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Toxicity assessment of Linezolid and the beneficial effects of human erythropoietin in mice

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ABSTRACT

Linezolid (LZD), an antibiotic launched in year 2000, but due to its bone marrow toxicity which results in to thrombocytopenia and red cell anemia, the market size is narrowed. The mechanism of such toxicity is still not very clear, so the present work focused to investigate the possible mechanism of bone marrow toxicity in progenitors of red blood cells and its treatment by using erythropoietin (EPO). Male albino mice administered doses of 100 and 200 mg/kg two times a day with 6 hours of interval, for 19 and 9 days, respectively. And beneficial effects of erythropoietin (EPO) were investigated. The results demonstrated that sensitive parameters of red cell anemia like PCE: NCE ratio and % retics were significantly decreased. Treatment of EPO resulted into stimulation of erythropoiesis in LZD exposed animals. At 100 mg/kg dose, increase in PCE: NCE ratio and % retics in EPO injected animals was significant as compared to stand alone LZD administered animals ($p<0.001$). There was also significant increase in RBC and HGB in same group due to EPO injection ($p<0.05$). These results give the evidence that LZD targeted precursor cells CFU-E, BFU-E and Erythroblasts in bone marrow and induced the conditions like red cell anemia. And plays beneficial role and stimulated the erythropoiesis in bone marrow to normalize the condition in mice. Therefore, this short term model can be used to fasten the screening of newer oxazolidinones class of antibiotics for bone marrow toxicity and the beneficial effects of EPO also can be screened.

Key words: Linezolid, myelosuppression, bone marrow toxicity, oxazolidinones, erythropoietin.

INTRODUCTION

Linezolid or “Zyvox” was discovered as an antibacterial drug to treat multidrug resistant *Staphylococcus aureus* (MRSA). Apart from its anti-MRSA activity, Zyvox also inhibits the growth of other bacterial pathogens, such as penicillin resistant pneumococci, vancomycin resistant enterococci (VRE), and *Enterococcus faecalis* [2] [19]. Clinical case reports also demonstrated the use of Zyvox in tuberculosis [27], nocardiosis [17] and anaerobic infections [3] [28].

Several preclinical safety studies of LZD reported the reversible toxic effects on bone marrow (FOI-linezolid, Pharmacia). United State Food and Drug Administration have already issued “Black Box” warning regarding the myelosuppression and thrombocytopenia caused due to LZD therapy in clinical use.. Different clinical trials and pivotal studies demonstrated that LZD induced hematological disorders, especially red cell anemia and

thrombocytopenia [9] [21]. Continuous efforts are made to overcome the toxicity caused by LZD treatment and several new generation oxazolidinones were synthesized in the context of structure toxicity relationships. Despite of those significant efforts, only two drugs have advanced the past human trials, namely radezolid from Rib-x Pharmaceuticals (CT, USA), and torezolid, which is developed by Trius Therapeutics (CA, USA) [1].

In vitro experiments have reported that LZD inhibits cell proliferation and delays the cellular metabolic activity by affecting the mitochondrial function [6]. In another report, it is reported that LZD inhibits protein biosynthesis of mitochondria which affects the production of ATP [16]. Considering these aspects, it is conceivable that LZD-associated bone marrow toxicity may be arisen due to the decreased metabolic activity and less production of ATP in bone marrow precursor cells which ultimately results in red cell anemia, thrombocytopenia or myelosuppression. Based on this scientific basis, this work focused on red cell anemia caused due to bone marrow toxicity associated with LZD. Additionally, the effects of EPO, a renal hormone, on bone marrow cells were also investigated as EPO acts on erythroid precursor cells CFU-E and BFU-E and erythroblast and stimulate erythropoiesis [12] [22] [23]. Action of EPO was investigated further in LZD induced bone marrow cells, by evaluating very specific parameters like PCE: NCE ratio in bone marrow and % retics in blood.

MATERIALS AND METHODS

Reagents and materials

LZD was procured from Symed Laboratories (Hyderabad, India). EPO injection (10,000 U/mL) was purchased from market (Trade name-Eprex, marketed by Janssen Cilag Ltd.), disposable syringes were procured from BD, to prepare dose formulations and reagents, and water for injection was used as diluent. Fetal bovine serum was purchased from Himedia, India. All other chemicals used were of highest purity and analytical grade.

Animal Management

Male Swiss mice weighing between 22-25g were used for this study. The temperature and relative humidity of the animal room was maintained at $22^0C \pm 3^0C$ and 30 to 70%, respectively. Illumination was controlled with 12 h dark and 12 h light cycle. All animals received sterilized water prepared by using Aquaguard RO filter. Animals were fed standard pellet diet. All above conditions were maintained throughout the experiment. All ethical practices were followed while performing experiments on animals [30].

Linezolid administration

Five groups of male mice comprising five animals in each group were housed in standard laboratory conditions (explain the condition). *Group I*, which was maintained as control, received vehicle (1% Tween 80 freshly prepared in water), *Group II* and *Group III* received linezolid at a dose of 100 mg/kg BW, while *Group IV* and *Group V* received linezolid at a dose of 200 mg/kg bw. Linezolid was administered at the above mentioned doses daily, two doses at 6 hours apart. Linezolid at 100 mg/kg dose was administered consecutively for 19 days, while linezolid at 200 mg/kg dose was administered consecutively for 9 days. The dosing of linezolid was terminated based on the severity of clinical signs and morbidity of the animals. The doses of linezolid were selected on the basis of our preliminary dose- response studies and the previous report [7].

Erythropoietin Injection

Erythropoietin (EPO) formulation was injected to *Group III* animals on day 17, 18 and 19 of the study, while *Group V* was injected with EPO on day 7, 8 and 9 of the study. EPO was prepared freshly by diluting stock solution (10000 U/mL) to 60 IU/mL in phosphate buffered saline, pH 7.2, containing 1% BSA (PB-BSA). The injection was administered by subcutaneous route at a dose of 0.5mL to each mouse at neck region using disposable 1mL syringe attached with $26^{1/2}$ disposable needle. EPO bioassay is well established assay in mice by single and multiple three administrations by counting the increase in reticulocytes in blood is examined [18].

Group I: Normal control [1% tween 80 as suspension in WFI, p.o.).
Group II: Linezolid (100 mg/kg/day in 1% tween 80 in WFI p.o. for 19 days).
Group III: Linezolid (100 mg/kg/day in 1% tween 80 in WFI, p.o. for 19 days) + EPO (30 IU/mouse s.c. daily for three days).
Group IV: Linezolid (200 mg/kg/day in 1% tween 80 in WFI p.o. for 9 days).

Group V: Linezolid (200 mg/kg/day in 1% tween 80 in WFI p.o. for 9 days) + EPO (30 IU/mouse s.c. daily for three days).

Clinical signs
Daily clinical signs were recorded from all groups. Clinical signs were observed two times in a day after each dose. Cage side observations were made to see the treatment related changes on behavior and any other clinical symptoms during the study.

Body weight measurement
The body weights were measured periodically during the entire study to know the treatment related changes on body weight gain. The treatment related changes in weight gain was compared between different groups compared to vehicle controls.

Bone marrow evaluation for PCE: NCE ratio
On termination of the treatment, femur bone was isolated and cleaned using 1ml disposable syringe attached with a disposable 23guage needle. Bone marrow was aspirated into 5ml glass tube containing 1mL FBS (Fetal Bovine Serum). Bone marrow cells produced single cell suspension into FBS by repeated passing through needle. Cells were centrifuged at 1000 rpm at 4^0C to form pellet and before making slides, pellet was disturbed and vortexed so that cells get separated and smear was prepared on clean grease free slides. Slides were stained using Giemsa stain to examine polychromatic (PCE) and normochromatic (NCE) erythrocytes. Total 1000 cells were counted to calculate PCE: NCE ratio [10].

Determination of blood parameters
Reticulocyte (Retics) counting was done by new methylene blue staining techniques, where anticoagulated blood was stained with new methylene stain and smears were prepared and dried on clean and grease free slides to examine under light microscope. Mature Red Blood Cells and Reticulocytes were counted under oil immersion (100x magnification). Total 1000 cells were counted and percent reticulocytes were calculated [13].

On termination of the treatment, blood was collected from retro-orbital sinus with the help of capillary in EDTA containing tube. Hematology parameters, such as WBC (white Blood Corpuscles), RBC (Red Blood Corpuscles), Platelets (Platelets), HGB (Hemoglobin), HCT (Hematocrit), MCV (Mean Cell Volume), MCH (Mean Cell Hemoglobin), MCHC (Mean Cell Hemoglobin Concentration) were measured using an automated hematology auto analyzer (Beckman, USA). The hematology parameters are routine and important while undertaking the toxicological studies [29].

Statistical analysis
The results were expressed as the mean ± SEM. Statistical differences between groups were assessed using the Newman-Keuls Multiple Comparison Test to calculate mean difference. q and p values were used to determine the difference between the groups. A probability level less than 0.05 were considered as significant.

RESULTS

Clinical signs and body weights
LZD at 100 mg/kg dose either alone or in combination with EPO (GII and GIII).
Both the groups LZD and LZD+EPO showed mild lethargy and overall decrease in cage activity from day 12 onwards and remained almost in this condition up to end of the treatment. In later group of animals, treatment of EPO improved the cage activity to some extent. There was mild decrease in gain in both of the groups from Day 7, but significant reduction in gain was noticed from day 14 onwards in both of the treated groups. Mild improvement in gain was noticed after EPO treatment on day 21 of the study. Though these change were biologically important, they were statistically insignificant ($p>0.05$). **(Fig 1)**.

LZD at 100 mg/kg dose either alone or in combination with EPO (GIV and GV).
Mild lethargy and overall decrease in cage activity was noticed in both LZD and LZD+EPO treated groups from day 4 of the study, while emaciated condition was noticed from day 9 onwards till end of the study. Poor body weight

gain was noticed on day 7, while loss in body weight was recorded on day11 of the study in both of the above groups. **(Fig 2)**.

Bone marrow evaluation for PCE: NCE ratio (GII to GIV)

We found a significant decrease in PCE: NCE ratio in LZD-treated animals compared to vehicle- treated control animals ($p<0.01$). These changes are considered to be impact of LZD treatment, as LZD has potential suppressive effects on red precursor cells. The co-exposure of LZD-treated animals to EPO resulted in significant increase in PCE: NCE ratio ($p<0.001$). At 200 mg/kg of LZD dose, also there was significant reduction of PCE: NCE ratio ($p<0.01$), which was more severe compared to the lower dose of LZD. But the treatment of EPO did not stimulate erythropoiesis in bone marrow and hence there was no increase in PCE: NCE ratio revealed. (**Figure 3 and 4).**

Determination of blood parameters (GII to GIV)

Percent Retics

The treatment of LZD produced significant reduction in the % retics in the blood ($p<0.001$), which indicates that LZD possesses properties of decreasing immature RBCs by suppressing erythropoiesis in bone marrow. In EPO treated animals, significant rise in relative population of retics in blood indicates that EPO can stimulate erythropoiesis in LZD treated animals ($p<0.001$). At 200 mg/kg dose of LZD, there was significant reduction in percent reticulocytes in both LZD alone and LZD + EPO groups ($p<0.001$), but the treatment of EPO didn't stimulated erythropoiesis by increasing in the percentage of reticulocytes in the blood. (**Figure 3 and 4).**

Red Blood Corpuscles

At 100 mg/kg dose, LZD caused reduction in the RBC count in the blood, which is considered as treatment-related changes, though statistical significance is not found ($p>0.05$). Treatment of EPO revealed increase in the total count of RBC in the blood. This indicates the stimulation of erythropoiesis in bone marrow after treatment with LZD ($p<0.05$). Here, we considered that EPO has positive effects on LZD-induced reduction in RBC due to suppression of bone marrow red cell proliferation. At 200 mg/kg dose, LZD did not produce significant reduction in RBC and the treatment of EPO stimulated the production of RBCs. **(Figure 5 and 6)**.

Hemoglobin

At 100 mg/kg dose, LZD treatment resulted in a statistically significant reduction in percent hemoglobin of blood ($p<0.05$) when compared to vehicle treatment. Hemoglobin is the major component of red cells and reduction of this protein is considered to be the significant contributor of red cell anemia. Treatment with EPO resulted in increase in the percent hemoglobin of blood which was correlated with the stimulation of erythropoiesis in bone marrow after treatment with LZD ($p<0.05$). EPO played a positive role in bringing up the percent hemoglobin through stimulation of erythropoiesis in LZD-treated animals. **(Figure 5 and 6).**

Hematocrit

LZD caused reduction in percent volume of RBC in the blood, which indicates a decrease the number of RBC in the blood. The decrease in HCT in LZD-treated animals though statistically non significant ($p>0.05$), but mild decreases in HCT is reported (change it give exact meaning). EPO played a positive role to normalize the LZD-induced reduction in HCT ($p<0.001$). The results were evident of stimulation of erythropoiesis and increase in the total volume increase of RBC in the blood.(reframe the sentence) The changes in HCT were not reported at 200 mg/kg dose of LZD or LZD +EPO-injected group. **(Figure 5 and 6).**

Red Blood Corpuscle Indices (Mean Cell Volume, Mean Cell Hemoglobin and Mean Cell Hemoglobin Concentration)

At both 100 and 200 mg/kg doses of LZD, there were no treatment-related changes in both the LZD and LZD+EPO groups, except a significant rise in MCV in LZD+EPO-treated group when compared to vehicle treatment ($p<0.05$). **(Figure 5 and 6).**

White Blood Corpuscles

There was no alterations noticed in WBC count at 100 mg/kg, also there were no any changes reported in EPO treated group, in both the groups the count was compared to control. The group of animals which received LZD the

dose of 200 mg/kg with EPO, there was reduction in WBC count ($p<0.01$), which was not considered treatment related change. **(Figure 7 and 8).**

Platelets

LZD at 100 mg/kg dose significantly reduced platelet count in blood as compared to vehicle control ($p<0.05$). While in LZD+EPO-treated group, where LZD was given 100 mg/kg dose, there was significant rise in the population of platelets in the blood when compared to stand alone LZD-treated group of 100 mg/kg dose. LZD at 200 mg/kg also, there was significant reduction in the PLT count ($p<0.001$), which was more severe than low dose of LZD. The treatment of EPO did not normalize the PLT count when injected to the animals which were exposed to 200 mg/kg dose of LZD. **(Figure 7 and 8).**

DISCUSSION

Linezolid was launched in year 2000 by trade name "Zyvox", with absolute bioavailability of 100% gives the option of treating first by intravenous and can be shifted to oral route to ease the treatment [14]. It has become primary choice for treating MRSA because of having less number of other effective medicines available, a few of like vancomycin which has limitations of having only intravenous route of administration [24]. Animal studies demonstrated that LZD causes the suppression of bone marrow cells particularly of erythroid lineage [26]. There are other evidences that LZD acts on erythroid precursor cells and suppress the erythropoiesis in bone marrow [4]. Based on these reports, the work was further helped to establish a mouse model to evaluate the LZD induced changes on erythroid progenitor cells or the model can be used to screen newer oxazolidinone entities may face consequences of suppressed erythropoiesis, anemia or reticulocytopenia. In the study PCE:NCE ratio was selected a sensitive marker to investigate the LZD induced effects on bone marrow red cells, given more understanding towards mechanistic approaches instead of only studying the % reduction in reticulocytes, as reported previously [11].

To support the findings with the exposure of LZD, the doses were selected to achieve higher therapeutic range of AUC, which can mimic the extremes of clinical scenario [13]. The work demonstrated that, at low dose the AUC was 280 mcg.hr/mL, which can be projected closure to the human AUC while treating resistant pathogens. In United State Food and Drug Administration Docket describing the preclinical summary, several studies showed the potential of LZD induced bone marrow hypocellularity and other hematological disorders. Further Hickey reported the mouse model to study the red cell anemia induced by LZD [11]. These reports further strengthen to design the study keeping mouse as a model and induce the red cell anemia by selecting new sensitive parameter like PCE: NCE ratio to study erythropoiesis and investigate the effects of EPO.

Treatment of EPO increases the production of polychromatic erythrocytes which is reflected by increase in PCE: NCE ratio in bone marrow [25]. The work was further investigated through mechanistic approach that EPO binds to precursor cells of bone marrow, which produce red cells [22]. The selection of parameters like PCE and retics gives proof of concept that LZD caused suppression of bone marrow precursor cells like CFU-E, BFU-E and erythroblasts etc. These precursor cells are having receptors for EPO to bind and to stimulate the erythropoiesis, therefore these result gives the evidences that LZD caused suppression of these precursor cells and EPO stimulated the erythropoiesis acting on CFU-E, BFU-E and erythroblasts through their receptors [22].

At high dose also same magnitude of impact noticed while examining the changes PCE in bone marrow and retics in the blood. The significant reduction in these parameters indicates that high dose, which is double of low dose was effective almost by 50% less duration of exposure, which proves the linearity of our experimental model. But due to lack of tolerance in such a high exposure of LZD, animal condition was worsen and there was systemic toxicity signs resulted into emaciation, severe weight loss and decreased activity and dehydration in animals. These complications were so high and systemic down turn of the body was not responding to EPO. Or it may be due to insufficient population or dysfunctional erythroid precursor which resulted into negative effects of EPO. Hence the injection of EPO didn't result the stimulation of erythropoiesis, and subsequently there were no reports on increase in population of PCE or % retics.

Apart from the main focus to investigate red cell anemia related changes, the work also demonstrated thrombocytopenia at both the doses, but was not kept as main finding of the study. This finding indicated that the

experimental model was sensitive enough, because thrombocytopenia is considered as most common finding in LZD treatment. The significant reduction in PLT may be due to anti proliferative activity of LZD on megakaryocytes or its precursor cells [13]. The work further suggested that precursors of PLT producing cells may have receptors of EPO, and binding resulted into stimulation of megakaryocytosis and hence PLT count was increased in blood, but further research is required to undertake this concept.

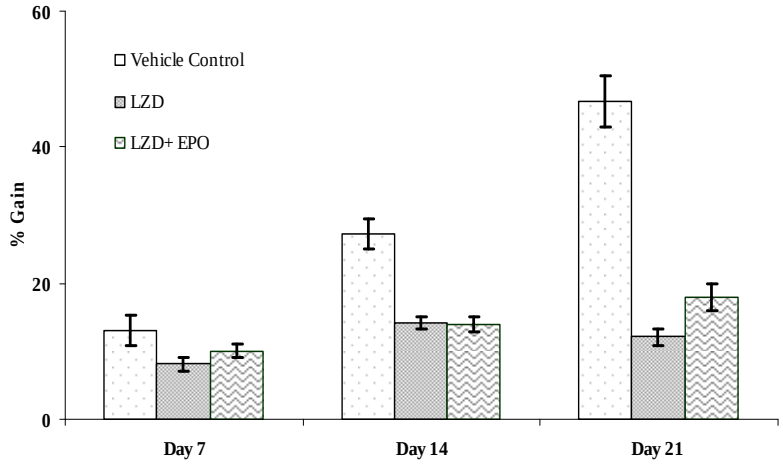

Figure 1: Body weight gain in percent on day 7, 14 and 21 of three groups (Vehicle control, LZD@100 mg/kg and LZD@100 mg/kg +EPO@30 IU/mouse). The percent gain calculated against day 1 of their respective treated groups. Results are Mean ± S.E. n=5.

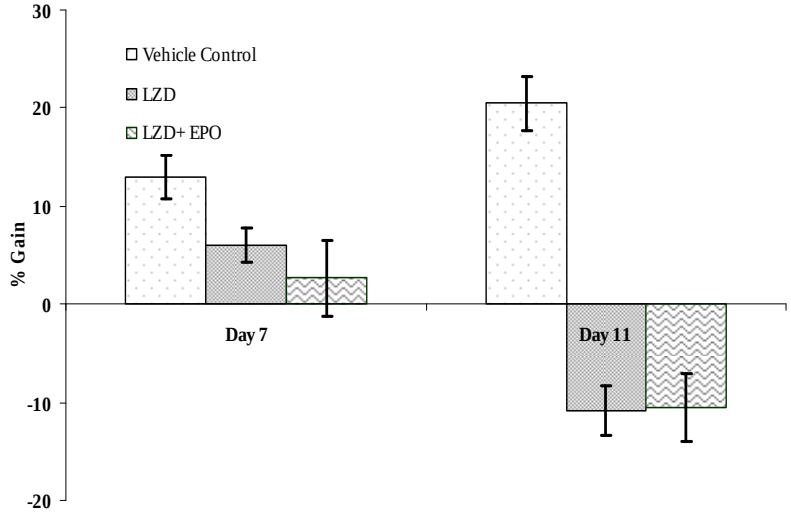

Figure 2: Body weight gain in percent on day 7 and 11 of three groups (Vehicle control, LZD@200 mg/kg and LZD@200 mg/kg +EPO@30 IU/mouse). The percent gain calculated against day 1 of their respective treated groups. Results are Mean ± S.E. n=5.

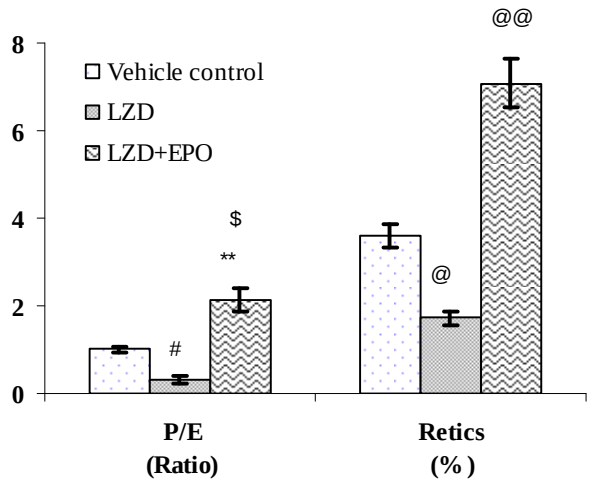

Figure 3: Bone marrow evaluation of PCE (Polychromatic erythrocytes) and NCE (normochromatic erythrocytes) ratio and % retics from blood. Chart represents three groups (Vehicle control, LZD@100 mg/kg and LZD@100 mg/kg +EPO@30 IU/mouse). Results are Mean ± S.E. n=5. #significantly different from control (p<0.01), **significantly different from stand alone LZD treated group (p<0.001), $significantly different from control group (p<0.001). @significantly different from control (p<0.001) and @@significantly different from stand alone LZD treated group (p<0.001).

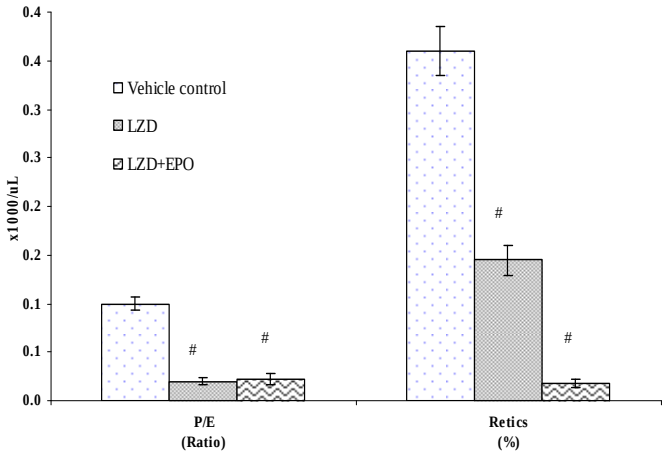

Figure 4: Bone marrow evaluation of PCE (Polychromatic erythrocytes) and NCE (normochromatic erythrocytes) ratio and % retics from blood. Chart represents three groups (Vehicle control, LZD@200 mg/kg and LZD@200 mg/kg +EPO@30 IU/mouse). Results are Mean ± S.E. n=5. #significantly different from control (p<0.001).

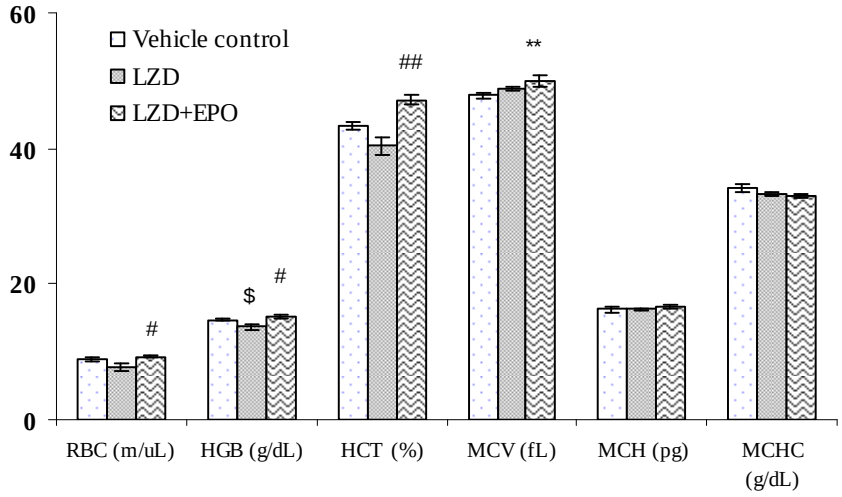

Figure 5: Hematology parameters estimate (RBC, HGB, HCT, MCV, MCH and MCHC). Chart represents three groups (Vehicle control, LZD@100 mg/kg and LZD@100 mg/kg +EPO@30 IU/mouse). Results are Mean ± S.E. n=5. $significantly different from control (p<0.05), **significantly different from control (p<0.05). #significantly different from stand alone LZD treatment group (p<0.05). ##significantly different from stand alone LZD treatment group (p<0.001).

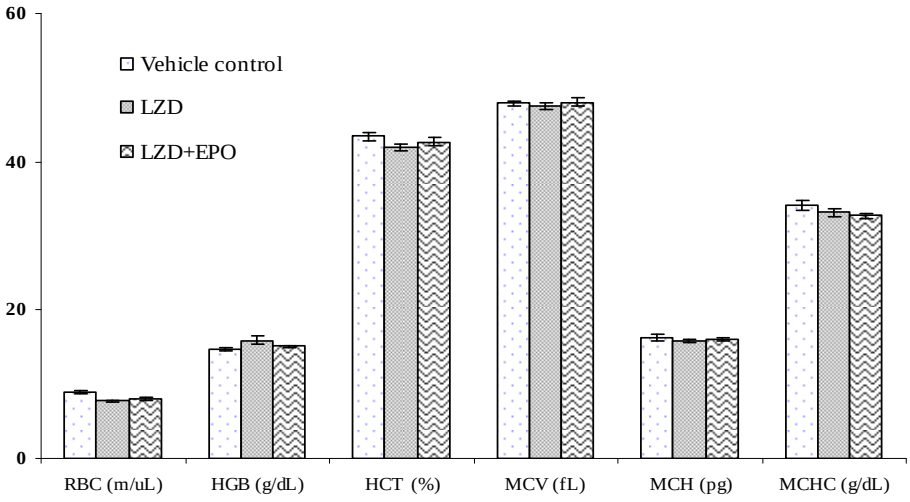

Figure 6: Hematology parameters (RBC, HGB, HCT, MCV, MCH and MCHC) estimates. Chart represents three groups (Vehicle control, LZD@200 mg/kg and LZD@200 mg/kg +EPO@30 IU/mouse). Results are Mean ± S.E. n=5.

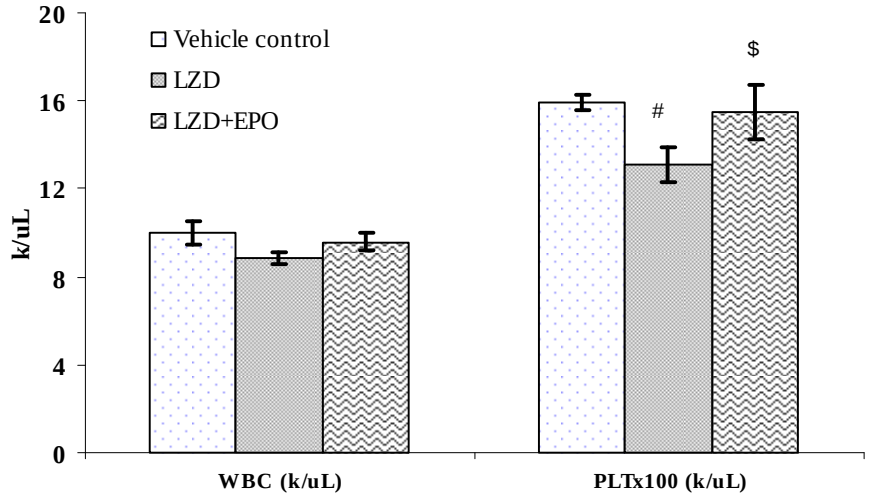

Figure 7: WBC and PLT estimates. Chart represents three groups (Vehicle control, LZD@100 mg/kg and LZD@100 mg/kg +EPO@30 IU/mouse). Results are Mean ± S.E. n=5. #significantly different from control (p<0.05). $significantly different from stand alone LZD treated group (p<0.05).

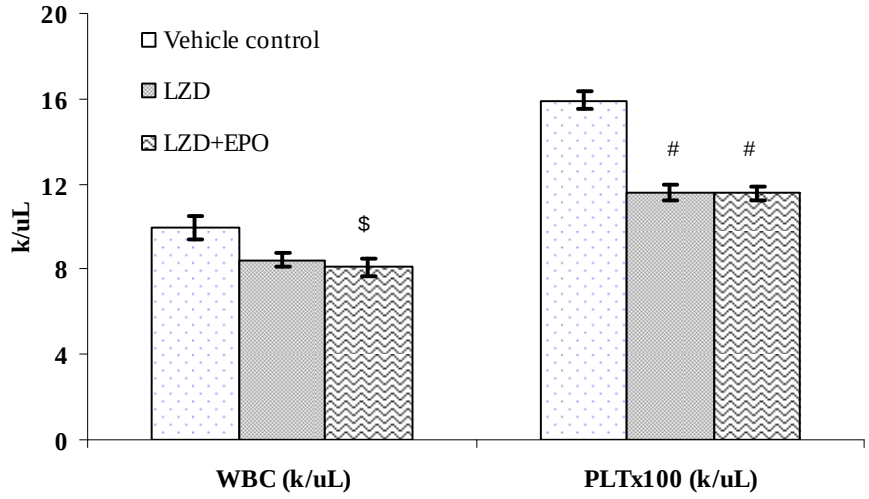

Figure 8: WBC and PLT estimates. Chart represents three groups (Vehicle control, LZD@200 mg/kg and LZD@200 mg/kg +EPO@30 IU/mouse). Results are Mean ± S.E. n=5. #significantly different from control (p<0.05). $significantly different from control (p<0.01).

CONCLUSION

Linezolid caused bone marrow suppression and dose dependant severity of the systemic toxicity in mice. The low dose given to mice achieved AUC, which was near to human therapeutic AUC and the elicited the condition like red cell anemia in mice. EPO can be better choice to add on therapy of LZD in the situation to treat red cell anemia.

Hence, more clinical research is required to establish the dose response, duration and safety of EPO administration. Use of EPO in proper monitored condition can be safe and it can be better option to cover the side effects arise due to Linezolid therapy.

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REFERENCES

[1]Adam RR, *Expert Rev Anti Infect Ther*, **2010**, 8.
[2]Babcock HM, Ritchie DJ, Christiansen E, Starlin R, Little R, Stanley S, *Clin Infect Dis*, **2001**, 32, 1373-1375.
[3]Behra-Miellet, J, Calvet L, Dubreuil L, *Int J Antimicrob Agents*, **2003**, 22, 28-34.
[4]Bernstein, WB, Trotta RF, Rector JT, Tjaden JA, Barile AJ, *Ann Pharmacother*, **2003**, 37, 517-520.
[5]Dennis L. Stevens, Daniel Herr, Harry Lampiris, John Lee Hunt, Donald H. Batts, Barry Hafkin, *Clinical Infectious Diseases*, **2002**; 34:1481–90.
[6]Duewelhenke N, Krut O, Eysel P, *Antimicrobial Agents and Chemotherapy*, **2007**, 51(1), 54-63.
[7]Emily J, Hickey CJ, Gill AS, Misura AF, Flattery GK, *Therapy*, **2006**, 3, 4.
[8]FOI-linezolid, Pharmacia.
[9]Green, SL., Maddox, JC., Huttenbach, ED, *J Amer Med Ass*, **2001**, 25, 1291-1293.
[10]Hayashi M, Tice RR, Macgregor JT, Aderson D, Blakey DH, *Mut Res*, **1994**, 312, 293-304.
[11]Hickey EJ, Gill CJ, Misura AS, Flattery AF, Abruzzo GK, *Therapy*, **2006**, 3, 521-526.
[12]Iscove N, Siever F, Witerhalter KH, *J Cell Physiol*, **1974**, 83, 309-320.
[13]Kazuaki Matsumoto, Ayumi Takeshita, Kazuro Ikawa, Akari Shigemi, Keiko Yaji, Yoshihiro, Shimodozono, Norifumi Morikawa, Yasuo Takeda, Katsushi Yamada, *International Journal of Antimicrobial Agents*, **2010**, 36, 179–181.
[14]Koepke JA, NCCLC and J.M. England, Methods for Reticulocyte Counting (Flow Cytometry and Supravital Dyes): *Approved Guideline. National Committee for Clinical Laboratory Standards*, Villanova, **1997**, ISBN-13: 9781562383022, Pages: 26.
[15]Matthew S Dryden, *J Antimicrob Chemother*, **2011**, 66 (4), iv7–iv15.
[16]Mc Kee, et al, *Ant Agents and chemother*, **2006**, 50, 2042-2049.
[17]Moylett EH, Pacheco SE, Brown-Elliott BA, et al, *Clin Infect Dis*, **2003**, 36, 313-318.
[18]Ramos AS, Schmidt CA, Andrade SS, Fronza M, Rafferty B, Dalmora SL, *Brazilian Journal of Medical and Biological Research*, **2003**, 36, 1561-1569.
[19]Rao N, White GJ, *Clin Infect Dis*, **2002**, 35, 902-903.
[20]Renslo AR, *Exp Rev Anti Infect Ther*, **2010**, 8, 565-574.
[21]Rubenstein E, Istruriz R, Stanford HC, et al, *Antimicrob Agents Chemother*, **2003**, 47, 1824-1831.
[22]Shuga J, Zhang J, Samson LD, Lodish HF, Griffith LG, *PNAS*, **2007**, 104(21), 8737–8742.
[23]Steve Elliott, Elizabeth Pham, Iain C, Macdougall, *Experimental Hematology*, **2008**, 36, 1573–1584.
[24]Stevens DL, Herr D, Lampiris H, Hunt JL, Batts DH, Hafkin B, *Clin Infect Dis*, **2002**, 34, 1481–90.
[25]SuzukiY, Nagae Y, Li J, Sakaba H, Mozawa K, Takahashi A, Shimizu H, *Mutagenesis*, **1989**, 4, 420-424.
[26]Vinh DC, Rubinstein E, *J Infect*, **2009**, 59, S59-S74.
[27]Vonder Lippe B, Sandven P, Brubakk O, *J Infect*, **2006**, 52, 92-96.
[28]Wareham DW, Wilks M, Ahmed D, Brazier JS, Millar M, *Clin Infect Dis*, **2005**, 40, 67-68.
[29]Kwasi Opoku Boadu, Samuel Kofi Tulashie, Michael Akrofi Anang, Jerome Desire Kpan, *European Journal of Experimental Biology*, **2011**, 1(2), 160-171.
[30]Samuel Fosu Gyasi, Esi Awuah, John A. Larbi1, George Asumeng Koffuor and Owusu-Afriyie Osei, *European Journal of Experimental Biology*, **2012**, 2 (3):683-689