Evaluation of Genotoxic and Lipid Peroxidative Potential of Ceftriaxone

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http://dx.doi.org/10.13005/bpj/1895

Lipid peroxidation can produce DNA adducts that can result in genotoxicity. It is involved in pathophysiology of various diseases and drug induced toxicities. Several cephalosporins are reported to cause chromosomal aberrations. Hence this study was planned to evaluate the genotoxic and lipid peroxidative potential of Ceftriaxone in Wistar rats. Ceftriaxone was given at the dose of 500 mg/kg body weight and 1000 mg/kg body weight intraperitoneally to Wistar rats. Genotoxicity was tested by performing in vivo micronucleus test. The frequency of micronucleated polychromatic erythrocytes (%MnPCEs) and polychromatic erythrocytes to normochromatic erythrocytes ratio (PCE:NCE) were estimated. Lipid peroxidative potential was assessed by estimating TBARS (Thiobarbituric acid reactive substance) levels in plasma, erythrocytes and tissue. The activities of antioxidant enzymes were also estimated. The data was analyzed using ANOVA and Dunnett’s test as post hoc. Ceftriaxone at both doses did not increase the % MnPCEs and PCE: NCE ratio in Wistar rats. Ceftriaxone at the dose of 500 mg/kg body weight has significantly altered TBARS levels in erythrocytes. But at a dose of 1000 mg/kg body weight, it has significantly increased plasma, erythrocyte and tissue TBARS levels. The activity of SOD was decreased significantly by ceftriaxone at both doses. The activity of GSH was decreased significantly by ceftriaxone at a dose of 1000 mg/kg body weight. Our study demonstrates that Ceftriaxone does not have the potential to cause genotoxicity. However, it does induce lipid peroxidation and alter the activities of antioxidant enzymes in Wistar rats.

Keywords: Ceftriaxone, genotoxicity, lipid peroxidation, Wistar rats.

Genotoxic compounds in food, environment and therapy can lead to permanent changes in the genetic material leading to mutagenicity and carcinogenicity. Lipid peroxidation and free radical generation can lead to irreversible damage to cellular structure and function. They are also involved in the pathophysiology of various diseases like rheumatoid arthritis, certain forms of cancer, hepatic and pulmonary diseases etc. Lipid peroxidation products can interact with cellular DNA and produce DNA adducts. These DNA adducts can damage DNA and cause genotoxicity. Lipid peroxidation also plays a role in various drug induced toxicities such as cardiotoxicity by...
doxorubicin, nephrotoxicity of gentamicin, renal injury by cisplatin, cyclophosphamide toxicity etc.

Ceftriaxone is an extensively prescribed third generation cephalosporin with expanded Gram negative coverage and good cerebrospinal fluid penetration. It is used commonly as it has long plasma half-life enabling single daily dosing and beta lactamase resistance. It is believed to have excellent safety profile. But the potential of cephalosporins such as cefixime, cefotaxime, cefaclor to induce aberrations in chromosomes has been described. In our previous studies, we have reported that ceftriaxone at a dose of 100 mg/kg BW and 200 mg/kg BW is not genotoxic but it has the potential to induce lipid peroxidation in mice. In continuation of our studies on safety of ceftriaxone, we have conducted the present study to evaluate the genotoxic and lipid peroxidative potential of Ceftriaxone at a higher dose (500 mg/kg BW and 1000 mg/kg BW) in Wistar rats.

MATERIALS AND METHODS

Chemicals

Ceftriaxone, giemsa stain, fetal bovine serum, May – Grunwald stain, GSH, NADH, tetramethoxy propane were bought from Sigma – Aldrich chemicals Pvt. Ltd., Bangalore, India. Heparin, thiobarbituric acid (TBA), trichloro acetic acid, 2, 4 – dinitro phenyl hydrazine, 1 – Choloro-2, 4 – dinitrobenzene, nitroblue – tetrazolium, phenazinemethosulphate were procured from Hi – media Laboratories, Mumbai, India. All other chemicals and solvents used were of analytical grade.

Animals

Wistar rats of both sexes, weighing 130-150g and 7-8 weeks old were procured from central animal house, Sri Kaliswari college, Sivakasi, India. They were 4 rats per cage and 12hr :12hr light dark cycle was maintained. Standard pellets and water were provided ad libitum. The care and maintenance of experimental animals complied with the The Indian National Science Academy (INSA) guidelines. Institutional Animal Ethics Committee approved the study protocol.

Experiment

Each study group had 6 Wistar rats. Group 1 served as control group and distilled water was injected intraperitoneally to these rats. Groups 2 – 5 were the test groups. Ceftriaxone was injected intraperitoneally at a dose of 500 mg/kg BW to group 2 and 3 rats and 1 g/kg BW to groups 4 and 5. All rats were sacrificed by cervical dislocation at different intervals. Bone marrow was harvested at 24 hr and 48 hr time point. Plasma, erythrocytes and tissue hemolysates were utilised for estimation of lipid peroxidation and various antioxidant activities.

In vivo micronucleus test

Bone marrow preparations were made as per the method of Schmid with modifications. May-Grunwald-Giems was used to stain the slides and the presence of micronuclei was studied. The number of micronuclei in polychromatic erythrocytes (MnPCE) per 2000 polychromatic erythrocytes (PCE) were counted per slide. The polychromatic to normochromatic erythrocytes (NCE) ratio (PCE/NCE) was calculated per 500 erythrocytes.

Biochemical estimations

Heparinized tubes were used to collect blood samples. Plasma was centrifuged. Tissue samples were washed and homogenized and various biochemical estimations were conducted. The formation of thiobarbituric acid reactive substances (TBARS) was an index of lipid peroxidation.

The method of Yagi was employed to estimate the Plasma TBARS levels. Deproteinised sample was treated with thiobarbituric acid at 90°C for 1 hour. The resultant pink colour gives a measure of TBARS. It was stated as nmol/ml.

Tissue TBARS was measured by the method advocated by Ohkawa et al. Colorimetric estimation of the colour formed by the reaction between thiobarbituric acid and lipid peroxidation breakdown products was done. It was stated as nmol/mg protein.

Erythrocyte TBARS was assayed by the procedure of Donnan. Erythrocytes were treated with 10% trichloroacetic acid(TCA) and resulting chromogen was estimated at 535nm. Their levels were stated as pmoles/mg Hb.

The plasma reduced glutathione (GSH) level was estimated as per the method of Beutler and Kelley. The chromogen formed after mixing plasma with TCA and treating the supernatant with disodium hydrogen phosphate and DTNB reagent
was determined at 412 nm. GSH activity was stated as mg/dl of plasma.

The procedure of Rotruck et al was used to determine the activity of glutathione peroxidase (GPx). Hemolysate was mixed with H$_2$O$_2$ and GSH and the remainder of GSH was mixed with DTNB and the resulting chromogen was measured. GPx activity was stated as U/g of erythrocyte lysate.

The method described by Kakkar et al was followed to assay Superoxide dismutase activity. The colour developed after 50% inhibition of formation of NADH phenazine methosulfate nitro blue tetrazolium (NBT) was read at 520 nm. SOD activity was stated as U/mg of hemolysate.

The catalase activity was assayed by Sinha’s method. It was based on the H$_2$O$_2$ utilization by the enzyme. The activity was stated as U/mg of hemolysate.

**Statistical analysis**

Results were expressed as mean ± SD. Data were analyzed using ANOVA followed by Dunnett’s test as post hoc. p<0.05 was considered as significant. Data was analysed by GraphPad prism version 6.05.

**RESULTS**

The % micronuclei induced after administration of ceftriaxone at 500mg/kg bw and 1000mg/kg bw at 24 hr and 48 hr harvest time and PCE:NCE ratio were statistically analyzed. The results from the various test groups were compared with the control group. The results of the in vivo micronucleus tests are presented in Table 1.

In the present study, ceftriaxone at the dose of 500 mg/kg BW did not increase the % MnPCE after 24 hr and 48 hr significantly as compared to control animals (P > 0.05) (Table 1). At the dose of 1000 mg/kg BW also there was no statistically significant increase in the % MnPCE (P > 0.05). (Table 1) The PCE:NCE ratio was also not significantly increased in ceftriaxone treated animals at both doses in comparison with control animals (P > 0.05) (Table 1).

Plasma, erythrocyte and tissue TBARS levels and activities of antioxidant enzymes were estimated in the control and experimental groups. TBARS formation was the index of lipid peroxidation. Plasma TBARS levels were expressed as nmol/ml. Erythrocyte TBARS values

| Treatment group                  | Harvest time (hrs) | % MnPCE (Mean ± SD) | PCE: NCE (Mean ± SD) |
|----------------------------------|--------------------|---------------------|----------------------|
| Group 1: Distilled water         | 24 hrs             | 1.01 ± 0.01         | 1.00 ± 0.01          |
| Group 2: Ceftriaxone (500 mg/kg) | 24 hrs             | 1.20 ± 0.08$^{**}$  | 1.07 ± 0.05$^{**}$   |
| Group 3: Ceftriaxone (500 mg/kg) | 48 hrs             | 1.38 ± 0.01$^{**}$  | 1.07 ± 0.03$^{**}$   |
| Group 4: Ceftriaxone (1000 mg/kg)| 24 hrs             | 1.60 ± 0.03$^{**}$  | 1.06 ± 0.01$^{**}$   |
| Group 5: Ceftriaxone (1000 mg/kg)| 48 hrs             | 1.69 ± 0.01$^{**}$  | 1.08 ± 0.04$^{**}$   |

Data expressed as mean±SD. n = 6; *p < 0.05, **p<0.01, ***p<0.001 (compared with control), NS— not significant.

| Treatment group                  | Sample collection time | Plasma TBARS (mean ± SD) | Erythrocyte TBARS (mean ± SD) | Tissue TBARS (mean ± SD) |
|----------------------------------|------------------------|---------------------------|-------------------------------|---------------------------|
| Group 1: Distilled water         | 24 hrs                 | 37 ± 2.2                  | 49 ± 1.3                      | 28 ± 1.89                 |
| Group 2: Ceftriaxone (500 mg/kg) | 24 hrs                 | 39 ± 1.2                  | 76 ± 1.87$^{***}$             | 23 ± 1.43                 |
| Group 3: Ceftriaxone (500 mg/kg) | 48 hrs                 | 41 ± 1.97                 | 83 ± 2.2$^{***}$              | 27 ± 2.3                  |
| Group 4: Ceftriaxone (1000 mg/kg)| 24 hrs                 | 79 ± 1.43$^{***}$         | 78 ± 3.02$^{***}$             | 49 ± 2.05$^{***}$         |
| Group 5: Ceftriaxone (1000 mg/kg)| 48 hrs                 | 76 ± 1.3$^{***}$          | 77 ± 1.13$^{***}$             | 47 ± 1.42$^{***}$         |

Data represented as mean±SD. n = 6; *p < 0.05, **p<0.01, ***p<0.001 (in comparison with control)
were expressed as pmoles/mg Hb. The tissue TBARS levels were estimated as nmol/mg protein. The values of GSH were expressed as mg/dl. The GPx activity was stated as U/g. The activity of SOD was expressed as U/mg. The CAT activity was stated as U/mg.

The effect of ceftriaxone on plasma, erythrocyte and tissue TBARS levels in rats are presented in Table 2. Ceftriaxone was considered as an inducer of lipid peroxidation if it increased the levels of plasma, erythrocyte as well as tissue TBARS significantly in comparison with control. In current study, ceftriaxone at the dose of 500 mg/kg body weight (Groups 2 and 3) has significantly altered TBARS levels in erythrocytes in comparison with control. (Table 2) Ceftriaxone at a dose of 1000 mg/kg body weight (Groups 4 and 5) has significantly (p<0.05) increased plasma, erythrocyte and tissue TBARS levels in comparison with control group. (Table 2)

The effect of ceftriaxone on antioxidant enzymes activities in rats is presented in Table 3. The activities of SOD was decreased significantly (p<0.05) by ceftriaxone at the dose of 500 mg/kg body weight (Groups 3 and 4). The activities of antioxidant enzymes – GSH and SOD were decreased significantly (p<0.05) by ceftriaxone at a dose of 1000 mg/kg body weight (Groups 4 and 5) as compared to control animals. (Table 3)

**DISCUSSION**

In the present study, the genotoxic and lipid peroxidative potential of Ceftriaxone at a dose of 500 mg/kg BW and 1000 mg/kg BW was evaluated in Wistar rats. Genotoxicity was tested by in vivo micronucleus test. The potential of Ceftriaxone to induce lipid peroxidation was assessed by measuring the levels of TBARS in the plasma, erythrocytes and tissues of Wistar rats. The activities of the antioxidant enzymes (GSH, SOD, CAT, GPx) was also measured. Micronucleus test is used to screen compounds that can cause breaks in the chromosome.\(^{25}\) The frequency of micronucleated polychromatic erythrocytes (% MnPCE) and PCE:NCE ratio were the parameters used to test for genotoxicity. Chromosomal damage is detected by an increase in % MnPCEs. In normal bone marrow, the PCE:NCE ratio is about 1:1.\(^{26}\) Reduction in this ratio due to cytotoxicity or formation of micronucleus and substantial DNA damages can lead to apoptosis.\(^{27}\) In our study, ceftriaxone at the dose of 500 mg/kg BW and 1000 mg/kg BW did not increase the % MnPCEs and PCE: NCE ratio at 24 and 48 hrs in Wistar rats. Hence Ceftriaxone has no genotoxic potential at these doses. This is in confirmation with our previous study where Ceftriaxone was tested at lower doses (100 mg/kg and 200 mg/kg).\(^{15}\) But there are some studies with contrasting reports such as studies by Metoviæ et al.,\(^{28}\) and Tanyildizi and Türk.\(^{29}\) These dissimilarities may be the result of different methods and different tissues used for genotoxicity screening.

Lipid peroxidation induced by several drugs may contribute to their toxicities. It is measured by estimating the TBARS formation. In our study, ceftriaxone at the dose of 500 mg/kg body weight has significantly altered TBARS levels in erythrocytes. But at a dose of 1000 mg/kg body weight, it has significantly increased plasma, erythrocyte and tissue TBARS levels. The activity of SOD was decreased significantly by ceftriaxone at both doses. The activity of GSH was decreased

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**Table 3. Effect of ceftriaxone on antioxidant enzyme levels in rat**

| Treatment group       | Sample collection time | GSH    | GPX    | CAT    | SOD    |
|-----------------------|------------------------|--------|--------|--------|--------|
| Group 1: Distilled water | 24 hrs                | 56 ± 1.28 | 46 ± 1.45 | 47 ± 1.53 | 89 ± 2.89 |
| Group 2: Ceftriaxone (500 mg/kg) | 24 hrs             | 54 ± 1.30 | 51 ± 2.30 | 54 ± 3.2  | 53 ± 1.57*** |
| Group 3: Ceftriaxone (500 mg/kg) | 48 hrs             | 51 ± 1.91 | 53 ± 1.73 | 57 ± 1.85 | 45 ± 2.12*** |
| Group 4: Ceftriaxone (1000 mg/kg) | 24 hrs           | 28 ± 1.82*** | 42 ± 1.61 | 47 ± 1.20 | 49 ± 1.65*** |
| Group 5: Ceftriaxone (1000 mg/kg) | 48 hrs           | 30 ± 1.51*** | 43 ± 1.72 | 45 ± 1.30 | 48 ± 1.34*** |

Data represented as mean±SD. n = 6; *p<0.05, **p<0.01, ***p<0.001 (in comparison with control)
significantly by ceftriaxone at a dose of 1000 mg/kg body weight. In our previous study, Ceftriaxone increased the TBARS levels at 200 mg/kg BW and decreased the activities of all antioxidant enzymes (GSH, GPx, CAT and SOD) at both doses (100 mg/kg and 200 mg/kg). Several studies have described similar findings—studies by Chakaraborty S et al. and Roy K et al.

Various studies have refuted these findings. Kaur B and Prakash A described the attenuation of oxidative stress by Ceftriaxone in MPTP model of Parkinson’s disease in rats. Akina S et al demonstrated the neuroprotective effect of ceftriaxone and selegiline mediated by antioxidant mechanisms in scopolamine induced cognitive impairment. Abdel-Daim MM & El-Ghoneimy A reported the free radical scavenging and antioxidant activity of Ceftriaxone in nephrotoxicity induced by deltamethrin in rats. Oxidative stress and apoptosis were reduced by ceftriaxone in neuropathic pain models in rats as reported by Amin B et al. In a study by Hussein AM, it was shown that Ceftriaxone reduces oxidative stress in Pentylene tetrozole kindled rats. Studies by Soni A et al. and Dwivedi VK et al. described that antioxidant enzyme activities were increased and lipid peroxidation was decreased by ceftriaxone in combination with Vancomycin and sulbactam respectively. These variations between the findings of our study and the above mentioned studies may be explained by the use of different animal models and methodologies.

CONCLUSION

Our study demonstrated that Ceftriaxone does not have the potential to cause genotoxicity. However, it does induce lipid peroxidation and alter the activities of antioxidant enzymes in Wistar rats. The role of this property in mediating the clinically encountered toxic effects needs to be elucidated. It is better to prescribe Ceftriaxone judiciously in clinical practice.

ACKNOWLEDGEMENT

We thank Dr. Kolanjiappan Kaliyaperumal for his guidance and Shrikanth Sharma, Dr. Renjith R and Kavitha T G for their help and support.

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