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Research Article

Role of Ascorbic Acid on Gentamicin Induced Alteration in Plasma Lipid Profile

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Abstract

Alteration in lipid profile is associated with cardiovascular disorders. Antibiotics may have the ability to alter plasma lipid profile. Keeping in mind this fact the study was carried out to evaluate the ability of the aminoglycoside antibiotic gentamicin to alter plasma lipid profile of rabbit blood plasma and to find out the role of ascorbic acid as a suppressor of lipid profile alteration. Animals divided into different experimental groups were treated with gentamicin and ascorbic acid. Results reveal that gentamicin has the capacity to alter plasma lipoid profile that was controlled by the well known antioxidant ascorbic acid.

Keywords: Lipid profile, cardiovascular disorders, gentamicin, ascorbic acid

Introduction

The term plasma lipid profile considers measurement of certain components of plasma including total cholesterol (TCh), high density lipoprotein cholesterol (HDL-Ch), triglycerides (Tg), low density blipoprotein cholesterol (LDL-Ch) and very low density lipoprotein cholesterol (VLDL-Ch). TCh consists of all the cholesterol present in different lipoproteins like high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL). HDL-Ch is believed to play a vital role in the removal of excess cholesterol from the blood vessel wall to the liver for excretion [1-2]. On the other hand, LDL-Ch (high cholesterol content) is responsible for the deposition of cholesterol in the inner wall of the artery, resulting in atherosclerosis. The neutral fat Tg are found in the tissue and blood. Adipose tissue is the main store house of calories and comprises of Tg. The large group of macromolecule VLDL-Ch is synthesized and secreted mainly by liver and intestinal mucosal cells, and contain large proprtion of Tg [3]. Tg may also have a role in the development of coronary heart disease (CHD) [4].

Many diseases are linked with elevated plasma lipid profile. Increased level of TCh, Tg, LDL-Ch, VLDL-Ch and decreased level of HDL-Ch [5] are found in diabetic patients. Elevated level of serum TCh, Tg, LDL-Ch and decreased level of HDL-Ch are found in CHD [6]. In patients with leukemia and Hodgkinis disease serum TCh, HDL-Ch and LDL-Ch level are found to be low but Tg level is elevated [7]. In case of AIDS patients disease progression is accompanied by a reduction in TCh, HDL-Ch and LDL-Ch level, and enhancement of Tg and VLDL-Ch levels [8]. Hyperlipidemia is seen in patients suffering from chonic kidney disease [9].

An aminoglycoside antibiotic, gentamicin has wide applications in different bacterial infections. It is a broad spectrum antibiotic and active against both gram-positive and gram-negative pathogens. It shows remarkable activity against P. aeruginosa and many gram-negative enteric bacilli [10]. In spite of its wide application, this antibiotic possesses remarkable adverse reactions like ototoxicity and nephotoxicity which are reported to be free radical associated and due to enhanced lipid peroxidation [11-16]. Alteration in plasma lipid profile may occur due to interaction of drug with lipid and drug-induced lipid peroxidation. Study shows that aminoglycoside antibiotics have the ability to induce lipid peroxidation as well as profile alteration [17-18]. It was also found from the study that gentamicin has the capacity to alter plasma lipid profile. Gentamicin treatment significantly increased plasma TCh, LDL-Ch, VLDL-Ch and Tg level in rats [19]. The antioxidant vitamin ascorbic acid not only has free radical scavenging property [20-23] but also has regulatory effect on plasma lipid profile alteration [17- 18, 24-28].

In this study, an attempt has been made to evaluate the lipid profile alteration potential of gentamicin in rabbit blood plasma and its suppression with ascorbic acid a well known antioxidant vitamin and a promising antihyperlipidemic agent. Administration of ascorbic acid with gentamicin will not only control its free radical mediated toxic potential but also will minimize gentamicin–induced, hyperlipidemia related, cardiovascular disorders.

Materials and Methods

Materials

Animal model selected was New Zealand White rabbit (Oryctolagus cuniculus). Gentamicin induced lipid profile alteration was studied by measuring plasma TCh, HDL-Ch, LDL-Ch, VLDL-Ch and Tg levels of rabbit blood. The design of study protocol was approved by Institutional Animal Ethical Committee.

Collection of blood from rabbits and estimation of lipid profile

After keeping in 18 h fasting condition the animals were divided into different experimental groups: control (C), drug treated (D), drug-antioxidant treated (DA), and only antioxidant treated (A). At a dose of 40 mg/kg body weight gentamicin was administered intramuscularly [29] to animal groups marked as D and DA. Ascorbic acid was also administered by same route at a dose of 40 mg/kg body weight [30] to animal group marked as DA and A. After 3 h and 24 h of drug and /or antioxidant administration, blood was collected from marginal ear vein of animals in labeled centrifuge tubes. It was centrifuged at 4000 g for 30 min and the clear plasma was used to determine lipid profile. The commercially available enzyme kits used for estimation of lipid profile were obtained from Span Diagnostics Limited, Surat, India.

Estimation of TCh

The estimation of TC was done by cholesterol oxidase (CHOD) – peroxidase aminoantipyrine phenol (PAP) method [31-32]. 10 µl of blood- serum was mixed with 1 ml of cholesterol reagent, containing Good's Buffer pH 6.7, cholesterol esterase, cholesterol oxidase, peroxidase, 4- aminoantipyrine and stabilizers. It was incubated at 37°C for 10 min. The measurement of absorbance was done at 505 nm against cholesterol reagent as blank. The content of TCh was determined from the standard curve prepared using cholesterol standard samples.

Estimation of HDL-Ch

Estimation of HDL Ch was performed using CHOD – PAP method [32]. Mixing of 200 µl of serum was done with 200 µl of precipitating reagent containing PEG 6000 (200 mM/L), stabilizer and preservative. The mixture was kept at room temperature for 10 min and centrifuged for 15 min at 2000 rpm and the clear supernatant was collected. To 100 µl of supernatant 1 ml cholesterol reagent was added and it was mixed and incubated at 37º C for 10 min. The absorbance of the mixture was measured at 505 nm. The concentration of HDL-Ch was determined from a standard curve prepared using HDL-Ch standard samples.

Estimation of LDL-Ch and VLDL-Ch

The content of LDL-Ch and VLDL-Ch in the samples was calculated using Friedewald's equations [33].

LDL-Ch content = TCh content – (Tg content / 5) – HDL-Ch content

VLDL-Ch content = Tg content / 5

Estimation of Tg

10 µl of serum was taken and it was mixed well with 1 ml of Tg mono reagent containing pipes buffer, 4-chlorophenol, magnesium, ATP, lipase, peroxidase, glycerolkinase, 4-aminoantipyrine, glycerol-3-phosphate oxidase, detergents, preservative and stabilizer. It was incubated at 37º C for 10 min. The absorbance of the solution was measured at 505 nm wave length [32, 34]. The content of Tg was determined from a standard curve prepared using Tg standard samples.

The percent changes in TCh, VLDL-Ch, LDL-Ch, Tg and HDL-Ch concentrations of different samples at different time intervals were calculated with respect to the corresponding control values.

Results and Discussion

Results of the study on gentamicin-induced alteration in plasma lipid profile and its control with ascorbic acid are shown in Table 1-5.

Table 1. Effect of ascorbic acid on gentamicin induced lipid profile alteration: percent change in TCh content

Time period	Animal set	Percent changes in TCh content			Analysis of variance and multiple comparison
		Samples			
		D	DA	A	
3hr	1	22.39 [a]	6.86 [b]	-10.85 [a]	F1 = 91.70 (df 2,8)
	2	18.35 [a]	9.91 [a]	-11.39 [a]	F2 = 0.15 (df 4,8)
	3	23.90 [a]	10.14 [a]	-16.72 [a]	Pooled variance
	4	16.20 [a]	7.89 [a]	-12.03 [a]	$(s^2)^*$ = 17.99
	5	24.69 [a]	10.22 [a]	-22.08 [a]	Critical difference (p=0.05)[#]
					LSD = 5.84
	Av	21.10	9.01	-14.61	Ranked means**
	(±se)	(±1.64)	(±0.68)	(±2.13)	(D) (DA) (A)
24h	1	6.11 [a]	2.52 [a]	-4.37 [a]	F1= 60.36 (df 2,8)
	2	8.43 [a]	3.98 [a]	-4.48 [a]	F2= 0.34 (df 4,8)
	3	6.58 [a]	1.78 [c]	-5.34 [a]	Pooled variance
	4	7.48 [a]	3.34 [a]	-4.55 [a]	$(s^2)^*$ = 3.87
	5	11.92 [a]	3.38 [d]	-8.46 [a]	Critical difference (p=0.05)
					LSD = 2.71
	Av	8.10	3.00	-5.44	Ranked means**
	(±se)	(±1.03)	(±0.38)	(±0.77)	(D) (DA) (A)

*Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at aP< 0.05, bP< 0.08, cP< 0.16, dP< 0.28. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate gentamicin-treated, gentamicin & ascorbic acid-treated and ascorbic acid-treated respectively. Av. = average of five animal sets; se = standard error (df = 4); df = degrees of freedom. *Error mean square. # Critical difference according to least significant difference (LSD) procedure (Ref. 197 and 198). **Two means not included within same parenthesis are statistically significantly different at P <0.05.*

Table 2. Effect of ascorbic acid on gentamicin induced lipid profile alteration: percent change in VLDL-Ch content

Time period	Animal set	Percent changes in VLDL-Ch content Samples D	DA	A	Analysis of variance and multiple comparison
3hr	1	31.69 [a]	9.90 [a]	-18.80 [a]	F1 = 60.47 (df 2,8)
	2	48.22 [a]	19.08 [a]	-39.69 [a]	F2 = 0.71 (df 4,8)
	3	41.42 [a]	29.27 [a]	-26.52 [a]	Pooled variance
	4	40.66 [a]	7.07 [a]	-26.55 [a]	$(s^2)^*$ = 88.89
	5	22.33 [a]	9.93 [a]	-26.44 [a]	Critical difference (p=0.05)[#]
					LSD = 12.99
	Av	36.87	15.05	-27.60	Ranked means**
	(±se)	(±4.48)	(±4.09)	(±3.37)	(D) (DA) (A)
24 h	1	9.90 [a]	0.66 [c]	-10.55 [a]	F1= 80.61 (df 2,8)
	2	21.60 [a]	9.55 [a]	-11.56 [a]	F2= 3.17 (df 4,8)
	3	22.63 [a]	8.26 [a]	-14.37 [a]	Pooled variance
	4	24.49 [a]	13.27 [a]	-12.85 [a]	$(s^2)^*$ = 15.12
	5	11.56 [a]	1.63 [b]	-14.87 [a]	Critical difference (p=0.05)
					LSD = 5.36
	Av	18.04	6.67	-12.84	Ranked means**
	(±se)	(±3.02)	(±2.40)	(±0.81)	(D) (DA) (A)

*Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at aP< 0.05, bP< 0.11, cP< 0.16. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate gentamicin-treated, gentamicin & ascorbic acid-treated and ascorbic acid-treated respectively. Av. = average of five animal sets; se = standard error (df = 4); df = degrees of freedom. *Error mean square. #Critical difference according to least significant difference (LSD) procedure (Ref. 197 and 198). **Two means not included within same parenthesis are statistically significantly different at P <0.05.*

Results show that the drug gentamicin has potential to alter plasma lipid profile. **Table 1** reveals that gentamicin could significantly increase TCh level which was suppressed on co-administration of ascorbic acid. Enhancement of VLDL-Ch content (**Table 2**) and LDL-Ch content (**Table 3**) was also done by gentamicin that was regulated significantly by ascorbic acid. Again, in case of Tg, results (Table 4) indicate that the drug gentamicin caused significant alteration in the plasma Tg level that is further controlled by ascorbic acid.

Table 3. Effect of ascorbic acid on gentamicin induced lipid profile alteration: percent change in LDL-Ch content

Time period	Animal set	Percent changes in LDL-Ch content Samples D	DA	A	Analysis of variance and multiple comparison
3hr	1	57.90 [a]	20.48 [b]	-28.03 [a]	F1 = 60.47 (df 2,8)
	2	32.82 [a]	21.16 [a]	-29.40 [a]	F2 = 0.71 (df 4,8)
	3	65.28 [a]	12.56 [e]	-50.24 [a]	Pooled variance
	4	54.64 [a]	38.95 [a]	-35.29 [a]	$(s^2)^*$ = 88.89
	5	60.03 [a]	23.94 [a]	-47.38 [a]	Critical difference (p=0.05)[#]
					LSD = 12.99
	Av	54.13	23.42	38.07	Ranked means**
	(±se)	(±5.60)	(±4.31)	(±4.57)	(D) (DA) (A)

Time period	Animal set	Percent changes in LDL-Ch content Samples D	DA	A	Analysis of variance and multiple comparison
24 h	1	16.92 [a]	12.53 [a]	-8.15 [a]	F1= 29.34 (df 2,8)
	2	21.54 [a]	13.68 [a]	-11.78 [a]	F2= 1.00 (df 4,8)
	3	12.06 [f]	3.80 [h]	-9.75 [d]	Pooled variance
	4	10.98 [a]	0.78 [i]	-9.93 [a]	$(s^2)^*$ = 39.94
	5	32.20 [a]	11.12 [g]	-17.36 [c]	Critical difference (p=0.05) [#]
					LSD = 8.71
	Av	18.74	8.38	-11.39	Ranked means**
	(±se)	(±3.85)	(±2.56)	(±1.59)	(D) (DA) (A)

*Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at aP< 0.05, bP< 0.08, cP< 0.09 , dP< 0.12, eP< 0.16, fP< 0.19, gP< 0.26, hP< 0.36, iP< 0.47. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate gentamicin-treated, gentamicin & ascorbic acid-treated and ascorbic acid-treated respectively. Av. = average of five animal sets; se = standard error (df = 4); df = degrees of freedom. *Error mean square. # Critical difference according to least significant difference (LSD) procedure (Ref. 197 and 198). **Two means not included within same parenthesis are statistically significantly different at P <0.05.*

Table 4. Effect of ascorbic acid on gentamicin induced lipid profile alteration: percent change in Tg content

Time period	Animal set	Percent changes in Tg content Samples D	DA	A	Analysis of variance and multiple comparison
3 h	1	31.69 [a]	9.90 [a]	-18.80 [a]	F1 = 60.47 (df 2,8)
	2	48.22 [a]	19.08 [a]	-39.69 [a]	F2 = 0.71 (df 4,8)
	3	41.42 [a]	29.27 [a]	-26.52 [a]	Pooled variance
	4	40.66 [a]	7.07 [a]	-26.55 [a]	$(s^2)^*$ = 88.89
	5	22.33 [a]	9.93 [a]	-26.44 [a]	Critical difference (p=0.05)[#]
					LSD = 12.99
	Av	36.87	15.05	-27.60	Ranked means**
	(±se)	(±4.48)	(±4.09)	(±3.37)	(D) (DA) (A)
24h	1	9.90 [a]	0.66 [c]	-10.55 [a]	F1= 80.61 (df 2,8)
	2	21.60 [a]	9.55 [a]	-11.56 [a]	F2= 3.17 (df 4,8)
	3	22.63 [a]	8.26 [a]	-14.37 [a]	Pooled variance
	4	24.49 [a]	13.27 [a]	-12.85 [a]	$(s^2)^*$ = 15.12
	5	11.56 [a]	1.63 [b]	-14.87 [a]	Critical difference (p=0.05)
					LSD = 5.36
	Av	18.04	6.67	-12.84	Ranked means**
	(±se)	(±3.02)	(±2.40)	(±0.81)	(D) (DA) (A)

*Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at aP< 0.05, bP< 0.33, cP< 0.39. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate gentamicin-treated, gentamicin & ascorbic acid-treated and ascorbic acid-treated respectively. Av. = average of five animal sets; se = standard error (df = 4); df = degrees of freedom. *Error mean square. # Critical difference according to least significant difference (LSD) procedure (Ref. 197 and 198). **Two means not included within same parenthesis are statistically significantly different at P <0.05.*

On the other hand, **Table 5** shows that gentamicin could significantly reduce plasma HDL-Ch concentration. Such ability of the drug is suppressed when ascorbic acid is co-administered.

Study clearly shows that there is an alteration in plasma lipid profile i. e., enhancement of TCh, VLDL-Ch, LDL-Ch, Tg and reduction in HDL-Ch levels induced by the antibiotic gentamicin. Our earlier study showed that aminoglycosides have lipid profile alteration ability [17-18]. Change in lipid profile and cardiovascular disorders are intimately associated and there are many evidences that support this fact [6, 35]. This study also indicates that ascorbic acid could control abnormal alteration in plasma lipid profile significantly. Many evidences are there that further strengthen the fact that ascorbic acid has antihyperlipidemic nature [17-18, 36].

Table 5. Effect of ascorbic acid on gentamicin induced lipid profile alteration: percent change in HDL-Ch content

Time period	Animal set	Percent changes in HDL-Ch content			Analysis of variance and multiple comparison
		Samples			
		D	DA	A	
3 h	1	-7.96 [a]	-4.41 [a]	6.42 [a]	F1 = 72.02 (df 2,8)
	2	-12.39 [a]	-4.37 [a]	9.11 [a]	F2 = 0.63 (df 4,8)
	3	-16.92 [a]	-5.75 [b]	13.05 [a]	Pooled variance
	4	-18.73 [a]	-7.73 [a]	8.89 [a]	$(s^2)^*$ = 10.60
	5	-16.15 [a]	-5.52 [d]	12.47 [a]	Critical difference (p=0.05)[#]
					LSD = 4.49
	Av	-14.43	-5.56	9.99	Ranked means**
	(±se)	(±1.92)	(±0.61)	(±1.23)	(D) (DA) (A)
24 h	1	-3.81 [a]	-2.67 [a]	2.94 [a]	F1= 24.89 (df 2,8)
	2	-10.02 [a]	-6.83 [a]	5.56 [a]	F2= 0.53 (df 4,8)
	3	-9.18 [a]	-4.31[a]	4.42 [a]	Pooled variance
	4	-4.75 [a]	-1.49 [c]	3.25 [a]	$(s^2)^*$ = 8.75
	5	-12.67 [a]	-4.49 [a]	7.97 [a]	Critical difference (p=0.05)
					LSD = 4.07
	Av	-8.09	-3.96	4.83	Ranked means**
	(±se)	(±1.66)	(±0.90)	(±0.91)	(D) (DA) (A)

*Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at aP< 0.05, bP< 0.08, , cP< 0.10, dP< 0.17. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate gentamicin-treated, gentamicin & ascorbic acid-treated and ascorbic acid-treated respectively. Av. = average of five animal sets; se = standard error (df = 4); df = degrees of freedom. *Error mean square. # Critical difference according to least significant difference (LSD) procedure (Ref. 197 and 198). **Two means not included within same parenthesis are statistically significantly different at P <0.05.*

Conclusion

Gentamicin is a frequently prescribed, parenterally administered, antibiotic to treat many bacterial infections, has not only lipid peroxidation induction potential but also lipid profile alteration capacity. There might be a correlation between gentamicin induced lipid peroxidation and profile alteration like other aminoglycoside antibiotics. Such potentials of gentamicin might be controlled well upon ascorbic acid co-administration. Thus, during selection of gentamicin as a potential drug candidate to treat bacterial infections, physicians should consider this fact and accordingly if they suggest antioxidant co-administration, maximum benefit might be obtained with minimal drug-induced hazards.

Conflicts of interest

The author dclares no conflicts of interest.

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