Title: CROSSLINKED HEMOGLOBIN: OXYGEN OFFLOADING, PLASMA RETENTION AND METABOLIC FATE

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Introduction. Crosslinked hemoglobin (HbXL) is a modified human hemoglobin, covalently bridged with a 2,3-DPG analog, 2-nor-2-forsylypyridoxal 5'-phosphate.\(^1\) Such a modification of hemoglobin by intramolecular crosslinking of the two dimers by this 2,3-DPG analog adds properties which are essential for a blood substitute: low oxygen affinity due to a "built-in 2,3-DPG effect" and a more stable molecule which cannot dissociate (due to the covalent intradimeric bond). These properties were studied in order to evaluate HbXL as a potential blood substitute.

Methods. Oxygen offloading by HbXL was measured in a modified Langendorff preparation of a paced isolated rabbit heart.\(^2\) A catheter with a fluid-filled balloon in the left ventricle was used to adjust preload to 15-20 torr and to monitor intraventricular pressure while a second catheter in the right atrium collected the effluent anaerobically. Plasma retention and metabolic fate of HbXL were determined in rats injected iv with HbXL or tritium-labeled HbXL. Plasma and urine hemoglobin concentrations were measured colorimetrically as cyanmethemoglobin, and radioactivity was measured by liquid scintillation counting. The molecular size of the \(^3\)H-labeled moieties eliminated via the kidneys and G.I. tract was estimated by filtering samples of urine and feces extracts through 10,000 MW cut-off membranes (Centricon-10, Amicon Corp.).

Results and Discussion. HbXL, which has a \(P_{50} = 47 \text{ torr}, \) offloaded \(40-60\%\) (depending on the heart rate) of its \(O_2\) to the heart when equilibrated with 95% \(O_2\) and 5% \(CO_2\) (saturation 95-100%) and 60-75% of its \(O_2\) when equilibrated with 25% \(O_2\), 5% \(CO_2\) and 70% nitrogen (saturation 80-85%). This indicated that HbXL would offload more oxygen to tissues than blood under identical conditions.

At a dose of 2 mg/g of body weight the initial plasma level of HbXL was about 3 g%. The plasma clearance appeared to be biphasic. About 1 g% was cleared relatively rapidly, with 1/2 of about 1 h, followed by a much slower, linear plasma decline at a rate of 1 g% in 8 h (fig.) HbXL was not excreted in the urine, in contrast to unmodified hemoglobin. These findings suggest a 1/2 of HbXL of 24 h at a clinically relevant starting plasma concentration of 5 g%.

\(^3\)H-HbXL injected iv (0.15 mg/g body weight) was not recovered as such in the urine and the plasma decay was similar to that found with nontagged HbXL. However, the label from \(^3\)H-HbXL was recovered in the urine as obviously metabolized fragments, MW ≤ 10,000. By 9 days about 50% of the label administered had been recovered in urine and about 20% was recovered in feces, in both instances as 10,000 MW or smaller breakdown products. Consequently, the amount of HbXL found in organs and muscle tissue 9 h after administration (about 35% of injected dose) decreased to about 5% of administered dose within the 9-day period. During this time period (9 days) after administration of \(^3\)H-HbXL almost 80% of the label was recovered in the urine and feces of the rats as smaller degradation fragments, indicating that HbXL is metabolized and eliminated from the body mainly via the kidney and gastrointestinal tract as breakdown products.

Conclusions. These findings show that HbXL has properties desirable for a blood substitute: it offloads oxygen efficiently, it has a sufficient intravascular retention, and it is metabolized and cleared from the body.

References:

\[y = 2.034 + (-0.028)x\]

HbXL plasma levels, in g%, after an iv dose of 2 mg/g of body weight.