenty-four healthy wistar rats were taken for the study. After acclimatization, rats were randomly divided into 4 groups (I, II, III and IV) and each group comprise 6 animals. Group-I served as control to which normal saline was administered. For sub-acute study rats of group-II and group-III were treated with amikacin (15mg/Kg BW) and aminoguanidine-hemisulphate (20mg/Kg BW)daily for 28 days intra-peritoneally, respectively. In group-IV rats, amikacin and aminoguanidine-hemisulphatewere co-administeredat their respective doses (Table 1).

Blood samples of about 2-4ml were collected from retro-orbital sinus of rats on zero, 15th and 29th days using capillary-tubes in aliquots containing anticoagulant heparin (strength 10 IU/ml of blood) and sodium EDTA were used for haematological estimation.

Statistical analysis

A standard statistical procedure was followed. The data collected during the experiment was subjected to analysis of variance under completely randomized design (CRD) and the level of significance was tested using Duncan Multiple Range Test (Duncan, 1955) at 5% ($P < 0.05$) level.

RESULTS AND DISCUSSION

The effect of amikacin, aminoguanidineand their co-administration after intra-peritoneal administration in wistar-rats shows a significant decrease in haematological parameters (Hb, PCV, TEC, TLC) on day 15th and 29th in amikacin treated wistar-rat of group-II as compared to day zero (Table 2,3,4,5) as found by Dinev *et al.* (2007) in goats administered amikacin for 5 days. The significant decrease in all these parameters is due to the infliction of stress in rats as a result of amikacin administration (Melillo, 2007; Jenkins, 2008). Although a significant increase in same parameters is found in aminoguanidine treated-rats of group III on day 15th and 29th as compared to day zero within same group. Preedy and Hammond (1991) also reported increase in all haematological parameters with daily administration of aminoguanidine. Such improvement in haematological indices is due to free radical scavenging effect of aminoguanidine. However, these indices were altered with drug exposure of amikacin. Such attenuation of altered haematological parameters by pretreatment with aminoguanidine is also reported by Mansour *et al.* (2002) in rats administered single dose of cisplatin, pretreated with aminoguanidine. However after the co-administration of amikacin and aminoguanidine, a significant decrease in parameters is found in group-IV wistar-rats on day 15th while a significant increase on day 29 as compared to day zero. The haematological parameters shows a significant decrease on day 15th and 29th in amikacin treated wistar

Table 1: The experimental design

Treatment Group(s) (n=6)	Dose (mg/kg)	Exposure-Period	Routes
Group-I (Control)	Normal saline	28 days	I.P.
Group-II (Amikacin sulphate)	15mg/kg/day	28 days	I.P.
Group-III (Aminoguanidine-hemisulphate)	20mg/kg/day	28 days	I.P.
Group-IV (Amikacin sulphate +Aminoguanidine-hemisulphate)	15mg/kg + 20mg/kg/day	28 days	I.P.

Table 2: Showing the effect of amikacin, aminoguanidine and their co-administration on Haemoglobin (g/dl) after intra-peritoneal administration in wistar-rats

Treatment Group(s)	Treatment period		
	Day zero (n=6)	Day fifteen (n=6)	Day twenty-nine (n=6)
Group-I (Control)	12.79 ± 0.42^{aA}	12.58 ± 0.37^{aB}	12.68 ± 0.30^{aB}
Group-II (Amikacin)	12.83 ± 0.13^{aA}	10.91 ± 0.27^{bC}	10.07 ± 0.11^{cC}
Group-III (Aminoguanidine)	13.02 ± 0.11^{cA}	13.96 ± 0.16^{bA}	14.79 ± 0.21^{aA}
Group-IV (Amikacin + Aminoguanidine)	12.64 ± 0.16^{aA}	11.23 ± 0.19^{Cc}	12.16 ± 0.09^{bB}

Values are in Mean± SE, Similar superscript do not differ significantly at 5% (P<0.05).
Capital superscripts represent significance between the groups.
Small superscripts represent significance within the groups.

Table 3: Showing the effect of amikacin, aminoguanidine and their co-administration on PCV (%) after intra-peritoneal administration in wistar-rats

Treatments Group(s)	Treatment-period		
	Day zero (n=6)	Day fifteen (n=6)	Day twenty-nine (n=6)
Group-I (Control)	43.69 ± 0.15^{aA}	43.63 ± 0.18^{aA}	43.83 ± 0.12^{aA}
Group-II (Amikacin)	43.42 ± 0.19^{aA}	42.67 ± 0.16^{bB}	42.22 ± 0.11^{bC}
Group-III (Aminoguanidine)	43.14 ± 0.21^{bA}	43.57 ± 0.17^{abA}	44.04 ± 0.12^{aA}
Group-IV (Amikacin + Aminoguanidine)	43.16 ± 0.18^{aA}	42.69 ± 0.21^{aB}	42.99 ± 0.17^{aB}

Values are in Mean± SE, Similar superscript do not differ significantly at 5% (P<0.05).
Capital superscripts represent significance between the groups.
Small superscripts represent significance within the groups.

Table 4: Showing the effect of amikacin, aminoguanidineand their co-administration on total Erythrocyte count (million/µl) after intra-peritoneal administration in wistar-rats

Treatment Group(s)	Treatment-period		
	Day zero (n=6)	Day fifteen (n=6)	Day twenty-nine (n=6)
Group-I (Control)	7.27 ± 0.04^{aA}	7.32 ± 0.04^{aB}	7.29 ± 0.06^{aB}
Group-II (Amikacin)	7.31 ± 0.04^{aA}	6.95 ± 0.03^{bC}	6.86 ± 0.05^{bC}
Group-III	7.29 ± 0.08^{bA}	7.64 ± 0.09^{aA}	7.71 ± 0.07^{aA}
Group-IV (Amikacin+ Aminoguanidine)	7.18 ± 0.04^{aA}	7.01 ± 0.03^{bC}	7.15 ± 0.03^{aB}

Values are in Mean± SE, Similar superscript do not differ significantly at 5% (P<0.05).
Capital superscripts represent significance between the groups.
Small superscripts represent significance within the groups.

Table 5: Showing the effect of amikacin, aminoguanidineand their co-administration on total Leukocyte count (million/µl) after intra-peritoneal administration in wistar-rats

Treatment Group(s)	Treatment-Period		
	Day zero (n=6)	Day fifteen (n=6)	Day twenty-nine (n=6)
Group-I (Control)	10.95 ± 0.160^{aA}	11.19 ± 0.10^{aB}	10.90 ± 0.23^{aB}
Group-II (Amikacin)	10.88 ± 0.21^{aA}	9.76 ± 0.17^{bC}	9.60 ± 0.16^{bC}
Group-III (Aminoguanidine)	11.04 ± 0.13^{cA}	12.31 ± 0.16^{bA}	13.74 ± 0.09^{aA}
Group-IV (Amikacin + Aminoguanidine)	10.98 ± 0.14^{aA}	9.92 ± 0.14^{bC}	10.69 ± 0.21^{aB}

Values are in Mean± SE, Similar superscript do not differ significantly at 5% (P<0.05).
Capital superscripts represent significance between the groups.
Small superscripts represent significance within the groups.

rats (group-II) as compared to control group (group-I). However the co-administration of amikacin and aminoguanidine shows a significant decrease on day 15th and significant increase on day 29th as compared to day 15th within the group-IV as compared to control rats (group-I).

CONCLUSION

The study suggests aminoguanidine hemisulfate has a protective effect on the haematological alterations as induced by the amkacin. This can be attributed to the counter-balancing act of amikacin leading to decrease and aminoguanidine resulting in increase in these parameters, revealing a non-significant change. Such improvement in haematological indices is due to free radical scavenging effect of aminoguanidine.

Acknowledgements

Authors are highly thankful to Director Research Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu (SKUAST-J), Dean Faculty of Veterinary Sciences SKUAST-J and HOD, Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Sciences SKUAST-J.

REFERENCES

Al-Shabanah, O.A., Alam, K.S., Nagi, M.N., Al-Rikabi, A.C. and Al-Bekairi, A.M., 2000. Protective effect of aminoguanidine, a nitric oxide synthase inhibitor, against carbon tetrachloride induced hepatotoxicity in mice. *Life Sci.,* **66:** 265–270.

Aoki, K., Ohmori, M., Takimoto, M., Ota, H. and Yoshida, T. 1997. Cocaine-induced liver injury in mice is mediated by nitric oxide and reactive oxygen species. *Eur. J. Pharmacol.,* **336**: 43–49.

Barclay, M.L. and Begg, E.J. 1994. Aminoglycoside toxicity and relation to dose regimen. *Adv. D. Reac. Toxico. Rev.*, **13**: 207-234.

Duncan, D. B. 1995. Multiple range and Multiple F-tests. *Biometrics.*, **11**:1-14.

Dinevi, T., Zapryanova, D. and Lashev, L. 2007. Changes in Some Blood Biochemical and Haematological Parameters in Goats after Aminoglycoside and Aminocyclitol. *Turkish J. Vet. Anim. Sci.*, **31**: 179-188.

Edson, R.S. and Terrell, C.L. 1999. The aminoglycosides. *Mayo Clin.Proc.,* **74**: 519-528.

Gardner, C.R., Heck, D.E. and Yang, C.S. 1998. Role of nitric oxide in acetominophen-induced hepatotoxicity in the rat. *Hepatology.,* **27**: 748–754.

Gilbert, D.N. 1995. Aminoglycoside.In:Mandell, G.L., Bennett, J. E., Dolin, R., (eds) Douglas and Bennett's Principles and practice of infectious diseases. 279-301. *New York: Churchill Livingston*.

Ihm, S.H., Yoo, H.J., Park, S.W. and Ihm, J. 1999. Effect of aminoguanidine on lipid peroxidation in streptozotocin induced diabetic rats. *Metabolism.,* **48**: 1141-1145.

Isaksson, B., Hanberger, H., Maller, R., Nilsson, L.E. and Nilsson, M. 1991. Synergistic post-antibiotic effect of amikacin and beta-lactam antibiotics on *Enterococcus faecalis*. *Antimicr. Age. Chem.,* **27**: 9-14.

Jenkins, J.R. 2008. Rabbit diagnostic testing. *J. Exo. Pet Med.,* **17**: 4-15.

Kubra, K., Semih, G., Safak, Eroz., Fyyaz, Ozdemir., Hulya, Ulusoy. and Sukru, U. 2009. Amkicin Induced Nephropathy; Is There Any Protective Way. *Ren Fail,* **29**: 23-27.

Mansour, M.A., Mostafa, A.M., Nagi, M.N., Khattab, M.M. and Al-Shabanah, O.A. 2002. Protective effect of aminoguanidine against nephrotoxicity induced by cisplatin in normal rats. *Comp. Biochem. Physio.*, **132**: 123–128.

Melillo A. 2007. Rabbit clinical pathology. *J. Exo. Pet Med.,* **16**:135-145.

Misko, T.P., Moore, W.M., Kasten, T.P., Nickols, G.A., Corbett, J.A., Tilton, R.G., McDaniel, M.L., Williamson, J.R. and Currie, M.G. 1993. Selective inhibition of the inducible nitric oxide syntheses by aminoguanidine. *European J. Pharm.*, **233**: 119-122.

Parlakpinar, H., Koc M., Polat, A.,Vardi, N., Ozer, M.K., Turkoz, Y. and Acet, A. 2004. Protective effect of aminoguanidine against nephrotoxicity induced by amikacin in rats. *Urol. Res.,* **32**(4): 278-282.

Preedy, V.R. and Hammond, B. 1991. An Investigation into the Effects of Aminoguanidine Treatment on the Plasma and Blood of Free-fed and Dietary-restricted Rats. *J. Phar. Pharm.,* **43**: 337-41.

Roberts, J.A. and Lipman, J. 2006. Antibacterial dosing in intensive care: pharmacokinetics,degree of disease and pharmacodynamics of sepsis. *Clin. Pharm.*, **45**: 755-773.

Sandhu, J.S., Sehgal, A., Gupta, O. and Singh, A. 2007. Aminoglycoside Nephrotoxicity Revisited. *J. Ind. Aca. Clin. Med.*, **8**: 331-333

Shiomi, M., Wakabayashi, Y. and Sano, T. 1998. Nitric oxide suppression reversibly attenuates mitochondrial dysfunction and cholestasis in endotoxemic rat liver. *Hepatology* **27**: 108–115.

Szabo, C., Ferrer-Sueta, G., Zingarelli, B., Southan, G.J., Salzman, A.L. and Radi, R. 1997.

Mercaptoethylguanidine and guanidine inhibitors of nitric-oxide synthase react with peroxynitrite and protect against peroxynitrite-induced oxidative damage. *J. Bio Che.*, **272**: 9030-9036.

Zaske, D.E., Evans, W.E., Schentag, J.J. and Jusko, W.J. 1992. Applied Pharmacokinetics.Principles of Therapeutic Drug Monitoring, 3rd edision Vancouver, WA: *App. Ther.,* **14:** 1-47.