Central Physiologic Mechanisms Which Augment Oxygen Release (Bohr Effect And 2,3-Dpg Binding) Are Preserved In The Presence Of Voxelotor At The Therapeutic Target Of 30% Hb Modification

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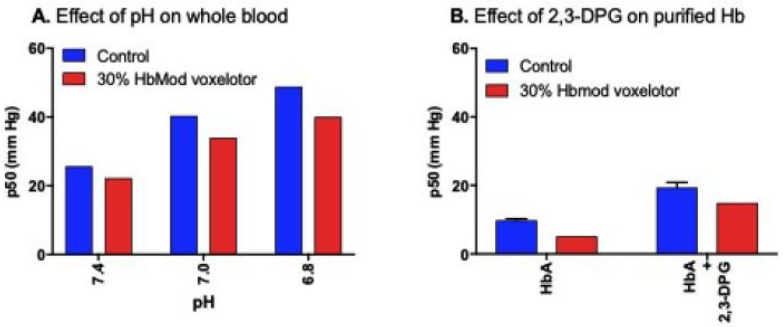
Background: Key physiologic mechanisms that drive O2 offloading to tissues in vivo include the Bohr effect and 2,3-DPG binding. The Bohr effect is the capacity of Hb to offload more O2 in hypoxic peripheral tissues in response to conditions of increased H+ concentrations (or acidosis). Similarly, the binding of 2,3-DPG to Hb stabilizes the deoxyHb state, reduces the Hb-O2 affinity and facilitates O2 release to tissues.

Aims: To investigate oxygen off-loading dynamics from voxelotor-modified hemoglobin in vitro under increased acidity or increased DPG content.

Methods: In this study, the Oxygen Dissociation Assay (ODA) and oxygen equilibrium curves (OECs) were used to determine the effect of voxelotor on Hb-O2 affinity in response to H+ and 2,3-DPG (5 mM). The ODA is a spectrophotometric assay that measures the time dependences of O2 release, while Hb-O2 affinities can be measured using OECs. Studies in the ODA were conducted with purified Hb (3 µM), while OEC determinations were conducted with both purified Hb (25 µM) and whole blood.

Results: Relative to Hb alone, voxelotor-modified Hb dose-dependently increased the proportion of oxygenated Hb (oxyHb) molecules during two hours of deoxygenation consistent with stabilization of hemoglobin in the oxy, or R- state. Under deoxygenated conditions, the percentage of oxygenated voxelotor-modified Hb decreased over time, demonstrating oxygen release (offloading) by voxelotor-modified Hb. With increased acidity (down to 6.8), there was an acceleration of oxygen release from voxelotor-modified Hb under deoxygenated conditions. This resulted in an increase in the p50 of voxelotor-modified blood and thus demonstrates a pHdependent decrease in the affinity of Hb for O2 in the presence of voxelotor at the therapeutic target Hb modification of 30% (Figure 1A). Similarly, the response of Hb to 2,3-DPG effect was not inhibited in the presence of voxelotor at the 30% modification target
(Figure 1B).



Summary/Conclusion: The results of this study indicate that the central physiologic mechanisms which augment oxygen release to metabolically active tissues, such as the Bohr effect and 2,3-DPG binding, are preserved when voxelotor is bound to Hb.