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Sub-Acute Toxicity Study of Etimicin Sulphate in Wistar Rat

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ABSTRACT
The aim of the present study was to evaluate subacute toxicity of etimicin sulphate on wistar rat. Results from the present study have elucidated that treatment of etimicin sulphate exerts no significant signs of toxicity at any dose level used in the study. Physical, biochemical as well as hematological parameters was unaltered throughout the study. Histopathology of all organs confirmed no significant alteration at any dose levels. The results of study have suggested there was no obvious toxicity observed with the treatment of etimicin sulphate. It was found to be safe alternative for various severe infections.

Keywords: Toxicity, Rat, Blood, Etimicin sulphate.

INTRODUCTION

Aminoglycoside antibiotics have long been used in antibacterial therapy. Despite their beneficial effects, aminoglycosides have considered nephrotoxic and ototoxic side effects. [1] Streptomycin was the first aminoglycoside introduced in 1944, followed by neomycin (1949), kanamycin (1957), gentamicin (1963), tobramycin (1968), amikacin (1972), and netilmicin in 1975. Tobramycin, gentamicin, amikacin and netilmicin are used in Gram-negative bacteraemic patients, while streptomycin is used as a mycobactericidal agent. The incidence of nephrotoxicity from aminoglycosides has increased from 2 to 3 % in 1969 to 20 % in the past decade. Despite nephrotoxicity and ototoxicity, the aminoglycosides are continuously being used in clinical practice because of their bactericidal efficacy, synergism with beta lactamagents, low cost, limited bacterial resistance, and a post-antibiotic effect. [2] However, netilmicin has low ototoxicity and nephrotoxicity. [3]

Etimicin sulfate is a new aminoglycoside. It is semi-synthetic water-soluble broad-spectrum antibiotics, is a derivative of gentamicin C1a, its mechanism is the inhibition of protein synthesis in susceptible strains, with efficient, safe, wide spectrum, less cross-resistance and other characteristics. [4-5]

It has been shown that for gentamicin-resistant strains, etimicin is still-sensitive; pairs of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus has good curative effect. [6]

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Scanty information is available on the toxicity and safety profile of etimycin sulphate drug. We therefore investigated whether etimicin sulphate treatment is associated with any kind of toxicities in preclinical setting using wistar rat as experimental model.

MATERIALS AND METHODS

Forty eight healthy wistar Strain rat (weighing 150-200 g) were divided into four groups (12 animals in each group) depending upon the dose of Etimicin. Each group is further sub divided into two groups depending on sex of rat having 6 animals each. Rat were housed in polycarbonate cages with bedding of husk, under hygienic conditions and acclimatized to the laboratory conditions for a period of seven days prior to initiation of dosing. Animals were kept in air conditioned rooms with 10-15 air changes per hour, temperature between 20-24°C having 12 h light and dark cycle and humidity between 30 to 70 %. Animals were given Nutrilab brand extruded pelleted mouse feed (Tetragon Chemie Pvt. Ltd. Banglore, India) and portable water *ad libitum*. Animals were given freshly prepared intravenous injection of Etimicin for 28 days. The mixture of Etimicin was prepared in 0.9 % NaCl injection before administration and was injected at following dose levels.

Group I 0 mg/kg
Group II 50 mg/kg
Group III 100 mg/kg
Group IV 200 mg/kg

Control group was injected 0.9 % NaCl only. Dosing was done approximately at the same time on each day. All the animals were observed for physical, biochemical and hematological alterations. Overnight fasted animals were sacrificed; blood and tissues samples were collected on 29th day. The Institutional animal ethics committee of Institute for

Toxicological Studies, Pune, India had approved the study protocol.

Hematological parameters

Hemogram was performed on ACT diff-2 Hematolgy Analyzer (Beckman Coulter India, Ltd., Mumbai, India).

Biochemical Parameters

Serum Gluatmic Oxaloacetic Transaminase (SGOT), Serum Gluatmic Pyruvic Transaminase activities (SGPT), Alkaline Phosphatase (ALP), Blood Urea Nitrogen (BUN) and plasma sugar levels were estimated on biochemistry analyzer using diagnostic kits (Transasia Biomedicals Ltd., Mumbai, India).

Histopathological examination

Liver, kidney, stomach, Heart and Lungs were removed from the sacrificed animals and were preserved in 10 % buffered formalin for histological examination.

Statistical analysis

Dunnett's test was used for the evaluation of data and $P < 0.05$ accepted as significant.

RESULTS

Physical parameters

No behavioral changes were observed throughout the dosing period. No significant change group mean body weight was observed in all the groups as compared to control group on 29^{th} day.

Hemogram

In male and female rat groups, no significant change was observed in hemoglobin (Hb), red blood cell counts (RBC), Rt (Reticulocyte), hematocrit (HCT), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell corpuscular hemoglobin concentration (MCHC) E (Eosiniophils) L (Leukocytes), M (Monocytes), N (Neutrophils), white blood cell (WBC) counts and platelet counts in all the treated groups as compared to control group (Table 1 & 2).

Biochemical parameters

In male and female rat groups, no significant change in SGOT, SGPT and ALP activities were observed in all the treated groups as compared to respective control group. No significant change in Serum proteins and Blood sugar levels were observed in both the groups (Table 3 & 4).

Histological examination

No significant treatment related histopathological changes were observed up to the dose of 200 mg/kg body weight.

DISCUSSION

Aminoglycoside antibiotics are the most commonly used antibiotics worldwide in the treatment of Gram-negative bacterial infections [7] Aminoglycoside antibiotics have the usual adverse effects of ototoxicity, renal toxicity, neuromuscular block, allergic reactions. [8] Their use is limited in the clinical practices due to side effect of ototoxicity and nephrotoxicity. [9-11] In order to combat these issues related to toxicity, a new aminoglycoside was successfully developed named etimicin sulphate. [12] Etimicin is active against most strains of the gram positive and gram negative bacteria. [13-14]

In present study no significant physical changes were observed during the study period of twenty eight days in all three etimicin sulphate treatment groups as compared to control. Body growth and increase in body weights of treated animals of either sex were of similar pattern as in control groups. The organ weight indicated no signs of toxicity.

Aminoglycosides have long been one of the commonest causes of drug-induced nephrotoxicity; about 10-20 % nephrotoxicity occurs during therapeutic courses. [7, 15] Aminoglycoside-induced nephrotoxicity is characterized by rises in serum creatinine, tubular necrosis and marked decreases in glomerular filtration rate and in the ultrafiltration coefficient. [7, 16] In addition, protein metabolism, the state of hydration, and the use of steroids influence the BUN. [17] Aminoglycosides are nephrotoxic because a small proportion of the administered dose (about 5 %) is retained in the epithelial cells lining the S1 and S2 segments of the proximal tubules after glomerular filtration. [18] We also evaluated kidney function related parameters and no significant differences were observed in BUN, glucose and proteins with respect to control (Table 3 and 4).

Blood was evaluated for hematological toxicity of etimicin sulphate. Hemogram was estimated and results had shown no significant changes on blood cell count and haemoglobin. The MCH and MCHC in etimicin sulphate treated group were similar to control. MCV was observed slightly lower with maximum dose in both sex which was statistically insignificant (Table 1 and 2).

Hepatotoxicity implies chemical-driven liver damage. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. [19] The first sign of damage to the liver is an increase in liver enzyme levels in the blood. When the liver is damaged, its enzymes are released into the bloodstream, where the levels can be measured by blood tests. Aminogycosides are also known to cause hepatotoxicity by increasing oxidative stress. To evaluate effect of etimicin on liver damage, liver specific parameters were estimated. It was found that etimicin sulphate had not altered ALP, SGOT and SGPT activities significantly, which confirmed no hepatotoxicity by Etimicin sulphate treatment of either sex as compared to the respective control group (Table 3 and 4). Histopathological analysis had shown no signs of toxicity in any of the organ in treatment groups as compared to etimicin sulphate. Thus histopathological studies also confirmed the safety data of other physiological, biochemical and heamatological parameters after etimicin sulphate treatment.

Table 1: Effect of sub acute dose of Etimicin on hemogram in male wistar rat

Parameters		Control	Etimicin (50 mg/kg)	Etimicin (100 mg/kg)	Etimicin (200 mg/kg)
Haemoglobin (g %)		15.54±1.55	15.55±1.16	15.20±1.88	13.58±1.76
Total RBC($X10^6$/cmm)		6.07±0.82	6.28±0.89	6.00±0.96	5.85±1.36
Rt (%)		1.05±0.22	1.18±.26	1.50±0.43	1.53±.42
HCT (%)		46.35±4.57	46.53±3.83	44.72±5.71	40.73±5.38
MCV (μm^3)		77.40±11.68	75.09±10.41	77.11±21.78	73.41±20.50
MCH (pg)		26±4.41	25.19±4.26	26.23±7.49	24.51±6.92
MCHC (%)		33.65±1.05	33.45±1.30	34.02±.80	33.38±1.33
Platelets ($X10^5$/cmm)		8.52±0.73	7.90±1.07	7.85±1.06	7.33±1.18
TotalWBC($X10^3$/cmm)		5.58±0.82	5.87±0.98	5.89±0.79	5.83±0.92
Differential %	N	20.50±1.87	22.50±1.87	22.17±1.47	22.50±1.87
	L	76.17±1.94	76±2.68	76.00±2.61	76±2.45
	E	2.67±1.21	1.83±0.98	1.83±0.98	1.83±0.75
	M	0.33±0.52	0.83±0.75	0.67±0.82	0.67±0.52

Table 2: Effect of sub acute dose of Etimicin on hemogram in female wistar rat

Parameters		Control	Etimicin (50 mg/kg)	Etimicin (100 mg/kg)	Etimicin (200 mg/kg)
Haemoglobin (g %)		15±1.93	15.03±1.23	13.77±2.27	14.85±2.42
Total RBC(X10^6/cmm)		5.52±0.85	5.22±0.74	5.83±1.01	5.98±0.78
Rt (%)		1.12±0.28	1.43±0.35	1.48±0.51	1.48±0.51
HCT (%)		44.07±6.54	44.53±3.93	41.77±6.01	43.53±7.09
MCV (µm^3)		81.44±16.91	86.03±9.80	74.14±20.67	74.75±20.42
MCH (pg)		27.75±5.47	29.12±3.35	24.53±7.53	25.50±7.06
MCHC (%)		34.02±1.04	33.86±0.83	32.89±1.08	34.13±1.05
Platelets (X10^5/cmm)		8.19±1.07	8.35±1.01	7.60±1.18	7.62±.91
TotalWBC(X10^3/cmm)		5.95±0.99	6.18±0.70	5.55±1.08	4.98±0.65
Differential %	N	21.33±2.16	22.50±1.87	22±1.90	22±1.41
	L	77.17±2.86	76±2.10	76.67±2.94	76±2.61
	E	2.17±1.17	1.83±1.47	1.83±0.98	2.33±1.21
	M	0.33±0.52	0.67±0.52	0.67±0.82	0.33±0.52

Table 3: Effect of sub acute dose of Etimicin on biochemical parameters in male wistar rat

	Group I	Group II	Group III	Group IV
Total Protein (g%)	6.37±1.30	6.32±.89	6.15±1.32	5.70±.87
BUN (mg%)	21.83±2.32	22.67±3.08	24.33±3.93	26.33±5.68
SGPT (IU/L)	49.67±8.16	54±9.38	59±8.10	59.33±12.55
SGOT (IU/L)	93.83±9.04	96.83±9.28	99.33±13.91	102.50±9.93
SAP (IU/L)	270.83±58.56	277.00±65.29	342.50±84.76	340.17±112.52
Blood Sugar (mg %)	95.17±5.85	92.67±7.37	96.67±5.24	96±7.56

Data is presented as mean ± Standard deviation (n=6 in each group)

Table 4: Effect of sub acute dose of Etimicin on biochemical parameters in female wistar rat

	Group I	Group II	Group III	Group IV
Total Protein (g %)	6.03±1.01	6.27±1.24	6.02±1.05	6.08±1.02
BUN (mg %)	20.83±2.32	21.83±2.14	25.67±3.72	25.83±5.27
SGPT (IU/L)	51.17±10.11	55.67±11.89	61.33±7.58	59.33±10.82
SGOT (IU/L)	94±6.16	103.50±11.50	97.83±9.33	102.50±12.96
SAP (IU/L)	256.33±78.38	301±62.65	332.17±96.12	330.50±91.52
Blood Sugar (mg %)	95.33±6.56	95.50±7.23	96.67±6.50	97.33±5.47

Data is presented as mean ± Standard deviation (n=6 in each group)

In conclusion, our results provide support for safety profile of this potential drug. The data suggest that Etimicin sulphate is safe even at maximum dose level and no significant effect was observed on any of physiological and biochemical parameters. Thus, etimicin sulfate injection is an effective safe antibiotic and possessing widely clinical application and worth for wide use.

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