Differential prooxidative effect of adult and fetal hemoglobin

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Human fetal hemoglobin assayed in a peroxidizing system shows an increased prooxidative effect when compared to human adult hemoglobin. This effect is related only to the oxyhemoglobin form since both fetal and adult methemoglobins did not show any prooxidative effect. The prooxidative effect of the oxyhemoglobin form is ascribed to its increased sensitivity to form superoxide free radicals when transformed to the methemoglobin form. It is proposed that the structure of the heme pocket of fetal oxyhemoglobin enhances free radical release when compared to adult oxyhemoglobin. This difference may be important in some hematological disorders presented by the newborn.

Oxyhemoglobin *Lipid peroxidation* *Methemoglobin* *Hemoglobin*

1. INTRODUCTION

Structural differences between adult and fetal hemoglobin are determined by the presence of α-β chains in adult hemoglobin (HbA) and α-γ chains in fetal hemoglobin (HbF). The oxygen affinity of both hemoglobins is very different, HbF having an increased saturation capacity compared to HbA at low oxygen tensions.

Human fetal erythrocytes are more susceptible to oxidative hemolysis than adult erythrocytes [1]. The antioxygenic capacity of fetal erythrocytes is different from that of adult red cells. Comparative studies of maternal and cord blood show that the activity of the enzymes catalase and glutathione peroxidase is significantly lower in fetal erythrocytes while that of the enzyme superoxide dismutase is equivalent to that found in maternal erythrocytes [2]. In addition, HbF exhibits an increased sensitivity relative to HbA to form methemoglobin [3] through the univalent reduction of oxygen leading to the formation of the prooxidant superoxide free radical [4].

This study was undertaken to investigate the prooxidant properties of adult and fetal human hemoglobin in its oxy- and methemoglobin states using a peroxidizing system formed by potassium linoleate.

2. EXPERIMENTAL

Samples were obtained from the cord blood of 10 full-term newborn infants and from the venous blood of their mothers (Hospital Paula Jara-Quemada, Santiago). All newborn were normal and their mothers presented no evidence of hematological diseases. Blood samples were collected in heparinized tubes, centrifuged at $1500 \times g$ for 10 min and the plasma and buffy coat removed by aspiration. Red cells were washed twice with saline and lysed by the addition of 1.5 vols distilled water. Cell debris was discarded by centrifugation at $6500 \times g$ for 15 min and the hemoglobin supernatant of each sample was fractionated by chromatography on CM-Sepharose to separate HbA and HbF. Fractionation was according to Congote and Kendall [5] based on the different elution pattern of both hemoglobins to an NaCl concentration gradient. After elution, fractions containing each hemoglobin type were collected, dialyzed

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against distilled water and concentrated by ultrafiltration using a dialysis tube (Spectrator membrane tubing 08-667A) under negative pressure at 5°C to 1/10 of its original volume. After concentration each fraction was suspended in phosphate-buffered saline (9 parts 0.9% NaCl, 1 part 0.1 mM KH_2PO_4, 50 mM EDTA; pH 7.4) to a final concentration of 10 mg Hb/ml. Hemoglobin concentration was determined as cyanmethemoglobin [6]. Oxyhemoglobin was prepared by bubbling O_2 (100%). Methemoglobin was formed by incubating a solution of hemoglobin with an equal volume of 1% $NaNO_2$ in the phosphate-buffered saline for 10 min at room temperature. Excess nitrite was removed by passing the methemoglobin solution through a Sephadex G-25 column in phosphate-buffered saline. Superoxide dismutase and catalase activity of the hemoglobin solutions was assayed according to Winterbourn et al. [7] and Beers and Sizer [8], respectively. Free Fe^{2+} in the hemoglobin solutions was determined using a diagnostic Merck test (Merckotest no. 3307). The potassium linoleate peroxidizing system was prepared as in [9] using a peroxide-free linoleic acid and the peroxidation initiated by adding the oxy- and methemoglobin solutions. Linoleate peroxidation was assessed by conjugated diene formation at 37°C and measured at 233 nm according to Hasse and Dunkley [10]. Results were expressed as nmol conjugated dienes/ml peroxidizing system using ϵ 2.52×10^9 l·mol^{-1} [11]. All reagents were obtained from Sigma (St. Louis, MO). Results are expressed as means ± SE and the significance of the differences between mean values was assessed by Student's *t*-test for unpaired data.

3. RESULTS AND DISCUSSION

3.1. *Separation of HbF and HbA from maternal and fetal cord blood hemolysates*

The separation of HbF and HbA from fetal to adult blood on CM-Sepharose is shown in fig.1. Both hemoglobins can be fractionated when an NaCl concentration gradient is applied to the column. The absorbance profile indicates the presence of two major fractions in both hemolysates, corresponding to the HbF and HbA components as described by Congote and Kendall [5]. The minor components of the HbF and HbA present in adult and fetal hemolysates respectively were discarded and the major fractions converted, after collection, to the oxy- and methemoglobin forms. Neither fractions presented any detectable free Fe^{2+} and were absent of superoxide dismutase and catalase activities.

3.2. *Comparative effect of HbF and HbA on the oxyhemoglobin and methemoglobin forms in the linoleate peroxidation system*

Fig.2 shows the prooxidant effect of HbF and HbA on their oxy forms in the linoleate peroxidizing system. Both hemoglobins elicited a concentration-dependent increase in linoleate peroxidation vs controls, HbF showing a significantly higher prooxidant effect than HbA. On the other hand, the results obtained with the methemoglobin forms of both fractions show that HbF and HbA elicited only a slight prooxidant effect, this being of a similar magnitude at the same hemoglobin concentrations (table 1). This effect is in accordance with Szebeni et al. [12] who described the absence of peroxidation in hemosomes containing methemoglobin and formed by unsaturated lipids.

The higher prooxidative effect of oxy HbF may be the result of its increased sensitivity to be converted to methemoglobin with the release of hydrogen peroxide and superoxide free radicals [13]. These radicals and the related hydroxyl free radical are involved in the initiation and propagation of unsaturated fatty acid peroxidation [14]. Linoleate peroxidation can be inhibited by superoxide dis-

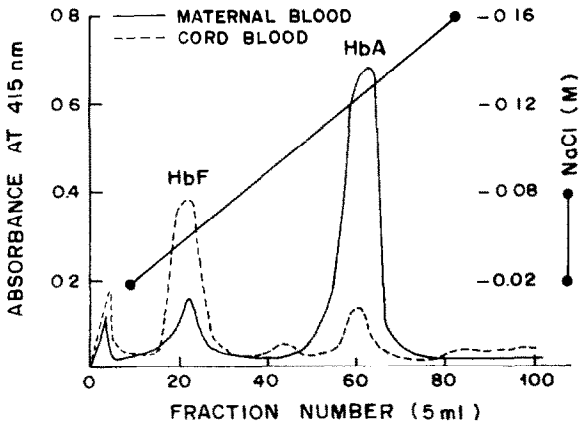

Fig.1. Separation of HbF and HbA from maternal and cord blood hemolysates by chromatography on CM-Sepharose. Elution profile represents a typical fractionation pattern.

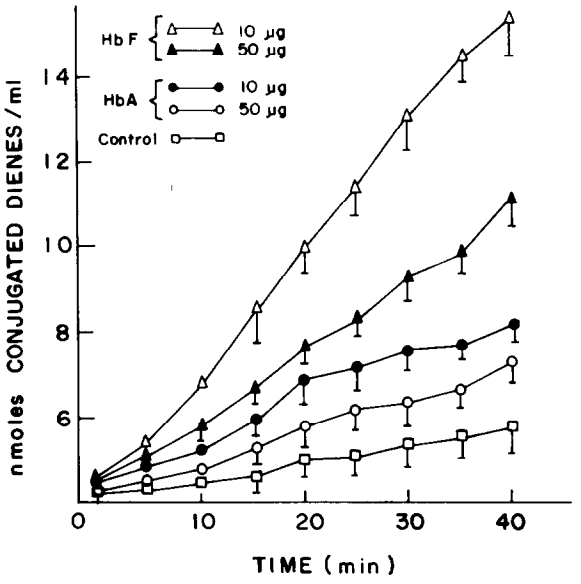

Fig.2. Comparative effect of different concentrations of HbF and HbA in the oxyhemoglobin form on linoleate peroxidation. Hemoglobin concentration is expressed as µg/ml peroxidizing system. Control values correspond to linoleate peroxidation obtained without oxyhemoglobin addition.

Table 1

Effect of the methemoglobin form of fetal hemoglobin (HbF) and adult hemoglobin (HbA) on linoleate peroxidation after 40 min incubation at 37°C

Addition/ml peroxidizing system	nmol conjugated dienes/ml peroxidizing system
HbF (10 µg)	6.2 ± 0.18
HbA (10 µg)	6.8 ± 0.22
HbF (50 µg)	7.1 ± 0.14
HbA (50 µg)	6.9 ± 0.08
Control	5.9 ± 0.42

Results represent the average of 6 experiments ± SE. Control values correspond to linoleate peroxidation obtained without methemoglobin addition. Differences between each value of HbF or HbA and the control are not significant

mutase, this effect being another probe of the participation of superoxide free radicals in the oxidative phenomenon [15].

As proposed by Carrel et al. [16], small fluctuations on the heme pocket of oxyhemoglobin may allow access of water molecules to the heme, resulting in the formation of methemoglobin and release of superoxide radicals. Any factor capable of causing increased flexibility or distortion of the heme pocket could result in increased methemoglobin formation. This situation has been observed with redox dyes (e.g. ascorbate, phenylhydrazine) and in some unstable hemoglobins [17]. We are aware of no report describing such an effect for fetal hemoglobin. Our results on the prooxidant effect of HbF sustain the view that the structure of the heme pocket of fetal hemoglobin may result in an enhanced effect on its methemoglobin conversion when compared to adult hemoglobin. This effect may be an important component in some hematological disorders observed in the newborn, especially in cases of premature delivery, where the cellular defenses against oxygen toxicity are not fully developed [2].

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