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# The Protective Effects of Pyridoxine on Linezolid-Induced Hematological Toxicity, Hepatotoxicity, and Oxidative Stress in Rats

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## What is already known on this topic?

- *The most important side effect of linezolid is hematological toxicity.*
- *In clinical practice, the effectiveness of simultaneous administration of pyridoxine plus linezolid is unclear.*

## What this study adds on this topic?

- *Few studies document the effects of pyridoxine use to prevent the hematological side effects of linezolid in children. Pyridoxine is preferred in antibacterial-resistant infections. Interestingly, in our study, leukopenia became more severe when used with pyridoxine. Our data showed that pyridoxine might have a protective effect on hepatotoxicity. More studies are needed to show the effects of chronic pyridoxine for treating pediatric patients.*

## ABSTRACT

**Objective:** Linezolid is often used to treat antibacterial-resistant infections. Linezolid can cause side effects. To date, the effectiveness of the simultaneous administration of pyridoxine and linezolid is unclear. Here we investigate the protective effect of pyridoxine on linezolid-induced hematological toxicity, hepatotoxicity, and oxidative stress in rats.

**Material and Methods:** The 40 male pediatric Spraque–Dawley rats were separated into 4 groups: control, linezolid, pyridoxine, and linezolid–pyridoxine. A complete blood count, liver function test, and measurements of antioxidant enzyme activities for superoxide dismutase, glutathione peroxidase, catalase, and lipid peroxidation were performed in blood before treatment and 2 weeks after administration of the treatment.

**Results:** White blood cell and hemoglobin counts for the linezolid group decreased, and the alanine aminotransferase level in the linezolid group increased compared to their respective baseline values. Post-treatment white blood cell decreased in the linezolid and linezolid–pyridoxine groups compared to those in the control group ($P < .001$). Alanine aminotransferase levels increased in the linezolid and linezolid–pyridoxine groups compared to those in the control group ($P < .001$ and $P < .05$, respectively). The activity of superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde levels increased in the linezolid group compared to the control group ($P < .001$, $P < .05$, $P < .001$, and $P < .001$, respectively). Linezolid plus pyridoxine treatment caused a significant decrease in malondialdehyde levels and superoxide dismutase, catalase, and glutathione peroxidase enzyme activities compared to the linezolid group ($P < .001$, $P < .01$, $P < .001$, and $P < .01$, respectively).

**Conclusion:** Pyridoxine may be an effective adjuvant agent for the prevention of linezolid toxicity in rat models.

**Keywords:** Linezolid, oxidative stress, pyridoxine, rat

## INTRODUCTION

Linezolid was the first member of a class of drugs called oxazolidinones. It was approved in 2000 for intravenous and oral clinical use by the US Food and Drug Administration.[1] Linezolid reaches high levels in many tissues (eye, synovial and cerebrospinal fluid, skin, bone, and lung). Therefore, it seems to be an alternative for the treatment of vancomycin-resistant gram-positive pathogens, particularly where prolonged courses of antimicrobial therapy are required.[2] Side effects of linezolid include headaches, diarrhea, and elevated transaminase levels. However, the most important side effects are those of myelosuppression,

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including anemia, leukopenia, and thrombocytopenia; moreover, in a study of 179 children, thrombocytopenia, anemia, and leukopenia were the most common side effects.[3,4] Pyridoxine has recently been shown to have highly efficient antioxidant properties, inhibit superoxide radicals, and prevent lipid peroxidation.[5,6] The protective effect of pyridoxine compared to the side effects of linezolid has been investigated in various studies on adults.[3,7-9] Here, we investigate the protective effects of pyridoxine on linezolid-induced hematological toxicity, hepatotoxicity, and oxidative stress in pediatric rats.

## MATERIALS AND METHODS

The study was conducted after ethical committee permission with approval number by Animal Experiments Ethical Committee of Süleyman Demirel University Faculty of Medicine (Protocol Number 21.07.2009-01). The animals were treated in accordance with the "Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals" guide prepared by the Süleyman Demirel University Ethical Committee about animals.

### Rats

Forty male pediatric Spraque–Dawley rats (8 weeks old, weighing 200-250 g) were divided into 4 groups: control (C, n = 10), linezolid (L, n = 10), pyridoxine (P, n = 10), and linezolid plus pyridoxine (LP, n = 10). For 14 days, by gavage twice a day, 1 mL of saline solution was administered to the C group. In addition, 125 mg/kg/day of linezolid was administered to the L group;100 mg/kg/day of pyridoxine was administered to the P group, and 125 mg/kg/day of linezolid and 100 mg/kg/day of pyridoxine to the LP group.

### Blood Samples and Biochemical Parameters

Blood samples were collected at baseline and 14 days post-treatment after the rats were anesthetized with ketamine (intraperitoneal injection of 80 mg/kg). At the beginning of the study, complete blood counts, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were studied. The normal values of the tests used in experimental animals in our study were as follows: hemoglobin: 11-19 g/dL, white blood cell (WBC): 11–19 g/L, platelet: 200-1500 × $10^3$/μL, alanine aminotransferase (ALT): 17.5-30.2 U/L, and aspartate aminotransferase (AST) 45.7-80.8 U/L.[10] Blood count values were measured using Beckman Coulter LH 750 (11800 S.W. 147 Avenue Miami, Florida, US, 33196), and biochemical analyses were measured using Olympus AU2700 (Shinjuku Monalith,2-3-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo 163-0914, Japan).

Hemolysate is prepared from the blood for the following antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) levels were also measured as previously described.[11-14] The rats were sacrificed at the end of the study.

### Statistical Analysis

The Statistical Package for Social Sciences (SPSS) software program version 15.0 (SPSS Inc.; Chicago, IL, USA) was used for blood and biochemical tests. Wilcoxon and Mann–Whitney *U* tests were performed. Malondialdehyde and antioxidant levels were compared with post hoc least significant difference. *P* value of <.05 was considered as statistically significant.

## RESULTS

The platelet, WBC, hemoglobin, AST, and ALT levels were determined to evaluate the effect of hematological and transaminase levels of linezolid and pyridoxine in rats (Table 1). White blood cell count and hemoglobin count of the L group and the WBC count of the P and LP groups were lower than their respective baseline values. Alanine aminotransferase levels in the L group were found to have increased compared to their baseline values. White blood cell counts were reduced in post-treatment in L, P, and LP groups compared to those in the C group, and leucocyte counts decreased in the LP group compared to those in the L group. However, there were no significant differences among the other groups. Alanine aminotransferase levels increased in the post-treatment L and LP groups compared to that of the C group ($P < .001$ and $P < .05$, respectively). Alanine aminotransferase levels increased in the L group compared to LP group ($P < .05$).

Erythrocyte CAT, SOD, and GSH-Px activities were evaluated in all groups, and levels of L group were significantly increased compared to the other groups. When the erythrocyte MDA levels were examined in all groups, the MDA level of the linezolid group was found to be significantly increased compared to the control group ($P < .0001$). No significant change was observed when comparing other groups to the control (Table 2).

**Table 1.** The Level of Hemoglobin, White Blood Cell, Platelet, AST, and ALT at the Beginning and End of the Study in Rat Groups

| | PLT (×$10^3$/μL) | | WBC (×$10^3$/μL) | | Hg (g/dL) | | AST (U/L) | | ALT (U/L) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Groups** | **Day 0** | **Day 14** | **Day 0** | **Day 14** | **Day 0** | **Day 14** | **Day 0** | **Day 14** | **Day 0** | **Day 14** |
| Control | 773.90 ± 131.7 | 900.30 ± 192.07 | 6.52 ± 1.52 | 6.73 ± 1.54 | 12.76 ± 2.87 | 13.13 ± 0.61 | 173.80 ± 24.87 | 173.00 ± 17.63 | 62.60 ± 11.72 | 70.80 ± 10.58 |
| Linezolid | 892.40 ± 183.41 | 798.60 ± 211.48 | 7.03 ± 1.40 | 3.01 ± 1.16**$^{\beta\beta\beta}$ | 14.18 ± 1.60 | 12.91 ± 0.72* | 171.40 ± 24.83 | 166.10 ± 39.45 | 75.50 ± 17.77 | 104.00 ± 22.75**$^{\beta\beta\beta}$ |
| Pyridoxine | 816.10 ± 245.74 | 732.90 ± 255.33 | 6.71 ± 1.02 | 3.38 ± 1.62**$^{\beta\beta\beta}$ | 13.17 ± 1.47 | 12.55 ± 1.56 | 173.90 ± 34.04 | 181.20 ± 31.84 | 72.20 ± 14.52 | 75.80 ± 17.64$^{\alpha\alpha}$ |
| Linezolid + pyridoxine | 850.20 ± 148.9 | 743.90 ± 282.52 | 6.87 ± 2.59 | 2.06 ± 1.15**$^{\beta\beta\beta\alpha}$ | 13.96 ± 1.33 | 13.19 ± 0.93 | 172.30 ± 16.91 | 172.00 ± 33.94 | 76.20 ± 13.15 | 83.80 ± 11.56$^{\beta\alpha}$ |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hg, hemoglobin; PLT, platelets; SD, standard deviation; WBC, white blood cell.
Results are expressed as mean ± SD, n = 10; Wilcoxon analysis performed day 0 versus day 14 (*$P < .05$) (**$P < .01$).
Mann–Whitney *U* test performed versus control group ($^{\beta}P < .05$) ($^{\beta\beta}P < .01$) ($^{\beta\beta\beta}P < .001$) versus linezolid ($^{\alpha}P < .05$) ($^{\alpha\alpha}P < .01$).

**Table 2.** The Levels of Antioxidant Enzymes and MDA Levels at the End of the Study

| | SOD (U/g Hb) | CAT (k/g Hb) | GSH-Px (U/g Hb) | MDA (nmol/g Hb) |
|---|---|---|---|---|
| Groups | Day 14 | Day 14 | Day 14 | Day 14 |
| Control | 4609.78 ± 153.79 | 2778.17 ± 322.19 | 1404.24 ± 266.01 | 70.80 ± 6.61 |
| Linezolid | 8757.18 ± 694.42$^{\beta\beta}$ | 3367.88 ± 706.33$^{\beta\beta}$ | 3122.07 ± 523.30$^{\beta\beta\beta}$ | 89.99 ± 8.84$^{\beta\beta\beta}$ |
| Pyridoxine | 5761.42 ± 1394.38$^{\beta\alpha\alpha\alpha}$ | 2729.00 ± 238.04$^{\alpha\alpha}$ | 1486.09 ± 535.04$^{\alpha\alpha\alpha}$ | 73.11 ± 5.98$^{\alpha\alpha\alpha}$ |
| Linezolid+Pyridoxine | 5304.80 ± 1584.70$^{\alpha\alpha\alpha}$ | 2798.12 ± 384.38$^{\alpha\alpha}$ | 1506.99 ± 553.72$^{\alpha\alpha\alpha}$ | 77.12 ± 9.05$^{\alpha\alpha}$ |

CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase.
Results are expressed as mean ± SD, n = 10.
Least significant difference test performed versus control group ($^{\beta}P < .05$) ($^{\beta\beta}P < .01$) ($^{\beta\beta}P < .001$) versus linezolid ($^{\alpha}P < .05$) ($^{\alpha\alpha}P < .01$) ($^{\alpha\alpha\alpha}P < .001$).

## DISCUSSION

Linezolid is a promising alternative for the treatment of infections caused by multiple drug-resistant bacterial strains. However, linezolid-related adverse events, specifically anemia and thrombocytopenia, limit its prolonged use, particularly in patients who have poor marrow reserves. Myelosuppression and immune-mediated mechanism explain these hematological side effects. A patient taking linezolid showed increased bone marrow iron saturation, erythroid aplasia, and vacuolated erythroblasts, demonstrating myelosuppression.[15] Berstein et al[16] documented a patient who developed thrombocytopenia and the presence of adequate, normal-appearing megakaryocytes after 7 days of linezolid therapy, suggesting immune-mediated thrombocytopenia. Recent studies have suggested that the oxidative stress markers were increased in linezolid-treated patients, suggesting that oxidative damage might be the underlying mechanism of induced thrombocytopenia.[17] In a study on adult rats, linezolid administration was correlated with significantly increased thrombocyte count levels after 15 days. Higher dosages of linezolid were correlated with significantly reduced levels of thrombocytes, WBC, and hemoglobin counts compared to the control group. Hematologic side effects and decreased antioxidative activity induced by linezolid were partly recovered by vitamin E.[18] In our study, anemia and thrombocytopenia were not observed in linezolid-administered groups at the end of the experiment, but the rate of leukopenia was 50% ($P < .005$). However, we observed a decrease in hemoglobin counts in the L group that was not statistically significant. Based on these results, hematological follow-up of patients with infection who were given linezolid and demonstrated borderline anemia should be carefully performed. We found no significant decrease in platelet counts after linezolid administration. In fact, the hematological effects in the published literature seem to occur more frequently in patients who received linezolid for 2 weeks or more; oral doses of linezolid up to 1200 mg have been reported to be well tolerated in humans.[15] However, since there is only one other published study in rats, the side effects of higher doses of linezolid should be investigated, with close attention to the hematologic effects of linezolid in rats. Studies have reported the use of pyridoxine in conjunction with linezolid to prevent or reduce linezolid side effects, particularly for patients with poor bone marrow. The administration of 50 mg pyridoxine (vitamin B6) orally once a day in 2 patients who had used linezolid for 9 months was found to help reserve anemia.[19] However, other studies found pyridoxine had no protective effect against the hematological side effects of linezolid.[3,8] Youssef et al[7] reported that pyridoxine might have an impact on anemia but did not prevent other hematological side effects. In the present study, we observed a decrease in hemoglobin count in rats that received linezolid alone. The decrease in hemoglobin counts was not observed in rats that received linezolid plus pyridoxine, indicating that pyridoxine might have been a protective effect. Interestingly, we also observed decreases in WBC counts for pyridoxine–linezolid-treated rats; this potential side effect has not been reported previously. Elevated transaminase levels have been reported as a side effect of linezolid. De Bus et al[20] presented an adult patient who developed concomitant lactic acidosis and severe liver failure after treatment with linezolid for 50 days due to an infected hip prosthesis. Our study observed elevated ALT levels similar to those previously reported, but we found no significant changes in AST levels. Alanine aminotransferase levels increased in the post-treatment L and LP groups compared to that of the C group; however, ALT levels increased in the L group compared to LP group. It showed that pyridoxine prevented the elevation of ALT levels.

We observed a significant increase in antioxidant enzyme activity and MDA levels in erythrocytes for rats treated with linezolid. Previous studies have shown that antioxidant enzyme activities generally decrease after oxidative damage. However, in our study, MDA levels and antioxidant enzyme activities were increased after the administration of linezolid. This suggested that the antioxidant system was activated to remove free radicals from linezolid. Although membrane damage occurs in erythrocytes, the lack of adequate hemoglobin levels was attributed to the absence of hemolysis. On the other hand, both antioxidant enzymes and MDA levels were not elevated in L and LP groups. These changes induced by pyridoxine have been shown to decrease the free radical production due to linezolid and/or free radicals formed by the help of pyridoxine.

The addition of pyridoxine to protect against the side effects of linezolid used in the treatment of gram-positive bacterial infections should be recommended to increase the effectiveness of treatment and prevent complications. Since the hematological toxic effects of linezolid limit its use against multidrug-resistance gram-positive pathogens, we believe that this study will be promising to guide future research.

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**Declaration of Interests:** The authors have no conflict of interest to declare.

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