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OXYGEN METABOLISM IN RABBIT BONE MARROW AND LIVER

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Summary: Oxygen metabolism has been quantified in rabbit bone marrow and liver. NADPH-Cytochrome c reductase activity in bone marrow microsomal and cytosol fractions was about 40% of that found in liver. Superoxide anion and peroxide generation were found to be present in both liver and bone marrow. Catalase and superoxide dismutase activity were measured in liver and in marrow preparations free of erythrocytes; while liver catalase activity was approximately twice that of bone marrow, very low superoxide dismutase activity was observed in erythrocyte free bone marrow homogenates.

The production and effects of toxic metabolites of oxygen, namely superoxide anion and peroxide, have been implicated in various mammalian tissues such as lung (1), and erythrocytes (2). Superoxide anion is produced by NADPH-cytochrome c reductase (EC 1.6.2.4) (3) and peroxide may be derived from the dismutation of superoxide anion. Furthermore, enzymes have been described which serve to detoxify these metabolites of oxygen; included are superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6) and glutathione peroxide (EC 1.11.1.7) (4).

The uncontrolled production of superoxide anion has been associated with lung damage (1) and erythrocyte hemolysis (2) and has been implicated in the bioactivation of catechols, such as α-methyl DOPA (5) and 2-hydroxyestrogens (6) to electrophilic arylating species, which have been shown to become covalently bound to hepatic macromolecules.

Since the bone marrow is a target for some drug and chemical toxicities, pathways for the formation and detoxication of oxygen metabolites have been

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investigated in this tissue and compared to that found in liver. We report herein the relative rates of superoxide anion and peroxide generation as well as the activities of NADPH-cytochrome c reductase, superoxide dismutase and catalase in rabbit bone marrow and liver.

MATERIALS AND METHODS

Animals and Tissue Preparation: Female white New Zealand rabbits weighing 1.4-1.8 kg were housed individually under a 12 hr light-dark cycle (lights on from 0600-1800 hours) and were allowed free access to a standard laboratory chow and water. Animals were anesthetized (ether), exsanguinated, the liver was promptly removed and perfused with ice-cold 1.15% KCl solution; 8-12 g of liver were homogenized with 4 vol. of 0.1 M phosphate buffer (pH 7.4) in a Dounce homogenizer. Bone marrow (2-4 g, wet weight), isolated from the femur and humerus was homogenized with 1.5 vol. of 0.1 M phosphate buffer (pH 7.4). Homogenates were centrifuged at 9000 x g at 4° for 15 min followed by centrifugation of the resulting supernatant at 105,000 x g for 60 min. The liver supernatant (cytosol fraction) was removed and the pellet (microsomal fraction) was suspended in phosphate buffer to a concentration of 6 mg protein/ml; the bone marrow pellet (approx. 8 mg protein) was suspended in 2 ml of phosphate buffer (pH 7.4). Cytosol and microsomal fractions were assayed for superoxide anion and peroxide generation as well as NADPH-cytochrome c reductase activity. Protein concentration was determined according to Lowry et al. (7) using crystalline bovine serum albumin as the standard.

Bone marrow whole homogenates were freed of erythrocytes by a modification of the method of Boyum (8). Sodium heparin (1200 units) was given intravenously 20 min prior to exanguination. Marrow, collected as described above and suspended in 20 ml of balanced salt solution (0.01% D-glucose, 50.0 μM $CaCl_2$, 0.1 mM $MgCl_2$, 14.5 mM tris, 0.54 mM KCl) containing 10 U heparin/ml was layered above 15 ml of Ficoll-Hypaque (ten parts Hypaque solution mixed with 24 parts 9% Ficoll solution) in a 50 ml plastic centrifuge tube. Erythrocytes were sedimented by centrifugation at 400 x g for 20 min. The upper layer containing erythrocyte free marrow cells was removed with a Pasteur pipet and placed in another plastic centrifuge tube (50 ml) and centrifuged at 400 x g for 10 min. The resulting supernatant was discarded, the marrow cells were suspended in 10 ml balanced salt solution and centrifuged at 200 x g for 10 min; the resulting supernatant was discarded and the marrow cells were suspended in 2 ml of buffer. Cells thus isolated were homogenized and used for measurement of catalase and superoxide dismutase activity. Microscopic examination showed less than 10% contamination by erythrocytes.

NADPH-cytochrome c reductase activity was measured by a modification of the method of Phillips and Langdon (9). Incubation mixtures containing 0.1 mg of microsomal or cytosol protein and 150 moles cytochrome c (Type VI, Sigma Chem. Co.) in 0.1 M phosphate buffer (pH 7.4) in a total volume of 3 ml were warmed to 37° and the increase in absorbance at 550 nm was recorded after the addition of 0.8 μmoles NADPH (Type III, Sigma Chem. Co.)

Superoxide anion and peroxide generation were assayed as described by Montgomery (10).

Catalase activity was measured according to the method by Beers and Sizer (11) as modified by Cohen et al. (12).

Superoxide Dismutase activity was measured as described by Misra and Fridovich (13) except that the reaction mixture was illuminated for 6 min at 25°.

TABLE 1

NADPH-Cytochrome c Reductase Activity and Superoxide and Peroxide Generation in Bone Marrow and Liver

Fraction	NADPH-cyt. c reductase (nmoles/min/ mg protein)	Superoxide generation (nmoles adrenochrome/ min/mg protein)Z	H_2O_2 generation (nmoles HCHO/ min/mg protein)
Bone marrow microsomes	61 ± 30[a]	1.2 ± 0.6	0.78 ± 0.05
Liver microsomes	158 ± 72	4.2 ± 1.6	3.74 ± 0.88
Bone marrow cytosol	24 ± 7	N.D.[b]	0.47 ± 0.18
Liver cytosol	55 ± 27	N.D.	0.29 ± 0.04

[a]Values are shown as the mean ± S.D., n=4.
[b]N.D. = not detectable.

RESULTS AND DISCUSSION

Table 1 shows the NADPH cytochrome c reductase activity of both bone marrow and hepatic microsomal and cytosol fractions isolated from female rabbits. Bone marrow microsomes contained 39% of the activity of liver microsomes while 44% of the cytosol activity was found in bone marrow cytosol. Total bone marrow NADPH-cytochrome c reductase activity was 10% of that found in liver (data not shown).

Superoxide anion and peroxide generating capacity of bone marrow and liver was also studied (Table 1). Neither bone marrow nor liver cytosol supported detectable superoxide anion production. The bone marrow microsomal fraction contained 29% of the activity found in the hepatic microsoomal fraction. Bone marrow microsomes contained 21% of the peroxide generating capacity of liver microsomes and bone marrow cytosol contained 162% of the activity of liver cytosol. Total bone marrow peroxide generation was 30% of that found in liver (data not shown).

The activities of superoxide dismutase and catalase in bone marrow and liver whole homogenates freed of contaminating erythrocytes are shown in Table 2. Although bone marrow catalase activity was 45% of that found in liver, bone

TABLE 2

Catalase and Superoxide Dismutase Activities in Bone Marrow and Liver Homogenates

Homogenate	Catalase activity (units/mg protein)[a]	Superoxide Dismutase (units/mg protein)[b]
Whole bone marrow	267 ± 73[c]	57 ± 11
Whole liver	595 ± 206	656 ± 115

[a]One unit of catalase is that amount of enzyme catalyzing the reduction of one μmole of H_2O_2/min/ml reaction mixture at 25° and pH 7.0.
[b]One unit of superoxide dismutase is that amount of enzyme causing an augmentation in the ΔA_{460} of 0.001 A/ml reaction mixture at 25° and pH 7.4.
[c]Values are shown as the mean ± S.D.; n=4.

marrow homogenates contained less than 10% of the superoxide dismutase activity observed in liver.

These results show that bone marrow has substantial capacity to metabolize oxygen to potentially toxic species, namely superoxide anion radical and peroxide (Table 1). Furthermore, although considerable catalase activity was present in bone marrow, relatively little superoxide dismutase activity could be detected in this tissue (Table 2). This low superoxide anion detoxification capacity suggests that bone marrow may be susceptible to superoxide anion-mediated injury. Such injury may be either the result of a direct effect of superoxide anion on bone marrow cells or the consequence bioactivation of xenobiotics or their metabolites by superoxide anion.

The superoxide anion-dependent bioactivation of α-methyl DOPA, dopamine, and 2-hydroxygestrogens in the liver (5,6,14) and brain (15) has been described. In addition, an organ specific deficiency of superoxide dismutase activity has been implicated in the pulmonary toxicity of paraquat (methyl viologen) (16).

The importance of these findings for chemically mediated bone marrow toxicity is currently being investigated. However, these findings do suggest that catecholic metabolites formed, for example, in the liver may exert toxic effects at extrahepatic sites such as bone marrow.

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