Effect of Cilostazol on the P50 of the Oxygen-Hemoglobin Dissociation Curve

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Abstract

Cilostazol is a drug used for the treatment of intermittent claudication caused by narrowing of the blood vessels and reduced oxygen supply, characterized by intense pain in the leg when walking. This study was designed to investigate the effect of cilostazol on the P50 of the oxygen hemoglobin dissociation curve. A total of eight healthy adult subjects were studied. Blood samples (0.5 mL) from each subject were mixed with 5, 10, and 20 μ L of the 0.5 mg/mL stock solution of cilostazol to give concentrations of 10, 20, and 40 μ g/mL equivalent to adult doses of 50, 100, and 200 mg, respectively. The control sample had no drug added. The oxygen hemoglobin dissociation curve of each sample was plotted and the P50 determined with a Hemox-Analyzer (TCS, Medical Products Division, Southampton, PA). The mean P50 for the control samples was 28.27 ± 0.43 mm Hg. The values of the samples exposed to 10, 20, and 40 μ g/mL cilostazol were 29.63 ± 0.66 , 30.15 ± 0.77 , and 31.66 ± 0.62 mm Hg, respectively. There was a statistically significant difference ($p < 0.01$) between the control and samples exposed to 40 μ g/mL cilostazol. This study suggests that cilostazol caused an increase in the release of oxygen from hemoglobin as shown in the P50 values. This effect was significant at the highest concentration of 40 μ g/mL.

Keywords

- cilostazol
- oxygen hemoglobin dissociation curve
- P50
- oxygen release

Cilostazol (Pletoz, Cipla Ltd., Mumbai, India) is a drug used to treat intermittent claudication, a condition caused by narrowing of the arteries supplying the legs with blood. The narrowing of the blood vessels and consequent reduction in oxygen availability is usually accompanied by pain. The pain associated with intermittent claudication worsens during walking and improves at rest. Intermittent claudication is reportedly the most common symptom of arterial insufficiency in peripheral artery disease.¹

Cilostazol is a selective inhibitor of the phosphodiesterase (PDE) III isoenzyme. PDE III is involved in the degradation of cyclic adenosine monophosphate (cAMP). Inhibition of the action of PDE III suppresses the breakdown of cAMP and thus increases the amount of cAMP in tissues.^{2,3} This dilates blood vessels and increases the flow of blood and, as a result, oxygen to the legs. In addition, the cAMP reduces platelet aggregation. Cilostazol is therefore of therapeutic benefit in

intermittent claudication. Long-term use of the drug reportedly increases walking distances in patients with peripheral artery disease.¹ Cilostazol is typically administered orally as 100 mg tablets twice daily and its absorption increases with a high fat meal.⁴

Cilostazol has been reported to normalize the level of erythrocyte 2,3 diphosphoglycerate in sucrose-fed Otsuka Long-Evans Tokushima (OLTEFT) fatty rats which had a reduction in 2,3 diphosphoglycerate.⁵ The metabolite 2,3 diphosphoglycerate in the erythrocyte shifts the oxygen hemoglobin dissociation curve to the right resulting in increased oxygen release.⁶ The oxygen hemoglobin dissociation curve is important clinically as it provides information on the relationship between partial pressure of oxygen and the oxygen saturation of arterial blood, which may have implications especially in circulatory diseases. The P50 is the partial oxygen pressure at which hemoglobin is 50% saturated

with oxygen; the average value reported with the Hemox-Analyzer (TCS, Medical Products Division, Southampton, PA) is 25.3 ± 1.5 mm Hg.⁷

Based on the ability of cilostazol to normalize the level of erythrocyte 2,3 diphosphoglycerate, it was hypothesized that cilostazol would shift the oxygen hemoglobin dissociation curve to the right and increase oxygen release from erythrocytes. This study was therefore designed to investigate the effect of cilostazol on the P50 of the oxygen hemoglobin dissociation curve of normal blood.

Material and Methods

Subjects

Eight healthy adult subjects with hemoglobin (HbAA) were studied. The informed consent of each subject was obtained before recruitment into the study. The study was performed according to the guidelines of the Helsinki Declaration. It was approved by the institutional ethics committee.

Drug Preparation

A 50 mg cilostazol tablet (Pletoz) was dissolved in 100 mL solution of dimethyl sulfoxide and water in a 1:9 ratio to form a stock solution of 0.5 mg/mL. Aliquots of 5, 10, and 20 μ L, respectively, were then pipetted from the stock solution into Eppendorf tubes with 0.5 mL of blood to give concentrations of 10, 20, and 40 μ g/mL, representative of adult doses of 50, 100, and 200 mg, respectively. The control tube had no drug added to the blood.

The mixtures were gently agitated and allowed to incubate for 20 minutes at room temperature (25°C) before measurements were made.

Blood Sample Collection

Around 3 mL of blood was collected from each volunteer via venepuncture of the antecubital vein into vacutainer tube containing K^+ EDTA (1.5 mg/mL) anticoagulant and kept at room temperature (25°C) until measurements were done.

Oxygen Hemoglobin Dissociation Curve

The Hemox-Analyzer, was used to plot the oxygen hemoglobin dissociation curves and determine the P50 values for the blood samples at 37°C.

The buffer solutions were prepared as described by the manufacturers. Briefly, 5 mL of hemoxy solution was mixed with 20 μ L of additive A, 10 μ L of additive B, and 10 μ L of an antifoaming agent supplied by the manufacturers. Test samples (50 μ L each) were added to the buffer solution and used to plot the oxygen hemoglobin dissociation curve. The P50 values were determined automatically by the Hemox-Analyzer. All tests were performed within 3 hours of sample collection.

Data Analysis

Data was analyzed by analysis of variance using Sigma Plot 11.0 (Systat Software Inc., San Jose, CA), and $p < 0.05$ was accepted as statistically significant. Values represent mean $\pm$ standard error of the mean ($n = 8$).

Results

The mean P50 for the control blood samples was 28.27 ± 0.43 mm Hg. For the samples incubated with 10 μ g/mL cilostazol (equivalent to 50 mg dose) the mean value was 29.63 ± 0.66 mm Hg, while for the samples incubated with 20 μ g/mL cilostazol (equivalent to 100 mg dose) it was 30.15 ± 0.77 mm Hg. Samples incubated with 40 μ g/mL cilostazol (equivalent to 200 mg dose) had a value of 31.66 ± 0.62 mm Hg. There was a statistically significant difference ($p < 0.01$) between the P50 values for the control and 40 μ g/mL cilostazol samples ($\rightarrow$ Fig. 1).

Discussion

The results from this study show that cilostazol caused a dose-dependent shift in the oxygen hemoglobin dissociation curve to the right, which suggests an increase in the release of oxygen from hemoglobin. This is reflected in the increase in the P50 values that was statistically significant at the highest concentration of 40 μ g/mL, which is equivalent to the 200 mg dose of cilostazol. This observation is in agreement with the study of Hotta et al where the level of erythrocyte 2,3 diphosphoglycerate in sucrose-fed OLTEFT fatty rats given 0.03% of cilostazol was significantly higher in comparison to those not administered the drug.⁵ In that model of diabetes, the platelet aggregation was increased and concentration of erythrocyte 2,3 diphosphoglycerate was decreased in comparison to normal rats. Cilostazol reduced platelet aggregation in addition to normalizing 2,3 diphosphoglycerate levels in the OLTEFT rats.⁵ The metabolite 2, 3 diphosphoglycerate in erythrocytes is known to shift the oxygen hemoglobin dissociation curve to the right, resulting in increased oxygen release.⁶ We suggest that the ability of cilostazol to increase oxygen release from erythrocytes in the present study is likely

Fig. 1 The graph shows the effects of 10, 20, and 40 μ g/mL of cilostazol (equivalent to doses of 50, 100, and 200 mg, respectively)

mediated by an increase in the concentration of 2,3 diphosphoglycerate.

The increase in oxygen release observed at 20 µg/mL equivalent to the normal therapeutic dose of 100 mg of cilostazol, though not statistically significant, is a useful effect. This correlates well with the therapeutic property of cilostazol to increase the flow of blood to the legs in peripheral artery diseases.⁸

Conclusion

This study provides evidence that in addition to reducing platelet aggregation and increasing blood flow to the legs via vasodilation, cilostazol has the added pharmacological action of increasing the release of oxygen from hemoglobin. This effect is expected to enhance the therapeutic role of the drug in the management of peripheral artery disease.

Conflict of Interest

There are no conflicts of interest, financial or otherwise.

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