4-1990

Pathophysiology of Hemorrhagic Shock and the Protective Effects of Antioxidants

Annette Elizabeth Fleckenstein

PATHOPHYSIOLOGY OF HEMORRHAGIC SHOCK AND THE PROTECTIVE EFFECTS OF ANTIOXIDANTS

by

Annette Elizabeth Fleckenstein

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Science
Department of Biological Sciences

Western Michigan University
Kalamazoo, Michigan
April 1990

PATHOPHYSIOLOGY OF HEMORRHAGIC SHOCK AND THE PROTECTIVE EFFECTS OF ANTIOXIDANTS

Annette Elizabeth Fleckenstein, M.S.

Western Michigan University, 1990

Considerable attention has focused on the role of free radicals in the pathophysiology of hemorrhagic shock. In this study, four pharmacological mechanisms for antagonizing free radical generation or reactions were compared in terms of their efficacy in attenuating post-hemorrhage (post-reinfusion) cardiovascular collapse. These included blocking arachidonic acid oxidation by cyclooxygenase (e.g., ibuprofen), inhibiting superoxide production by xanthine oxidase (e.g., oxypurinol), chelating iron (e.g., desferal), and inhibiting lipid peroxidation (e.g., U74006F and U78517G, The Upjohn Company, Kalamazoo, Michigan).

Cardiovascular function, cerebral blood flow, arterial blood gases, serum glucose, and plasma vitamin E were examined in a hemorrhage/reperfusion model using urethane-anesthetized rats. U74006F and U78517G attenuated the progressive cardiovascular collapse characteristic of hemorrhagic shock whereas ibuprofen, desferal, and oxypurinol were ineffective. Protection against the progressive decline in cerebral blood flow associated with hemorrhagic shock was observed only in U74006F-treated rats.

ACKNOWLEDGEMENTS

I wish to express sincere appreciation for his encouragement and counsel to Dr. Edward Hall under whose direction at The Upjohn Company, Kalamazoo, Michigan, this research was conducted. Special appreciation is expressed also to my advisor and committee chairman, Dr. Leonard Beuving, for his guidance and support throughout my course of study. Appreciation is expressed to Miss Sally Smith for her guidance throughout the course of my research; to Dr. John McCall, and Dr. Mark Braughler for their assistance in conducting this research; and to my committee member, Dr. Cecil McIntire, for reviewing this thesis.

Finally, my deepest gratitude is extended to my parents and my brother, without whose love and support this thesis might not have been completed.

Annette Elizabeth Fleckenstein

Order Number 1339827

Pathophysiology of hemorrhagic shock and the protective effects of antioxidants

Fleckenstein, Annette Elizabeth, M.S.

Western Michigan University, 1990

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

TABLE OF CONTENTS

Table of Contents--Continued

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Statement of the Problem

Traumatic hemorrhage can lead to a dramatic decrease in effective circulating blood volume such that ventricular filling is diminished thereby leading to severe hypotension. Compensatory vasoconstriction by the release of epinephrine and norepinephrine from the adrenal medulla and peripheral adrenergic nerve terminals, respectively, is inadequate once mean arterial pressures fall below 50 mm Hg. At this pressure, tissue perfusion is inadequate to meet metabolic demands. This is of critical importance when hypoperfusion affects the heart and brain; either situation can initiate the progressive cardiovascular collapse that is a hallmark of the hemorrhagic shock scenario.

Conventional treatment of traumatic hemorrhage consists of replacement of circulating volume and administration of inotropic agents to enhance cardiac output and improve tissue blood flow. In hemorrhagic shock, however, a progressive decline in cardiovascular function often occurs and may even be exacerbated when conventional transfusions are administered. While several factors likely contribute to this collapse, considerable attention has been focused on the pathophysiological role

of oxygen radical and lipid peroxidative injury to the myocardium and to medullary cardiovascular control centers.

Free Radical Generation in Ischemia and Reperfusion

Controlled reactions involving low levels of highly reactive oxygen species are essential for normal physiological function. The reintroduction of oxygen into ischemic/hypoxic tissue may, however, result in an uncontrolled burst of free radical generation (Granger, Hollwarth, & Parks, 1986). In reperfusion of the ischemic myocardium, excessive oxygen radical production, presumably exceeding endogenous defense capabilities, has been both directly (Kramer, Arroyo, Dickens, & Weglicki, 1987; Misra, Weglicki, Abdulla, & McCay, 1984; Zweir, Flaherty, & Weisfeldt, 1987) and indirectly (Bernier, Hearse & Manning, 1986; Horneffer & Gardner, 1984; Zweir, Rayburn, Flaherty, & Weisfeldt, 1986) demonstrated. Oxygen free radical generation in the reperfused brain has similarly been reported (Cao, Carney, Duchon, Floyd, & Chevion, 1988; Kirsh, Phelan, Lange, & Traystman, 1987). At least five possible means for generating free radicals in ischemia-reperfusion situations have been described. These include mitochondrial electron transport chain uncoupling (Demoupolos, 1973), xanthine oxidase activation (McCord, 1985), prostaglandin biosynthesis (Watson & Ginsberg, 1988), granulocyte infiltration (Simpson, Fantone, &

Lucchesi, 1988), and catecholamine oxidation (Jewett, Eddy, & Hochstein, 1989).

Under ordinary conditions (i.e., in the presence of an adequate oxygen supply), ubiquinone or coenzyme Q exists as a semiquinone free radical within the electron transport chain (Ruzicka, Beinert, Schilper, Dunham, & Sands, 1975). While small amounts of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) leakage from the mitochondria normally occurs (Patole, Swaroop, & Ramasarma, 1986; Turrens, Alexandre, & Lehninger, 1985), the semiquinone radical is in large measure tightly controlled by being coupled to the reduction of O_2 to H_2O. However, when tissue oxygen supplies are diminished as seen in severe hemorrhagic hypotension, electron transport factors dissociate (Demopoulos, 1973) and ubiquinone is no longer tightly controlled (Demopoulos, Flamm, Pietronigro, & Seligman, 1980). Ubiquinone can be reduced via a one-electron transfer to form the ubisemiquinone radical. This molecule can, in turn, be re-oxidized by whatever molecular O_2 is present and in so doing increased levels of O_2^- are generated (Fisher, 1988). Furthermore, because ubiquinone is lipid soluble, its dissociation from the chain and the subsequent generation of an uncontrolled free radical species potentiates damage within the lipid-rich inner mitochondrial membrane in which ubiquinone normally resides (Mellors & Tappel, 1966).

Ischemic damage to the mitochondria also results in decreased oxidative phosphorylation and a slowing or stopping of adenosine triphosphate (ATP) generation. Concomitantly, adenosine monophosphate (AMP) accumulates and is then catabolized into adenosine, inosine, and finally hypoxanthine (Jennings, Reimer, Hill, & Mayer, 1981; Kleihues, Kobayashi, & Hossman, 1974; McCord, 1985). Diminished ATP concentrations promote failure of membrane ionic pumps and thereby increased intracellular levels of calcium (Siesjo, 1981; Siesjo, 1984). Calcium ions activate cytosolic proteases which may, in turn, convert endogenous xanthine dehydrogenase into xanthine oxidase (Jarasch, Bruder, & Heid, 1986; McCord, 1985). Upon reperfusion and the accompanying increase in oxygen tension, xanthine oxidase utilizes molecular oxygen as an electron acceptor in the conversion of hypoxanthine into xanthine and in so doing produces O_2^- and H_2O_2 (McCord, 1985; Simpson, Fantone, & Lucchesi, 1988).

The abnormally high intracellular calcium ion concentrations that frequently occur in muscle and nervous tissue during hemorrhagic hypotension-induced ischemia can trigger free radical generation by a second method; the activation of phospholipase A_2 and subsequent liberation of arachidonic acid. Enzymatic oxidation of released arachidonic acid can lead to biosynthesis of O_2^- by one of two major pathways. First, O_2^- can be generated in the

cyclooxygenase catalyzed conversion of arachidonic acid into prostaglandin G_2. In the subsequent reduction of its hydroperoxide group by prostaglandin hydroperoxidase to yield prostaglandin H_2, enzyme-centered radical intermediates are formed which can oxidize NADH or NADPH into their free radical counterparts. When these nucleotide radicals react with molecular oxygen, O_2^- is again formed (Watson & Ginsberg, 1988). Second, O_2^- can be produced via a side-chain reaction involving PGH synthetase and lipoxygenase when in the presence of NADH or NADPH (Kukreja, Kontos, Hess, & Ellis, 1986).

Initiation of the 5-lipoxygenase pathway of the arachidonic acid cascade not only triggers O_2^- production but the synthesis of such chemotactic factors as hydroperoxyeicosatetraenoic acid (HPETE) as well. Moreover, O_2^- reacts with a yet to be characterized extracellular (plasma) precursor to produce chemotactic activity (Petrone, English, Wong, & McCord, 1980). Neutrophils and other phagocytes are thereby attracted to ischemic tissue. Various stimuli, including tissue reperfusion, will activate NADPH oxidase, the respiratory system of these phagocytes. The activation of this enzyme results in an increase in the O_2 consumption of phagocytes referred to as a "respiratory burst" (Rossi, Della Bianca, Grzeskowiak, & Zeni, 1986). Oxidase catalyzed one electron reductions of oxygen at the expense of NADPH will generate

O_2^- (Babior, Curnette, & Okamura, 1988). Superoxide is the key to a series of reactions in which large quantities of oxidants including H_2O_2, oxidizing radicals (the hydroxyl radical, $\cdot OH$), and oxidized halogens (chloramines, OCl^-) are produced (Babior et al., 1988).

With ischemia, as seen in the traumatic hemorrhage scenario, comes the release, via sympathetic neurotransmission and from the adrenal medulla, of catecholamines. The autoxidation of catecholamines has been implicated as a source of oxygen radicals in myocardial ischemia-reperfusion injury (Singal, Kapur, Dhillon, Beamish, & Dhalla, 1982). However, the autoxidation of catecholamines at physiological pH occurs too slowly to be a major oxygen radical source. It has been suggested, therefore, that catalyzed oxidation of catecholamines involving metal ions and/or enzymes might be significant sources of oxygen radicals (Jewett, Eddy, & Hochstein, 1989). In the presence of iron, epinephrine, norepinephrine, and dopamine can undergo an oxidation reaction wherein O_2^- is formed (Misra & Fridovich, 1972). The oxidation of epinephrine by mechanisms involving iron proteins has been documented (Green, Mazur, & Shorr, 1956). Further, the degradation of catecholamines by the enzyme monoamine oxidase resulting in the formation of H_2O_2 is another potential source of oxygen radicals (Marker, Weiss, Silides, & Cohen, 1981).

Each of the five free radical generating mechanisms described above produces $O_2^{\cdot-}$. Moreover, the autoxidation of Fe^{II} to Fe^{III} will also result in $O_2^{\cdot-}$ formation. In a superoxide-dismutase catalyzed reaction, $O_2^{\cdot-}$ is converted into H_2O_2 which, under the influence of the enzyme catalase, is converted into H_2O. In solution, $O_2^{\cdot-}$ exists in equilibrium with the hydroperoxyl radical ($HO_2^{\cdot}$). Under the acidic conditions found in the hemorrhagic hypotension scenario, $HO_2^{\cdot}$, the more lipid soluble and better oxidizing/reducing agent of the pair (Bielski, Arudi, & Sutherland, 1983), predominates and hence the potential for interaction with membranes is enhanced. Further, $HO_2^{\cdot}$ will dismutate to hydrogen peroxide (H_2O_2) far more readily than will $O_2^{\cdot-}$ under acidotic conditions since the rate constant for the former is 10^8 times greater than that for the dismutation of $O_2^{\cdot-}$ to H_2O_2 (Braughler & Hall, 1989).

Hydrogen peroxide (H_2O_2), in the presence of free iron, can produce at least two different highly reactive radical species. Upon reacting with Fe^{II}, H_2O_2 will form the hydroxyl radical (Fenton's reaction) or the ferryl ion (Fe^{III}-OH). Also, when $O_2^{\cdot-}$ reacts with H_2O_2 by means of the Haber-Weiss reaction, $^{\cdot}OH$ is formed. Both $^{\cdot}OH$ and Fe^{III}-OH are extremely powerful oxidants which can react readily with a variety of substances including DNA, proteins, and lipids.

Pathogenesis of Lipid Peroxidation

Perhaps the most important molecular targets for free radical attack are lipids. Oxygen radicals can affect lipids by initiating peroxidation; a process equivalent to fat becoming rancid. Peroxidation consists of the direct reaction of lipid with oxygen free radicals to form semi-stable peroxides and other free radical intermediates. A chain of subsequent autocatalytic free radical reactions results which can profoundly disrupt the integrity of the cell membrane phospholipid bilayer. Moreover, enhanced permeability of membranes to calcium attributable to Fe^{++} initiated lipid peroxidative damage has been observed (Braughler et al., 1987). While it is unclear as to which oxygen radical species actually initiates these events (Braughler, Duncan, & Chase, 1986; Braughler & Hall, 1989; Minotti & Aust, 1987), evidence of lipid peroxidative chain reactions playing an important role in ischemic and traumatic cell injury is considerable (Goldberg et al., 1984; Kurihara, 1985; Watson et al., 1984).

Oxygen radicals, because of an unpaired electron in their outermost orbitals, react readily with the phospholipid components of cellular membranes. They attack unsaturated fatty acids by removing an allylic hydrogen situated between adjacent double bonds and thereby create a carbon-centered lipid radical ($L^{\cdot}$). This radical reacts with oxygen dissolved within membranes to form a lipid

peroxyl radical (LOO·). The peroxyl radical can, in turn, react with other lipid molecules to generate a lipid hydroperoxide (LOOH) and another carbon centered L·. This second generation of L· can react with dissolved oxygen as described above to trigger further peroxidative sequences (Braughler et al., 1989; Demopolous et al., 1980; Ursini, 1986).

Lipid hydroperoxide molecules can react with either Fe^{II} or Fe^{III} to yield lipid peroxyl radicals or lipid alkoxyl radicals (LO·), respectively. Either product can react with polyunsaturated fatty acids and thereby serve as a third source of carbon-centered lipid radicals. When alkoxyl radicals are the reactants, alcohol molecules (LOH) are formed. When peroxyl radicals react, lipid hydroperoxide molecules capable of further reactions with iron, are the final products (Aust & Svingen, 1982; Braughler et al., 1989; Demopoulos et al., 1980; Ursini, 1986).

Free iron is readily available in the ischemic-hypoxic cell environment seen during hemorrhagic hypotension. Under normal physiological conditions, it is sequestered intracellularly by the protein ferritin and extracellularly by the protein transferrin. Under the acidotic conditions encountered in ischemia or in reactions involving O_2^-, iron can be liberated from ferritin (Braughler & Hall, 1989; Thomas, Morehouse, & Aust, 1985). Hemoglobin can also

release its iron upon reacting with lipid hydroperoxides or H_2O_2 (Gutteridge, 1986).

Pathophysiological Effects of Free Radicals

Once initiated, free radical propagation can affect injury by a variety of mechanisms. In addition or secondary to lipid peroxidation, free radicals can damage proteins by altering component amino acids or by enhancing their susceptibility to hydrolysis (Wolff, Garner, & Dean, 1986). Latent forms of collagenase and gelatinase can be activated by free radical-mediated oxidative mechanisms. Oxygen radical species can inactivate proteinase inhibitors and thereby render previously controlled lysosomal proteases unchecked (Werns, Shea, & Lucchesi, 1985). However, perhaps the most significant of the hemorrhagic shock phenomenon is the membrane injury produced by lipid peroxidation as described above.

Peroxidation of myocardial membrane lipids increases their fluidity and permeability (Okabe et al., 1988). Enhanced permeability can lead to an excessive influx of calcium ions which, in turn, can promote injury in at least three ways (Simpson et al., 1988). First, calcium ions activate various proteases and can thereby cause tissue destruction. Second, calcium may activate phospholipases and in so doing increase levels of arachidonic acid metabolites and thereby affecting cell injury. Finally,

enhanced calcium influx can activate ATP-ase and thereby drain myocyte energy stores. In any event, myocardial function is compromised.

Superoxide radicals and their metabolites can also jeopardize cardiac function because they decrease calcium transport by cardiac sarcoplasmic reticulum (Okabe et al., 1988). Sarcoplasmic reticulum injury-enhanced intracellular calcium ion concentrations can contribute to the formation of rigor complexes and hence the contracture which heralds irreversible myocardial ischemia (Okabe et al., 1988).

Not only is myocardial tissue jeopardized by free radical production, but so too is the cerebral vasculature. During hemorrhagic hypotension, cerebral blood flow is compromised. Upon reinfusion, the reintroduction of molecular oxygen into previously hypoperfused brain tissue may trigger a "burst" of free radical production (Cao et al., 1988). Oxygen radical-induced injury to the cerebral vasculature may explain the progressive decline in cerebral blood flow that has been observed post-reinfusion. Diminished cerebral blood flow to the brainstem cardiovascular centers could explain, at least in part, the progressive decline in cardiovascular function associated with hemorrhage-reperfusion injury.

Free radical injury to the peripheral vasculature may also play a role in post-reinfusion cardiovascular

deterioration. Augmented adhesion of granulocytes to blood vessels has been described during the ischemic impairment of blood flow (Grogaard, Gerdin, & Arfors, 1985). Numerous stimuli can trigger these cells to undergo a "respiratory burst" which exposes endothelial tissue to O_2^-, H_2O_2, HOCl, and $OH^{\cdot}$ generated thereby (Freeman, Rosen, & Barber, 1986). As a result, cell membrane integrity and fluidity may be compromised. Since the lipid hydroperoxides, products of free radical reactions, inhibit the ability of blood vessel walls to secrete prostacyclin, the critical balance between prostacyclin and thromboxane can be disrupted (Demopoulos, 1980; Hall & Wolf, 1986). The resulting micro-occlusions intensify local ischemia and thereby contribute to the overall collapse.

Attenuation of Free Radical Injury

Under normal circumstances, free radical damage in vivo is precluded by endogenous antioxidants and enzymatic radical scavengers. However, during severe ischemic hypoxia, antioxidant concentrations fall thereby creating susceptibility to free radical damage. Decreased superoxide dismutase and glutathione levels during myocardial ischemia have, for example, been repeatedly demonstrated (Freeman & Crapo, 1982; Guarnieri, Flamigni, & Caldərera, 1980; Rao & Mueller, 1983). By pharmacological administration of endogenous free radical scavengers such

as superoxide dismutase or catalase, attenuation of myocardial (Otani et al., 1984; Werns et al., 1985) and cerebral (Kirsh et al., 1987; Snelling, Ackerman, Dean, North, & Traystman, 1987) reperfusion injury has been attained.

The most important lipid-soluble antioxidant in both cell membranes and plasma, alpha-tocopherol (vitamin E) is frequently quantitated as an index of lipid peroxidation in vivo. Dietary vitamin E deficiency increases the susceptibility of biomembranes to oxidative injury (Tappel & Zalkin, 1959; Yoshida, Busto, & Watson, 1985). The molecule donates a hydrogen atom to polyunsaturated lipid radicals in the initiation step of lipid peroxidation thereby preventing a chain-like propagation of free radical reactions. Beneficial effects of vitamin E administration attributed to its antioxidant properties have been demonstrated in spinal cord injury (Hall & Wolf, 1986), subarachnoid hemorrhage (Travis & Hall, 1987), and myocardial hypoxia/reoxygenation models (Guarnieri, Ferrari, Visioli, Caldarera, & Nayler, 1978).

The Potential for Antioxidant Treatment

Given the catastrophic consequences of hemorrhagic shock and the probable role of free radicals in this phenomenon, the potential for antioxidant treatment becomes apparent. In this study, five compounds known to inhibit

some aspect of the free radical-mediated lipid peroxidative pathway were tested: U74006F, (21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16a-methyl-pregna-1,4,9-(11)-triene-3,20-dione, monomethane sulfonate; The Upjohn Co., Kalamazoo, Michigan); U78517G, (2H-1-benzopyran-6-ol,2-((4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl) methyl)-3,4-dihydro-2,5,7,8-tetramethyl-dihydrochloride; Upjohn Co.); desferal, (desferrioxamine mesylate; Ciba-Geigy, Basel, Switzerland); oxypurinol (Sigma Chemical Company, St. Louis, Missouri); and ibuprofen (The Upjohn Co., Kalamazoo).

The aminosteroid U74006F, a compound previously shown to attenuate hemorrhagic shock-associated pathophysiology in cats (Hall et al., 1988), is a potent inhibitor of lipid peroxidation in vitro (Braughler et al., 1988). Like vitamin E, U74006F affects this inhibition by scavenging $LOO^{\cdot}$ (Braughler et al., 1988; Braughler et al., 1989). However, other pseudo-antioxidant actions for U74006F have been demonstrated including an ability to reduce the release of arachidonic acid from cultured cells in response to iron-induced lipid peroxidation (Braughler et al., 1988). In addition to increasing membrane stability (Braughler et al., 1988; Braughler et al., 1989), U74006F has been shown to prevent transendothelial migration of activated neutrophils (Kitt et al., 1989) and to inhibit 5-lipoxygenase activity (Bach & Brashler, 1989).

In U78517G, the steroid portion of U74006F is replaced with the radical-scavenging tetramethylchroman ("trolox") portion of d-alpha tocopherol. The resulting amalgamation of the trolox with the bispyrrolidinopyrimidine of U74006F yields a molecule with demonstrated antioxidant and cerebroprotective capability in central nervous system injury and ischemia (Hall et al., 1989).

Desferal, oxypurinol, and ibuprofen each prevent O_2^- injury but by distinctly different mechanisms. In addition to being a O_2^-/·OH scavenger, desferal is a potent inhibitor of ·OH production (Sanan, Sharma, Balveer, Sanan, & Wadhera, 1989). The oxidation of Fe^{++} coupled to the decomposition of LOO· or LO· is an important reaction in lipid peroxidation. By virtue of its iron chelating abilities, desferal may also prevent the iron-catalyzed decomposition of lipid hydroxyl radicals into radical species. Oxypurinol, the active metabolite of allopurinol, acts as an inhibitor of xanthine oxidase and thereby suppresses a major route for O_2^- generation. Oxypurinol is also an extremely potent scavenger of ·OH radicals (Moorhouse, Grootveld, Halliwell, Quinlan, & Gutteridge, 1987). Ibuprofen inhibits cyclooxygenase activity and the subsequent production of prostaglandins PGE_2 and $PGF_{2\alpha}$ (Oyanagui, 1978). In so doing, ibuprofen inhibits O_2^- production (Kantor, 1979).

In this study, the protective effects of these

antioxidants on cardiovascular function, cerebral blood flow, arterial blood gases, and serum glucose in a hemorrhage/reperfusion injury model has been explored.

MATERIALS AND METHODS

General Methodology

Male Sprague-Dawley rats fed and watered ad libitum weighing 356 ± 4 grams (mean ± standard error (S.E.)) were anesthetized with urethane (Sigma Chemical Co., 1.8mg/kg, ip, in 9% saline with 0.15 mg/kg supplements given if needed prior to initial blood flow measurements). Using a heating pad, the animals' rectal temperatures were maintained at 36.7 ± 0.2 degrees Celsius. The right femoral artery and vein were each cannulated with PE-50 tubing for monitoring cardiovascular function (i.e., mean arterial (MAP), systolic, diastolic, and pulse pressures, heart rate) and for administering drug/vehicle, respectively. The right carotid artery was cannulated for blood withdrawal using PE-90 tubing.

After establishing stable cardiovascular function, cortical blood flow was measured using the hydrogen clearance technique (see below). Then, following the administration (iv) of 0.1ml stock sodium heparin solution (The Upjohn Co.) and over an interval of approximately five minutes, each rat was hemorrhaged by spontaneous withdrawal of blood through the carotid cannula into an elevated glass bottle. The MAP was held at 43-45 mmHg for two hours by adjusting the height of the blood level in the bottle-

reservoir so that it exerted that same pressure against the mean arterial pressure. As the animal attempted to autoregulate during the hemorrhage, it shed or withdrew blood spontaneously from the bottle. Cortical blood flow was measured 60 minutes after the onset of hemorrhage.

Beginning approximately 8 minutes prior to reinfusion and over a 5 minute period, vehicle or test drug was administered. Shed blood was thereafter restored via the carotid artery at an approximate rate of 1 ml/minute. Cardiovascular function was monitored after 5 minutes and then at 15 minute intervals post-reinfusion using a Grass Model 79 polygraph. Cortical blood flow was measured at 30, 60, and 120 minutes post-reinfusion.

Prior to hemorrhage and at 120 minutes post-reinfusion, arterial pH and blood gases (pO_2 and pCO_2) were measured using an Instrumentation Laboratories System 1301 blood-gas analyzer. Arterial glucose was measured using a One Touch™ Blood Glucose Monitoring System. Coincidently, serum samples were obtained for vitamin E analysis and stored at -80 degrees Celsius. In addition, serum samples were obtained at the time of death from any rat which did not survive either the hemorrhage period or the 120 minute period post-reinfusion.

Cerebral Blood Flow Determination

The hydrogen clearance technique was use to measure

cortical blood flow (Farrar, 1987; Young, 1980). A 0.002 inch-diameter platinum-iridium wire insulated with PE 10 tubing was inserted stereotaxically through a burr hole which was placed 5 mm posterior to the coronal suture and 3 mm to the left of the sagittal suture. The blood flow electrode was polarized to +350 mV with respect to a subcutaneous electrode. Hydrogen was administered under 130 mm Hg pressure via a tube placed directly in front of the animal's nostrils but far enough away and for a short enough time period so as not to create significant hypoxia. The following parameters were measured before and after hydrogen administration and shown not to have deteriorated as its result: mean arterial, systolic, diastolic, and pulse pressures, heart rate, arterial glucose, arterial blood gases (pO_2 and pCO_2) and pH.

The current generated by the oxidation of H_2 at the electrode tip was monitored using a two-channel polarograph with the results being recorded on a Kipp and Zonen BD41 recorder. Following the inspiration of H_2, the current declined exponentially as the cortical tissue content of H_2 fell. Using the recorded H_2 clearance curves, cortical blood flow values were derived using the Fick principle equation:

$$\text{blood flow} = (0.693)(t/2)^{-1}(100)$$

where t/2 is the time in minutes for the current to decay

by half the peak value and 0.693 is the natural log function constant (Hall & Travis, 1988). Cortical blood flow was expressed in ml/100 g tissue/minute.

Vitamin E Determination

Vitamin E concentrations were determined by high performance liquid chromatography with electrochemical detection (460 Waters, Milford, Massachusetts) via methodology modified from Natale, et al., 1988. Fifty microliter serum samples were diluted in 50 ul of saline and then deproteinized using 100 ul absolute ethanol. Vitamin E and its derivatives were then extracted into 500 ul hexane. A 300 ul sample of the hexane layer was evaporated under argon and the residue was reconstituted with 1 ml methanol before injection into the chromatograph. In order to quantify the data, vitamin E (Sigma Chemical Co., St. Louis, Missouri) was used for standard curve generation.

Drug Preparation

U74006F was dissolved (5mg/ml) in a citrate buffer (3.8 mg/ml citric acid, 0.94 mg/ml sodium citrate dihydrate, 4.7 mg/ml NaCl). U78517G and ibuprofen were each dissolved in saline (5mg/ml). Desferal and oxypurinol were each prepared in saline at concentrations of 25mg/ml or 12.5mg/ml. Drug/vehicle was administered via the femoral vein at the following dosages: U74006F, 10mg/kg;

U78517G, 10mg/kg; ibuprofen, 10mg/kg; desferal, 25mg/kg or 50mg/kg; oxypurinol, 25mg/kg or 50mg/kg. Comparison of the saline and citrate vehicles revealed no detectable differences in effect.

Statistical Analysis

Differences between vehicle and drug-treated animals were statistically analyzed over the entire time course using repeated measures analysis of variance. Paired *t*-tests were also employed to compare individual values post-reinfusion with appropriate pre-hemorrhage data.

RESULTS

Pathophysiology of Hemorrhagic Shock

As each rat compensated for hemorrhage by reducing its vascular capacity (i.e., via sympathetic venoconstriction), blood was transferred into the bottle/reservoir. Rats less able to maintain this compensatory response spontaneously withdrew blood from the bottle earlier in the hemorrhage leaving less blood in the reservoir when the time came for scheduled reinfusion. Animals into which less was left to reinfuse at the end of the hemorrhage period tended to deteriorate more rapidly after reinfusion than their counterparts with greater reinfusion volume. Hence it was important to match vehicle with drug-treated animals of similar reinfusion volumes. To achieve this, vehicle-treated animals were divided into two groups: Group I (n=7) and Group II (n=8) with mean reinfusion volumes of 7.5 $\pm$ 0.5 and 5.1 $\pm$ 0.6 ml, respectively.

Figure 1 displays MAP over the course of the experiment in Group I vehicle-treated rats. Replacement of shed blood restored cardiovascular function as measured by heart rate, systolic, diastolic, pulse and mean arterial pressure to levels comparable to those prior to hemorrhage. However, cardiovascular function deteriorated soon thereafter as also shown in Table 1. By 15 minutes post-

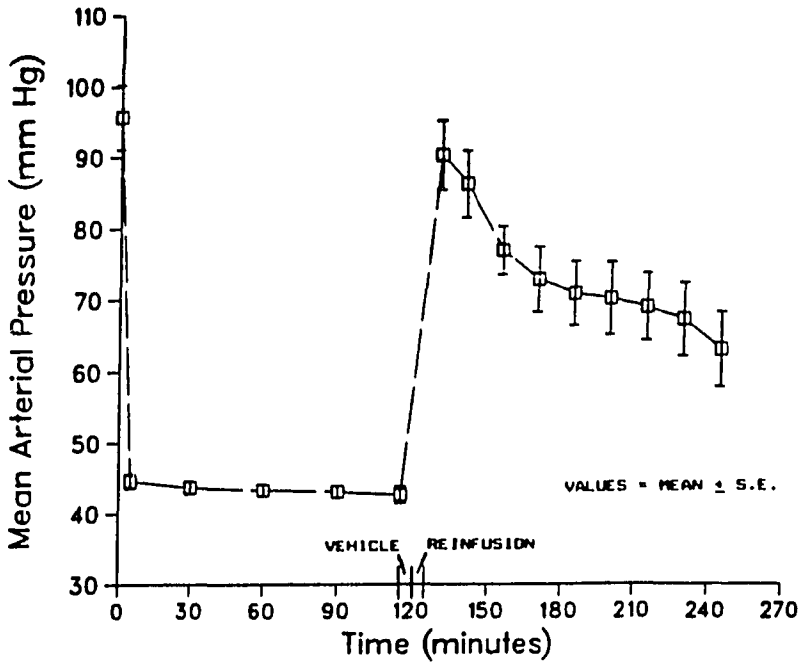


Figure 1. Mean Arterial Pressure Over the Course of the Experiment in Vehicle-Treated Rats (Group I).

reinfusion, mean arterial and systolic pressures had fallen whereas heart rate had risen to statistically significant levels compared to pre-hemorrhage values. By 30 and 60 minutes post-reinfusion, diastolic and pulse pressures, respectively, had also fallen significantly. By 120 minutes post-reinfusion, MAP had fallen from a pre-hemorrhage 95.7 ± 4.6 mm Hg to 62.9 ± 5.1 mm Hg ($p<0.0002$). Serum glucose concentrations had likewise fallen significantly although arterial pH, pCO_2, and pO_2 did not differ significantly from pre-hemorrhage values. There were no deaths in this vehicle treated group.

Table 1

Physiological Parameters Measured in Urethane-Anesthetized Rats Treated With Vehicle Just Prior to Reinfusion of Shed Blood

	Prior to Hemorrhage	60 Minutes into Hemorrhage	Minutes Post-Reinfusion 5	15	60	120
Mean Arterial Pressure (mmHg)	96 ± 5	43 ± 1**	90 ± 5	86 ± 5*	71 ± 4**	63 ± 5**
Systolic Pressure (mmHg)	139 ± 7	77 ± 5**	127 ± 9	124 ± 7*	110 ± 8**	92 ± 8**
Diastolic Pressure (mmHg)	73 ± 5	31 ± 2**	70 ± 4	66 ± 4	57 ± 5**	52 ± 5**
Pulse Pressure (mmHg)	64 ± 3	46 ± 4**	57 ± 8	58 ± 3	54 ± 4**	41 ± 3**
Heart Rate (beats/minute)	389 ± 14	437 ± 21*	417 ± 8	440 ± 10**	450 ± 10**	442 ± 9**
Serum Glucose (mg/dl)	156 ± 13					91 ± 8**
pH	7.39 ± 0.01					7.38 ± 0.03
pO_2	80.6 ± 2.6					80.3 ± 4.9
pCO_2	44.6 ± 1.8					39.2 ± 2.9

Note. n=7. Values = Mean ± Standard Error of the Mean.

* P<0.05 by paired-test comparison with pre-hemorrhage data.

** P<0.01 by paired-test comparison with pre-hemorrhage data.

Anti-Shock Efficacy of Lipid Peroxidation Inhibition: U74006F and U78517G

Figure 2 illustrates that over the 2 hour time course post-reinfusion of shed blood, U74006F maintained MAP better than did vehicle-treatment. Table 2 shows that at 5 and 15 minutes post-reinfusion in U74006F-treated rats, mean arterial, systolic, and diastolic pressures were actually significantly greater than those observed prior to hemorrhage. Heart rate and pulse pressure were not, however, significantly altered by the reinfusion of shed blood. At 30 minutes post-reinfusion and through the end of the experiment, heart rate was elevated whereas the remaining cardiovascular parameters were not altered significantly from pre-hemorrhage values. Serum glucose levels were significantly lower at 120 minutes post-reinfusion than those prior to hemorrhage. Neither the mean glucose level at 120 minutes post-reinfusion nor its mean decline over the course of the experiment differed statistically from that observed in the corresponding vehicle-treated animals. No significant alteration of arterial pH, pCO_2, or pO_2 was observed relative to pre-hemorrhage values (data not shown). No animals expired in the U74006F-treated group.

Figure 3 displays MAP expressed as percent of initial (i.e., pre-hemorrhage) values over the course of the experiment for Group I vehicle, U74006F and U78517G treated

Table 2

Physiological Parameters Measured in Urethane-Anesthetized Rats Treated With Drug or Vehicle Just Prior to Reinfusion of Shed Blood

	Prior to Hemorrhage	Minutes Post-Reinfusion 5	15	60	120
Mean Arterial Pressure (mmHg)					
Vehicle I[a]	96 ± 5	90 ± 5	86 ± 5*	71 ± 4**	63 ± 5**
U74006F[b]	93 ± 6	114 ± 4**	113 ± 6**	90 ± 5	79 ± 6*
U78517G[c]	84 ± 2	104 ± 6*	97 ± 5	81 ± 6	73 ± 6
Oxypurinol[d]	93 ± 6	93 ± 7	87 ± 5	74 ± 6*	67 ± 6**
Systolic Pressure (mmHg)					
Vehicle I	139 ± 7	127 ± 9	124 ± 7*	110 ± 8**	92 ± 8**
U74006F	128 ± 5	154 ± 3**	152 ± 6**	128 ± 6	112 ± 8
U78517G	121 ± 5	154 ± 9**	146 ± 8*	124 ± 10	107 ± 10
Oxypurinol	123 ± 11	128 ± 14	121 ± 10	107 ± 12	93 ± 13*
Diastolic Pressure (mmHg)					
Vehicle I	73 ± 5	70 ± 4	66 ± 4	57 ± 5**	52 ± 5**
U74006F	75 ± 6	94 ± 6**	93 ± 7**	72 ± 5	64 ± 5*
U78517G	64 ± 2	81 ± 5*	74 ± 5	62 ± 6	57 ± 5
Oxypurinol	72 ± 5	75 ± 6	70 ± 3	59 ± 5*	54 ± 5*
Heart Rate (beats/minute)					
Vehicle I	389 ± 14	417 ± 8	440 ± 10**	450 ± 8**	442 ± 9**
U74006F	386 ± 16	384 ± 11	413 ± 10*	453 ± 15**	436 ± 14**
U78517G	420 ± 20	378 ± 20*	412 ± 19	435 ± 19	425 ± 16
Oxypurinol	400 ± 16	420 ± 14	432 ± 8	472 ± 11**	465 ± 13**

Table 2--Continued

	Prior to Hemorrhage	Minutes Post-Reinfusion			
		5	15	60	120
Pulse Pressure (mm Hg)					
Vehicle I	64 ± 3	57 ± 8	58 ± 3	54 ± 4**	41 ± 3**
U74006F	53 ± 3	60 ± 6	59 ± 7	56 ± 7	48 ± 6
U78517G	56 ± 6	73 ± 8**	72 ± 8	62 ± 9	50 ± 8
Oxypurinol	51 ± 7	53 ± 8	51 ± 8	48 ± 8	39 ± 8
Serum Glucose					
Vehicle I	156 ± 13				91 ± 8**
U74006F	149 ± 8				111 ± 7*
U78517G	143 ± 9				118 ± 14
Oxypurinol	141 ± 15				106 ± 9

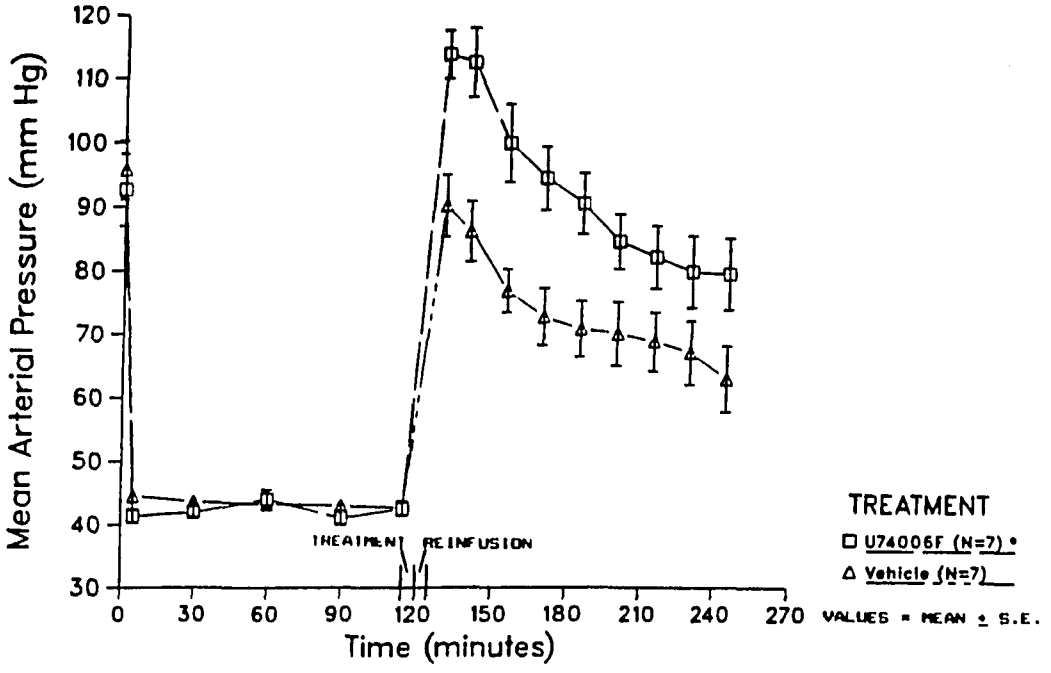
Note. Values = Mean ± Standard Error of Mean.

a;n=7, reinfusion vol. = 7.5 ± 0.5 ml. b;n=7, reinfusion vol. = 7.7 ± 0.4 ml.

c;n=6, reinfusion vol. = 7.2 ± 0.8 ml. d;n=6, reinfusion vol. = 7.3 ± 1.0 ml.

* P<0.05 and ** P<0.01 by paired test comparison with pre-hemorrhage data.

+ P<0.05 by repeated measures analysis of variance comparing vehicle versus treated groups.

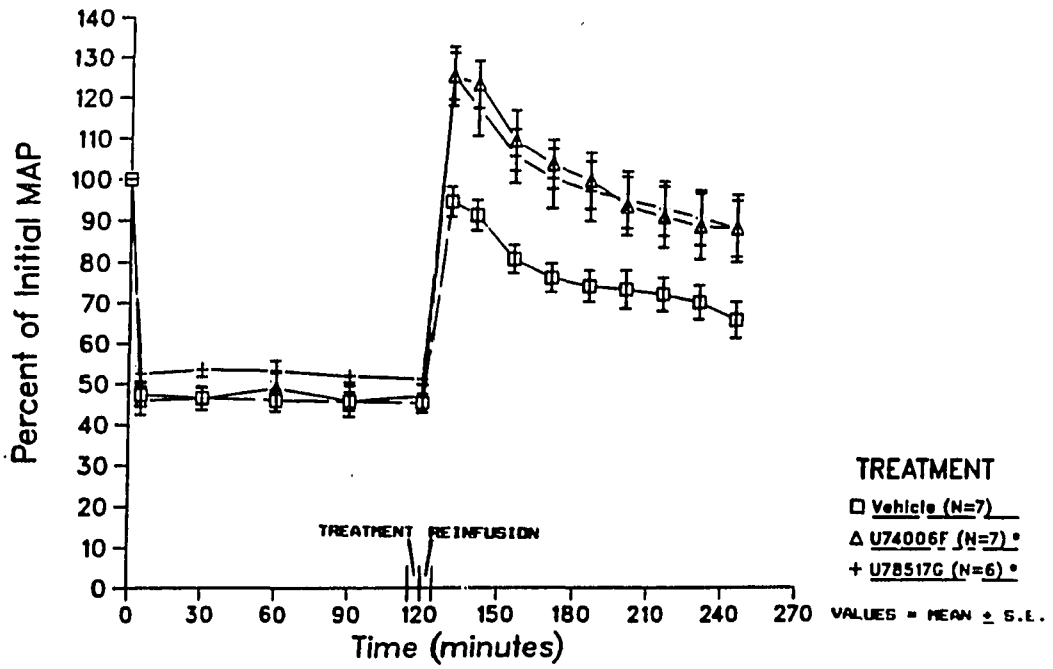


* Significantly different from vehicle ($P<0.01$) over the time course post-reinfusion (minutes 125-245) using repeated measures analysis of variance.

Figure 2. Comparison of the Effects of Vehicle or U74006F Treatment on Mean Arterial Pressure Post-Reinfusion.

rats. An examination of the time course post-reinfusion shows that the U74006F and U78517G curves are essentially superimposed. As was the case with U74006F, administration of U78517G better maintained MAP over the course of the experiment relative to vehicle-treatment (see Figure 3). Heart rate, mean arterial, systolic, diastolic, and pulse pressures in U78517G treated rats 5 minutes post-reinfusion were significantly greater than corresponding values prior to hemorrhage (see Table 2). However, from 30 minutes

post-reinfusion through the end of the experiment, these parameters no longer differed significantly from corresponding pre-hemorrhage values. Nor was the substantial decline in serum glucose over the course of the experiment statistically significant (see Table 2). Arterial pH, pO_2, and pCO_2 at 120 minutes post-reinfusion did not differ significantly from pre-hemorrhage values (data not shown).



* Significantly different from vehicle ($P<0.005$) over the time course post-reinfusion (minutes 125-245) using repeated measures analysis of variance.

Figure 3. Comparison of the Effects of Vehicle, U74006F, or U78517G Treatment on Mean Arterial Pressure Post-Reinfusion.

Anti-Shock Efficacy of Xanthine Oxidase Inhibition: Oxypurinol

Figure 4 displays MAP over the course of the experiment in Group I vehicle and oxypurinol-treated rats. Oxypurinol treatment (25mg/kg), as evidenced by the similarity between the oxypurinol and vehicle curves, did not prevent the decline in cardiovascular function post-reinfusion. Mean arterial, systolic, and diastolic pressure fell significantly whereas heart rate rose over the course of the experiment despite oxypurinol treatment (see Table 2). A substantial although not statistically significant decline in serum glucose levels was also observed. Neither the mean glucose level at 120 minutes post-reinfusion nor its mean decline over the course of the experiment differed statistically from that observed in the corresponding vehicle-treated animals. Arterial pH, pO_2, and pCO_2 at 120 minutes post-reinfusion did not differ significantly from pre-hemorrhage values (data not shown). Increasing the dosage of oxypurinol to 50mg/kg also failed to attenuate diminished glucose or cardiovascular function post-reinfusion (data not shown). No rats expired in the oxypurinol-treated group.

Anti-Shock Efficacy of Iron Chelation: Desferal

Figure 5 displays MAP over the course of the experiment in Group II vehicle and desferal (50mg/kg)

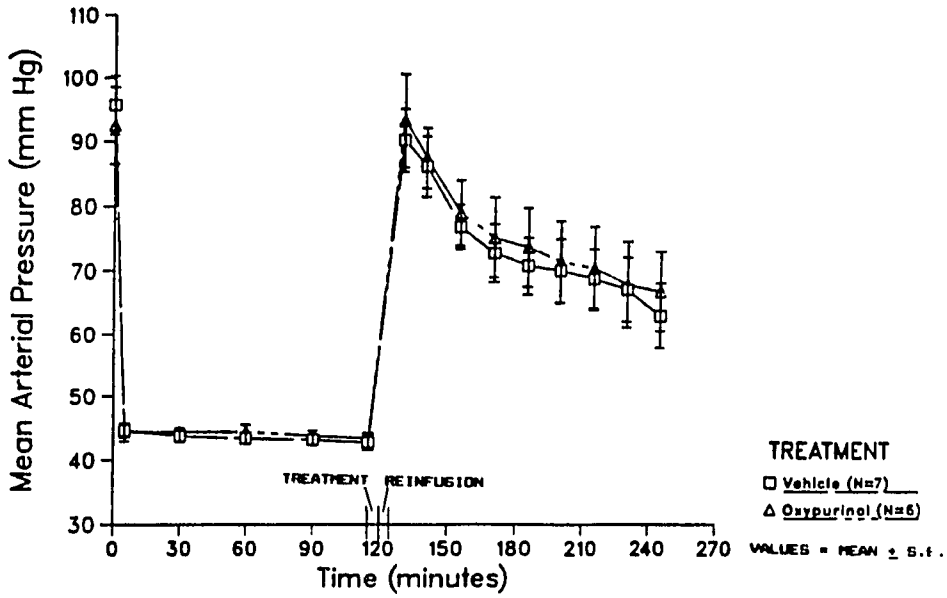


Figure 4. Comparison of the Effects of Vehicle or Oxypurinol Treatment on Mean Arterial Pressure Post-Reinfusion.

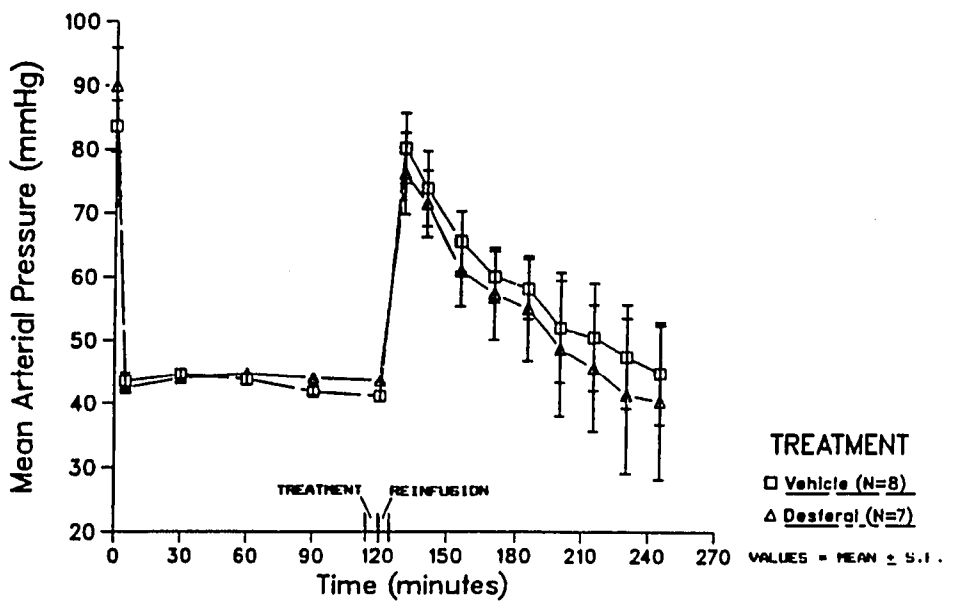


Figure 5. Comparison of the Effects of Vehicle or Desferal Treatment on Mean Arterial Pressure Post-Reinfusion.

treated rats. Table 3 shows that in vehicle-treated rats, reinfusion restored mean arterial, systolic, diastolic and pulse pressures to near pre-hemorrhage values. However, by 30 minutes post-reinfusion, each of these parameters had deteriorated significantly. Serum glucose levels fell significantly over the course of the experiment. Heart rate was elevated significantly throughout the post-reinfusion period. Arterial pH, pO_2, and pCO_2 of surviving rats at 120 minutes post-reinfusion did not differ significantly from pre-hemorrhage values (data not shown). Death during the second hour post-reinfusion occurred in 1/8 vehicle-treated animals.

Desferal treatment failed to attenuate falling MAP as evidenced by the similarity between the vehicle and desferal curves (see Figure 5). Furthermore, desferal, like oxypurinol, did not prevent significant declines in systolic, diastolic, and pulse pressure nor did it attenuate the elevation of heart rate. Serum glucose levels fell substantially over the course of the experiment in a manner not statistically different from that observed in vehicle-treated animals. Arterial pH, pO_2, and pCO_2 of surviving rats at 120 minutes post-reinfusion did not differ significantly from pre-hemorrhage values (data not shown). Lowering the dosage of desferal to 25mg/kg because 50mg/dl appeared to be slightly detrimental in terms of MAP relative to vehicle control yielded no apparent benefit

Table 3

Physiological Parameters Measured in Urethane-Anesthetized Rats Treated With Vehicle or Drug Just Prior to Reinfusion of Shed Blood

	Prior to Hemorrhage	Minutes Post-Reinfusion 5	15	60	120
Mean Arterial Pressure (mmHg)					
Vehicle II[a]	84 ± 4	80 ± 6	74 ± 6	58 ± 5**	45 ± 8**
Desferal[b]	90 ± 6	76 ± 6*	71 ± 5**	55 ± 8**	40 ± 12**
Ibuprofen[c]	84 ± 5	82 ± 10	71 ± 10	54 ± 8*	36 ± 12**
Systolic Pressure (mmHg)					
Vehicle II	114 ± 6	110 ± 6	102 ± 8	88 ± 8*	61 ± 13**
Desferal	120 ± 7	101 ± 7*	96 ± 6**	77 ± 10**	52 ± 15**
Ibuprofen	120 ± 6	114 ± 16	100 ± 16	79 ± 13*	50 ± 17**
Diastolic Pressure (mmHg)					
Vehicle II	65 ± 3	60 ± 4	54 ± 4	43 ± 4*	34 ± 8**
Desferal	74 ± 6	61 ± 6*	58 ± 5*	48 ± 7**	34 ± 10**
Ibuprofen	64 ± 5	66 ± 8	57 ± 8	44 ± 6	28 ± 9**
Heart Rate (beats/minute)					
Vehicle II	390 ± 14	426 ± 9*	450 ± 12**	425 ± 19**	430 ± 8**
Desferal	423 ± 15	466 ± 25	484 ± 23	463 ± 31*	466 ± 17
Ibuprofen	405 ± 23	417 ± 19	443 ± 19	403 ± 19	440 ± 4
Pulse Pressure (mmHg)					
Vehicle II	50 ± 5	50 ± 4	48 ± 5	45 ± 4	23 ± 4**
Desferal	45 ± 6	40 ± 4	38 ± 4	24 ± 6*	18 ± 5**
Ibuprofen	56 ± 2	48 ± 8	44 ± 8	36 ± 7*	22 ± 8**
Serum Glucose (mg/dl)					
Vehicle II	159 ± 11				86 ± 10**
Desferal	134 ± 7				85 ± 5
Ibuprofen	159 ± 8				93 ± 16

Note. Expired animals were calculated as zero for mean arterial, systolic, diastolic, and pulse pressure; only living rats were entered for heart rate and serum glucose means. No rat died prior to 60 minutes post-reinfusion.

Values = Mean ± Standard Error of Mean.

a:n=8. Reinfusion vol. = 5.1 ± 0.6 ml. b:n=7. Reinfusion vol. = 4.9 ± 1.0 ml.

c:n=6. Reinfusion vol. = 5.3 ± 0.7 ml.

* $P<0.05$ and ** $P<0.01$ by paired test comparison with pre-hemorrhage data.

(data not shown). Death during the second hour post-reinfusion occurred in 2/7 of the desferal (50mg/kg) treated rats.

Anti-Shock Efficacy of Cyclooxygenase Inhibition: Ibuprofen

Figure 6 displays MAP over the course of the experiment in Group II vehicle and ibuprofen-treated rats. Ibuprofen-treatment failed to attenuate falling MAP as evidenced by the similarity between the vehicle and ibuprofen curves. Ibuprofen likewise failed to prevent significant declines in systolic, diastolic, and pulse pressure nor did it prevent an elevation of heart rate. Serum glucose levels fell substantially over the course of the experiment in a manner not statistically different from that observed in vehicle-treated animals. Arterial pH, pO_2, and pCO_2 of surviving rats at 120 minutes post-reinfusion did not differ significantly from pre-hemorrhage values (data not shown). Death in the second hour post-reinfusion occurred in 2/6 of the ibuprofen-treated animals.

Cerebral Blood Flow Measurements

Table 4 shows cerebral blood flow measurements associated with vehicle, U74006F, U78517G, and oxypurinol treated rats at various times during the experiment. This data is displayed in Figure 7. Among surviving rats in

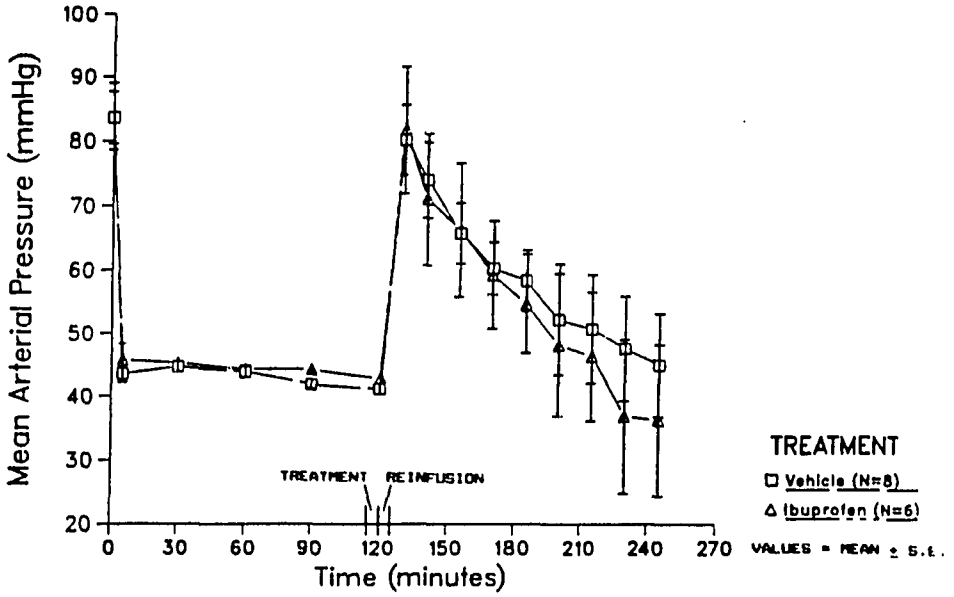


Figure 6. Comparison of the Effects of Vehicle or Ibuprofen Treatment on Mean Arterial Pressure Post-Reinfusion.

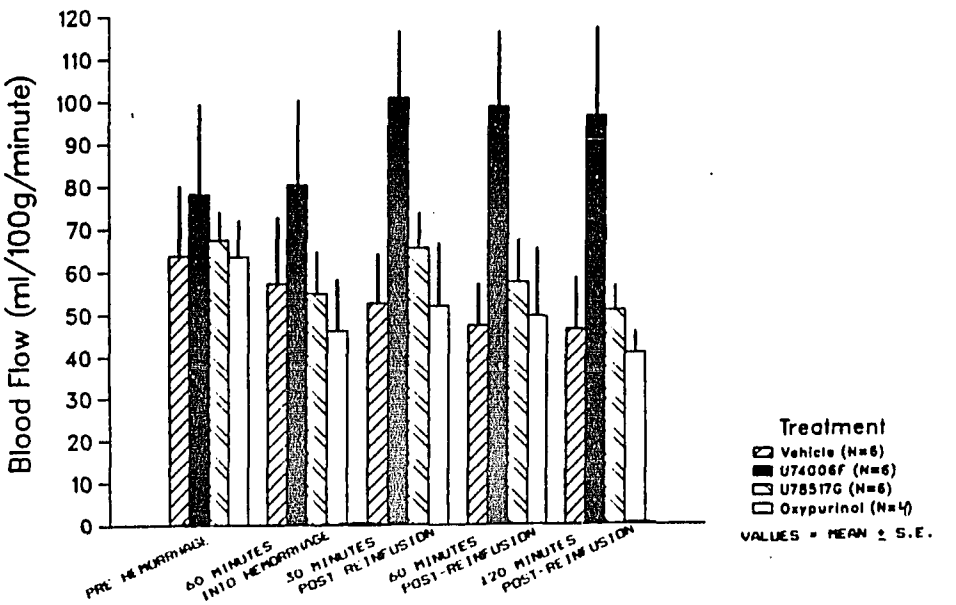


Figure 7. Comparison of the Effects of Vehicle, U74006F, U78517G, or Oxypurinol Treatment on Cerebral Blood Flow Post-Reinfusion.

Table 4

Cerebral Blood Flow in Urethane-Anesthetized Rats Which Survived the 120 Minute Period Post-Reinfusion

Treatment	Prior to Hemorrhage	60 Minutes into Hemorrhage	Minutes Post-Reinfusion		
			30	60	120
Vehicle[a]	63.6 ± 16.2[@]	57.0 ± 15.3	52.4 ± 11.4	47.1 ± 9.7	46.1 ± 12.2
U74006F[b]	77.9 ± 21.2	80.0 ± 20.0	100.5 ± 15.6	98.3 ± 17.7	96.1 ± 20.8*
U78517G[c]	67.3 ± 6.5	54.7 ± 9.9	65.2 ± 8.2	57.2 ± 9.8	50.6 ± 5.9
Oxypurinol[d]	63.4 ± 8.4	46.0 ± 12.1	51.7 ± 14.6	49.2 ± 15.8	40.5 ± 5.0
Vehicle II[e]	61.7 ± 8.3	56.6 ± 2.2	53.8 ± 11.9	50.5 ± 9.7	45.2 ± 10.7
Desferal[f]	52.5 ± 5.1	43.9 ± 2.8	42.0 ± 5.3	37.5 ± 4.1	40.5 ± 5.0

@ ml/100g/minute

Note. Drug or vehicle was administered just prior to reinfusion of shed blood. Values = Mean ± Standard Error of the Mean.

a;n=6. b;n=6. c;n=6. d;n=4. e;n=6. f;n=4.

* P<0.01 by repeated measures analysis of variance comparing vehicle versus drug treatment with data expressed as percent of pre-hemorrhage values.

which cerebral blood flow was measured, blood flow was diminished 15.5 ± 3.8% (n=41) after 60 minutes of hemorrhagic hypotension. The mean blood flow in each of the drug-treated groups both prior to and 60 minutes into the hemorrhage did not differ significantly from that of vehicle-treated rats.

Upon reinfusion of shed blood into vehicle-treated rats, cerebral blood flow relative to pre-hemorrhage values decreased. Neither U78517G nor oxypurinol treatment significantly attenuated this decline. Administration of U74006F, however, not only prevented the decline but also

significantly enhanced blood flow relative to vehicle treatment ($p<0.01$ by repeated measures analysis of variance comparing vehicle versus U74006F treatment with data expressed as percent of pre-hemorrhage values). Table 4 also illustrates that desferal-treatment (50mg/kg) relative to its appropriate control failed to protect against diminished cerebral blood flow. Complete data concerning ibuprofen treatment was unavailable although preliminary results suggested that ibuprofen administration did not preserve cerebral blood flow.

Vitamin E Measurements

Of the 63 rats in this study, 12 (19%) were unable to survive the hemorrhage period. Vitamin E levels in these 12 rats at the time of death were significantly lower ($p<0.0003$) than pre-hemorrhage values, having fallen from 9.1 ± 0.5 to 6.0 ± 0.3 ug/ml plasma. Table 5 presents vitamin E data for animals which survived the hemorrhage period. Substantial declines in vitamin E were seen in each of the treatment groups. No direct linear relationship between either pre-hemorrhage or final (i.e., 120 minute post-reinfusion) vitamin E concentrations and final MAP was observed. Nor is there a direct relationship between either final MAP or change in MAP and change in serum vitamin E levels. None of the drugs attenuated vitamin E losses within the animals.

Table 5

Serum Vitamin E Levels in Urethane-Anesthetized Rats Treated With Drug or Vehicle Just Prior to Reinfusion

Treatment	N	Prior to Hemorrhage (mg/dl)	Final Vitamin E (mg/dl)	Mean % Decline
Vehicle I	6	7.6 ± 0.6	6.5 ± 0.6**	14.9
U74006F	7	9.5 ± 0.7	8.2 ± 0.7**	13.8
U78517G	6	9.6 ± 1.2	7.1 ± 0.5	22.2
Oxypurinol	6	10.5 ± 1.0	8.0 ± 0.7**	23.4
Vehicle II	7	8.3 ± 0.4	6.4 ± 0.5**	23.7
Desferal	7	9.0 ± 0.4	7.0 ± 0.6**	22.6
Ibuprofen	5	8.0 ± 1.0	5.9 ± 0.4	17.9

Note. Final vitamin E refers to values at 120 minutes post-reinfusion or at the time of death. No animals died during the hemorrhage period.

Values = Mean ± Standard Error of the Mean.

** $P<0.01$ by paired-test comparison with pre-hemorrhage data.

DISCUSSION

Considerable attention has focused on the role of free radicals in the pathophysiology of hemorrhagic shock. Oxygen free radical and lipid peroxidative injury to the myocardium as well as to the cerebral and peripheral vasculature may contribute substantially to the progressive cardiovascular collapse which hallmarks the hemorrhagic shock scenario. In this study, four pharmacological mechanisms for antagonizing free radical generation or reactions have been explored in terms of their comparative efficacy in attenuating post-hemorrhage (post-reinfusion) cardiovascular collapse. These included attenuating arachidonic acid oxidation by cyclooxygenase (e.g., ibuprofen), inhibition of xanthine oxidase-mediated superoxide radical production (e.g., oxypurinol), iron chelation (e.g., desferal), and inhibition of lipid peroxidation (e.g., U74006F and U78517G).

In response to ischemic insult, arachidonic acid is released from cell membranes. Inhibition of this release would attenuate oxygen radical pathology by curtailing prostaglandin synthase activity and thereby squelching a significant source of oxygen radicals. Moreover, reduced activity of cyclooxygenase, a component of the prostaglandin synthase complex, would decrease levels of

injurious eicosanoids which have been implicated in the pathophysiology of shock (Chaudry & Baue, 1982; Hall et al., 1988). U74006F has been demonstrated to reduce the release of arachidonic acid from cultured cells in response to lipid peroxidation (Braughler et al., 1988). Hence, the demonstrated benefits of U74006F in this study might be ascribed, at least in part, to its membrane stabilizing effects. However, ibuprofen, an inhibitor of cyclo-oxygenase activity, failed to attenuate the cardiovascular collapse observed in this study thereby suggesting that cyclooxygenase-catalyzed events may not be of paramount importance to the hemorrhagic shock scenario.

In addition to arachidonic acid oxidation, at least four means exist whereby oxygen radicals are generated: mitochondrial electron transport chain uncoupling, catecholamine autoxidation, granulocyte infiltration, and xanthine oxidase activation. Oxypurinol, an active metabolite of allopurinol, inhibits xanthine oxidase activity thereby eliminating one of the above sources. Although protective effects directly attributable to this abatement in myocardial ischemia situations have been described (see Introduction above), oxypurinol failed to attenuate the post-hemorrhagic cardiovascular collapse observed in this study. A likely explanation is that although oxypurinol eliminates one O_2^- source it does not preclude O_2^- formation by the other four. This suggests

that although xanthine oxidase may play some role it is not necessarily the major one in the pathophysiology of hemorrhagic shock.

The involvement of Fe^{++} in free radical/lipid peroxidative and hemorrhagic shock pathology has been reported (see Introduction above). Free iron can trigger the generation of numerous deleterious radical species including hydroxyl radicals (Fenton's reaction), ferryl ions (Fe^{III} -OH), O_2^-, lipid peroxyl radicals or alkoxyl radicals. Administration of desferal, a potent iron chelator, might presumably inhibit each of these reactions. Moreover, its ability to directly scavenge O_2^-/OH· would further preclude free radical damage. Desferal has been shown previously to improve the survival rate and speed of recovery as well as to reduce the degenerative liver changes associated with severe hemorrhagic shock (Sanan et al., 1989). However, in this study, desferal was not effective in attenuating post-hemorrhage cardiovascular collapse suggesting that although iron-related phenomena may play a role it is not necessarily the most important issue in the presently studied ischemia/reperfusion scenario.

Oxygen radicals, regardless of their source, cause much of their cellular damage via initiation of membrane lipid peroxidation. Upon reacting with membrane unsaturated fatty acids, they trigger the production of a

carbon centered lipid radical. This lipid radical, in turn, can react with available oxygen to form a lipid peroxyl radical which can result in the formation of still more lipid radical species. This propagation can continue leading to cellular destruction. An effective way to prevent this amplification would seem to be to scavenge the initial lipid radical species. Both U74006F and U78517G are potent lipid peroxyl scavengers (Braughler & Pregenzer, 1989; Hall et al., 1989) and it was upon administration of either of these two compounds that the post-hemorrhage cardiovascular collapse observed in this study was attenuated.

The attenuation of post-hemorrhagic cardiovascular collapse by U74006F has been previously reported in a cat model (Hall et al., 1988). Its efficacy was hypothesized to result, at least in part, from protection of the brainstem cardiovascular centers. Indeed in this study, a progressive decline in cerebral blood flow beginning just after reperfusion was observed in vehicle, desferal, and oxypurinol treated groups; each of which also exhibited post-reinfusion cardiovascular collapse. Animals treated with U74006F did not, however, display declining cerebral blood flow but instead, a slight although not statistically significant enhancement over the 2 hour period post-reinfusion. Similar effects on cerebral blood flow upon administration of U74006F have been documented previously

(Hall & Travis, 1988; Hall & Yonkers, 1988). Hence, the support of cardiovascular function could be based on a preservation of cerebral blood flow such that brainstem cardiovascular center perfusion and therewith sympathetic outflow is maintained. This does not, however, preclude the possibility that direct protection of the heart or peripheral vasculature might also play a role in attenuating the ischemia/reperfusion injury. In fact, treatment with U78517G attenuated the cardiovascular collapse post-reinfusion as did U74006F. Unlike the latter, however, U78517G failed to protect cerebral blood flow over the time course post-reinfusion. Hence protection of the heart or the peripheral vasculature may be of primary importance in terms of U78517G efficacy.

Given that free radical generation and the resulting injury begins almost immediately following the reintroduction of oxygen into ischemic tissue, great damage to the cardiovascular system of the animals in this study must have been initiated immediately upon reinfusion of shed blood. In a system not compromised by free radical production, reinfusion of a relatively large blood volume over a short time span (i.e., under the conditions of this study) would have been expected to induce at least a short period of hypertension. A significant, albeit temporary, elevation of MAP observed in U74006F and U78517G animals which were treated prior to reinfusion. This treatment

presumably spared the onslaught of free radical damage brought on by reperfusion. This hypertensive phenomena was not, however, observed in vehicle, oxypurinol, desferal, or ibuprofen treated animals indicating perhaps that free radical injury and cardiovascular collapse had already begun prior to the cardiovascular measurements taken 5 minutes post-reinfusion.

The efficacy of both U74006F and U78517G have been attributed, at least in part, to their vitamin E-like scavenging of LOO·. However, serum vitamin E concentrations were not preserved in either U74006F or U78517G-treated rats suggesting that U74006F and U78517G attenuate the cardiovascular collapse by means not necessarily akin to vitamin E-like scavenging. Substantial declines in serum vitamin E over the course of the experiment were associated with each of the vehicle and drug-treated groups. Large vitamin E losses were likewise seen in animals which died during the hemorrhage period. The loss of this antioxidant is consistent with the premise that antioxidant molecules are rapidly consumed in ischemia/reperfusion injury.

Figure 8 summarizes a hypothesized scheme illustrating the role of oxygen radicals in the hemorrhage/reperfusion scenario. In brief, severe hemorrhagic hypotension and subsequent tissue hypoxia will trigger dissociation of the electron transport chain and thereby enhance the leakage of

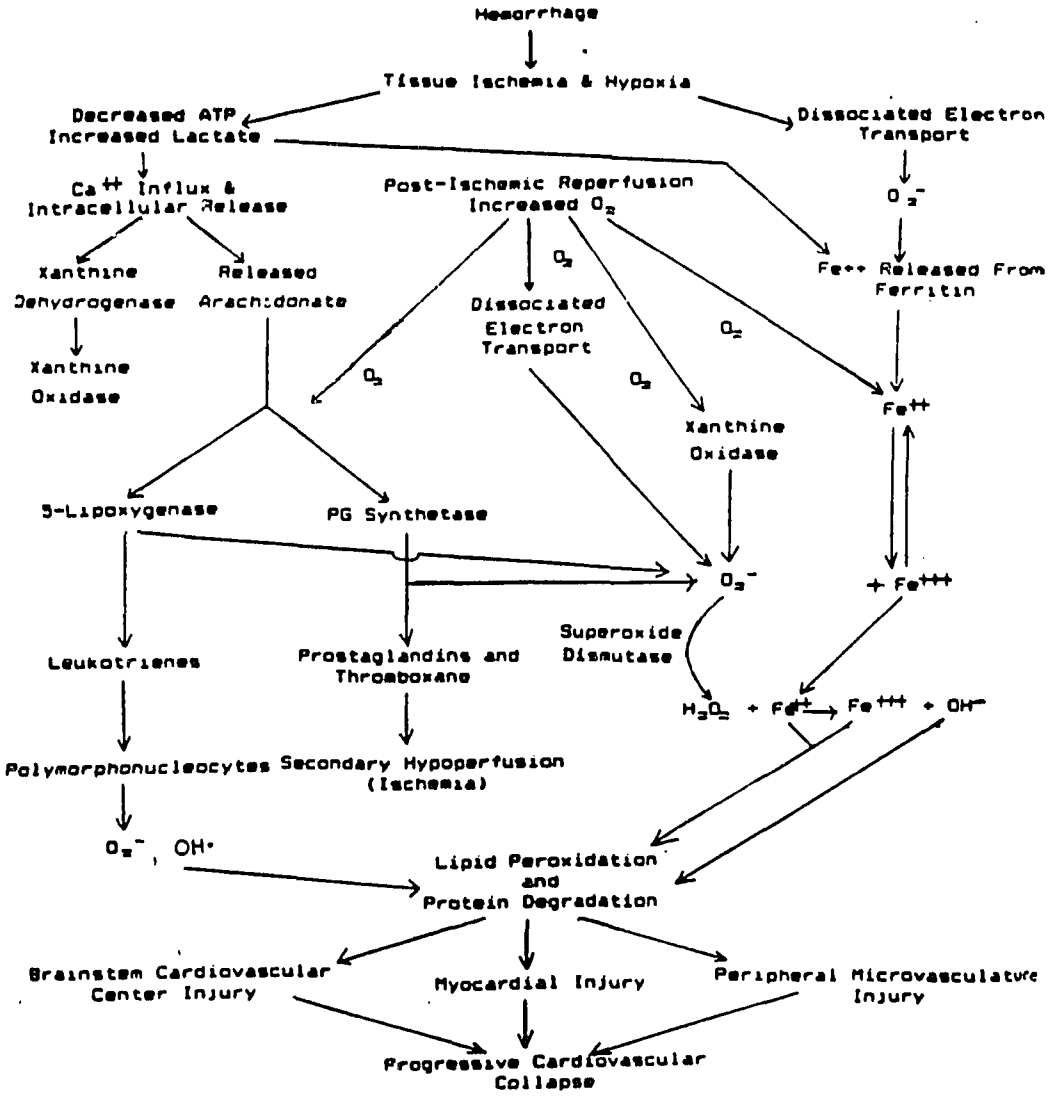


Figure 8. Hypothetical Pathogenesis of Ischemia-Reperfusion Injury.

O_2^-. Elevated lactic acid and depleted ATP are likewise associated with tissue ischemia. Under these latter conditions, Ca^{++} influx and intracellular release is enhanced thereby leading to both the conversion of xanthine dehydrogenase to xanthine oxidase and to the release of

arachidonic acid. Both the acidotic conditions associated with high lactic acid and the presence of O_2^- favor the release of Fe^{++} from ferritin. Hence, reinfusion of shed blood into ischemic tissue reintroduces oxygen to tissue primed with elevated Fe^{++}, dissociated electron transport, and activated xanthine oxidase. As described previously, an increase in oxygen tension under each of these conditions will trigger the formation of O_2^-. Moreover, O_2^- can be generated by the conversion of released arachidonic acid into prostaglandins and thromboxane by prostaglandin synthase or into leukotrienes by 5-lipoxygenase, respectively. The vasoconstricting effects of thromboxane and certain prostaglandins can lead to secondary hypoperfusion while the chemotactic properties of leukotrienes can lead to further superoxide generation by polymorphonucleocytes. Regardless of their source, O_2^-will react in a superoxide dismutase catalyzed reaction to form H_2O_2 which, in turn, will react with Fe^{+++} to form the highly reactive $\cdot OH$. The generation of hypoxic free radicals triggers lipid peroxidation and the resulting damage to the myocardium, the brainstem cardiovascular center, and the peripheral vasculature thereby leading to the progressive cardiovascular collapse associated with hemorrhagic shock.

Given the above scenario, it would be expected that by antagonizing the generation of free radical species, hemorrhagic shock might be attenuated. However, iron

chelation, xanthine oxidase, and cyclooxygenase inhibition by desferal, oxypurinol, and ibuprofen, respectively, failed to attenuate the post-reinfusion cardiovascular collapse observed in this study. A reasonable explanation is that administering any one of these drugs independently eliminates only one of several potential oxygen radical sources. However, by administering U74006F or U78517G, not the individual superoxide sources but instead the lipid radical species that they generate in common are scavenged. Hence, the ability of these lipid radical scavengers to attenuate the cardiovascular collapse after blood reinfusion suggests a role for lipid peroxidation in the pathophysiology of hemorrhagic shock.

APPENDIX

Appendix A

Research Protocol Clearance From Institutional Animal Care and Use Committee

INVESTIGATOR CERTIFICATION

Title of Project Pathophysiology of Hemorrhagic Shock: Protective Effects of Anti-Oxidants.

If any of the above procedures are changed, I will submit a new protocol.

I understand that any failure to comply with the *Animal Welfare Act*, the provisions of the *DPHS Guide for the Care and Use of Laboratory Animals* and requirements set down by the IACUC may result in the suspension of my animal studies.

Annette E. Fleckenstein — *Signature: Principal Investigator*

BMED — *Department*

11/21/88 — *Date*

REVIEW BY THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

_____Disapproved __X__Approved _____ Approved with the provisions listed below

Provisions:
or
Explanation

Leonard Bacaner — *IACUC Chairperson*

11-22-88 — *Date*

Researcher's Acceptance of Provisions:

NA — *Signature: Principal Investigator*

Date

NA — *IACUC Chairperson Final Approval*

Date

Approved IACUC Number 0026

Revised June, 1988

BIBLIOGRAPHY

Aust, S.D., & Svingen, B.A. (1982). The role of iron in enzymatic lipid peroxidation. Free Radical Biology and Medicine, 5, 1-26.

Babior, B.M., Curnette, J.T., & Okamura, N. (1988). The respiratory burst oxidase of the human neutrophil. In B. Halliwell (Ed.), Oxygen Radicals and Tissue Injury: Proceedings of an Upjohn Symposium (pp.43-48). Bethesda, MD: Federation of American Societies for Experimental Biology.

Bach, M.K., & Brashler, J.R. (1989). Studies of synergism between inhibitors of the 5-lipoxygenase pathway. In P.Y. Samuelsson, K. Wong, & F.F. Sun (Eds.). Advances in Prostaglandin, Thromboxane, and Leukotriene Research, 19, New York: Raven Press LTD.

Bernier, D.J.H., & Manning, A.S. (1986). Reperfusion-induced arrhythmias and oxygen-derived free radicals. Circulation Research, 58, 331-340.

Bielski, B.H.J., Arudi, P.L., & Sutherland, M.W. (1983). A study of the reactivity of perhydroxyl radical/superoxide ion with unsaturated fatty acids. Journal of Biological Chemistry, 258, 4759-4761.

Braughler, J.M. (1988). Calcium and lipid peroxidation. In B. Halliwell (Ed.), Oxygen Radicals and Tissue Injury: Proceedings of an Upjohn Symposium (pp.99-104). Bethesda, MD: Federation of American Societies for Experimental Biology.

Braughler, J.M., Chase, R.L., Neff, G.L., Yonkers, P.A., Day, J.S., Hall, E.D., Sethy, V.H., & Lahti, R.A. (1988). A new 21-aminosteroid antioxidant lacking glucocorticoid activity stimulates adrenocorticotropin secretion and blocks arachidonic acid release from mouse pituitary tumor (AtT-20) cells. Journal of Pharmacology and Experimental Therapeutics, 244, 423-427.

Braughler, J.M., Duncan, L.A., & Chase, R.L. (1986). The involvement of iron in lipid peroxidation: Importance of ferric to ferrous ratios in irritation. Journal of Biological Chemistry, 261, 10282-10289.

Braughler, J.M., & Hall, E.D. (1989). Central nervous system trauma and stroke: I. Biochemical considerations for oxygen radical formation and lipid peroxidation. Free Radical Biology and Medicine, 6, 289-301.

Braughler, J.M., Hall, E.D., Jacobsen, E.J., McCall, J.M., & Means, E.D. (1989). The 21-aminosteroids: Potent inhibitors of lipid peroxidation for the treatment of central nervous system trauma and ischemia. Drugs of the Future, 14(2), 143-152.

Braughler J.M., & Pregenzer, J.F. (1989). The 21-aminosteroid inhibitors of lipid peroxidation: Reactions with lipid peroxyl and phenoxy radicals. Free Radicals in Biology and Medicine, 7, 125-130.

Braughler, J.M., Pregenzer, J.F., Chase, R.L., Duncan, L.A., Jacobsen, E.J., & McCall, J.M. (1987). Novel 21-aminosteroids as potent inhibitors of iron-dependent lipid peroxidation. Journal of Biological Chemistry, 262, 10438-10440.

Cao, W., Carney, J.M., Duchon, A., Floyd, R.A., & Chevion, M. (1988). Oxygen free radical involvement in ischemia and reperfusion injury to the brain. Neuroscience Letters, 88, 233-238.

Chaudry, I.H., & Baue, A.E. (1982). Overview of hemorrhagic shock. In R.A. Cowley & B.F. Trump (Eds.) Pathophysiology of Shock, Anoxia, and Ischemia (pp. 203-219). Baltimore, MD: Williams and Wilkins.

Demopoulos, H.B. (1973). The free radical pathology. Federation Proceedings, Federation of American Societies for Experimental Biology, 32, 1859.

Demopoulos, H.B., Flamm, E.S., Pietronigro, D.D., & Seligman, M.L. (1980). Free radical pathology and microcirculation. Acta Physiologica Scandinavica, 492(suppl.), 91-119.

Farrar, J.K. (1987). Hydrogen clearance technique. In J.H. Wood (Ed.), Cerebral Blood Flow (pp.275-287). New York: McGraw-Hill Book Company.

Fisher, A. (1988). Intracellular production of oxygen-derived free radicals. In B. Halliwell (Ed.), Oxygen Radicals and Tissue Injury: Proceedings of an Upjohn Symposium (pp.34-42). Bethesda, MD: Federation of American Societies for Experimental Biology.

Freeman, B.A., & Crapo, J.D. (1982). Free radicals and tissue injury. Laboratory Investigations, 47(5), 412.

Freeman, B.A., Rosen, G.M., & Barber, M.J. (1986). Superoxide perturbation of the organization of vascular endothelial cell membranes. Journal of Biological Chemistry, 261(14), 6590-6593.

Goldberg, W.J., Watson, B.D., Busto, R., Kurchner, H., Santiso, M., & Ginsberg, M.D. (1984). Concurrent measurement of (Na+,K+)-ATPase activity and lipid peroxides in rat brain following reversible global ischemia. Neurochemical Research, 9, 1737-1747.

Granger, D.N., Hollwarth, M.E., & Parks, D.A. (1986). Ischemia-reperfusion injury: Role of oxygen-derived free radicals. Acta Physiologica Scandinavia, 548(suppl.), 47-63.

Green, S., Mazur, A., Shorr, E. (1956). Mechanism of the catalytic oxidation of adrenalin by ferritin. Journal of Biological Chemistry, 220, 237-255.

Grogaard, B., Gerdin, B., & Arfors, K.E. (1985). Involvement of neutrophils in the cortical blood flow impairment after cerebral ischemia in a rat. Proceedings of the 4th International Conference of Superoxide and Superoxide Dismutase, Rome.

Guarnieri, C., Ferrari, R., Visioli, O., Caldarera, C.M., & Nayler, W.G. (1978). Effect of alpha-tocopherol on hypoxic-perfused and reoxygenated rabbit heart muscle. Journal of Molecular and Cellular Cardiology, 10, 893-906.

Guarnieri, C., Flamigni, F., Caldarera, C.M. (1980). Role of oxygen in the cellular damage induced by re-oxygenation of hypoxic heart. Journal of Molecular and Cellular Cardiology, 12, 797-808.

Gutteridge, J.M.C. (1986). Iron promoters of the Fenton reaction and lipid peroxidation can be released from hemoglobin by peroxides. FEBS Letters, 201, 291-295.

Hall, E.D., Braughler, J.M., Yonkers, P.A., Pazara, K.E, Smith, S.L., Schneider, D.I., Van Doornik, F.J., & Jacobsen, E.J. (1989). U78517F, a second generation lazaroid with potent anti-oxidant and cerebro-protective activity in models of CNS injury and ischemia. Journal of Neurotrauma,6, 213-214.

Hall, E.D., & Travis, M.A. (1988). Effects of the nonglucocorticoid 21-aminosteroid U74006F on acute cerebral hypoperfusion following experimental subarachnoid hemorrhage. Experimental Neurology, 102, 244-248.

Hall, E.D., & Wolf, D.L. (1986). A pharmacological analysis of the pathophysiological mechanisms of posttraumatic spinal cord ischemia. Journal of Neurosurgery, 64, 951-961.

Hall, E.D., & Yonkers, P.A. (1988). Attenuation of post-ischemic cerebral hypoperfusion by the 21-aminosteroid U74006F. Stroke, 19, 340-344.

Hall, E.D., Yonkers, P.A., & McCall, J.M. (1988). Attenuation of hemorrhagic shock by the nonglucocorticoid 21-aminosteroid U74006F. European Journal of Pharmacology, 147, 299-303.

Horneffer, P.J., & Gardner, T.J. (1984). Oxygen free radical scavengers reduce infarct size on reperfusion. Circulation, 70(suppl II.), 371.

Jarasch, E.D., Bruder, G., & Heid, H.M. (1986). Significance of xanthine oxidase in capillary endothelial cells. Acta Physiologica Scandinavia, 48(5), 39-46.

Jennings, R.B., Reimer, K.A., Hill, M.L., & Mayer, S.E. (1981). Total ischemia in dog hearts in vitro. Circulation Research, 49, 892-900.

Jewett, S.L., Eddy, L.J. & Hochstein, P. (1989). Is the autoxidation of catecholamines involved in ischemia-reperfusion injury? Free Radical Biology and Medicine, 6, 185-188.

Kantor, T.G. (1979). Ibuprofen. Annals of Internal Medicine, 91, 877-882.

Kirsh, J.R., Phelan, A.M., Lange, D.G., & Traystman, R.J. (1987). Reperfusion induced free radical formation following global ischemia. Pediatric Research, 21, 202A.

Kitt, T.M., Luderer, J.R., Shappell, S.B., Mitchell, J.R., Taylor, A.A., & Smith, C.W. (1989). The effect of U74006F, a novel aminosteroid, on human neutrophil function. The FASEB Journal, 3(3), abstract 2144.

Kleihues, P., Kobayashi, K., & Hossman, K. (1974). Purine nucleotide metabolism in the cat brain after one hour of complete ischemia. Journal of Neurochemistry, 23, 417-425.

Kramer, J.H., Arroyo, C.M., Dickens, B.F., & Weglicki, W.B. (1987). Spin-trapping evidence that graded myocardial ischemia alters post-ischemic superoxide production. Free Radical Biology and Medicine, 3, 153-159.

Kukreja, R.C., Kontos, H.A., Hess, M.L., & Ellis, E.F. (1986). PGH synthase and lipoxygenase generate superoxide in the presence of NADH or NADPH. Circulation Research, 59, 612-619.

Kurihara, M. (1985). Role of monoamines in experimental spinal cord injury in rats. Journal of Neurosurgery, 62, 743-749.

Marker, H.S., Weiss, C., Silides, D.J., & Cohen, G. (1981). Coupling of dopamine oxidation (monoamine oxidase activity) to glutathione oxidation via the generation of hydrogen peroxide in rat brain homogenates. Journal of Neurochemistry, 36, 589-593.

McCord, J.M. (1985). Oxygen-derived free radicals in post-ischemic tissue injury. New England Journal of Medicine, 312, 159-163.

Mellors, A., & Tappel, A.L. (1966). Peroxidation by ubiquinone and ubiquinol. Journal of Biological Chemistry, 241, 4353.

Minotti, G., & Aust, S.D. (1987). The requirement for ferric initiation of lipid peroxidation by ferrous hydrogen peroxide. Journal of Biological Chemistry, 262, 1089-1104.

Misra, H.P., & Fridovich, I. (1972). Superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry, 247, 3170-3175.

Misra, H.P., Weglicki, W.B., Abdulla, R., & McCay, P.B. (1984). Identification of a carbon-centered free radical during reperfusion injury in the ischemic rat heart. Circulation, 70(suppl. II), 371.

Moorhouse, P.C., Grootveld, M., Halliwell, B., Quinlan, J., & Gutteridge, J.M.C. (1987). Allopurinol and oxypurinol are hydroxyl radical scavengers. FEBS Letters, 213, 23-28.

Natale, J.E., Schott, R.J., Hall, E.D., Braughler, J.M., & D'Alecy, L.G. (1988). Effect of the aminosteroid U74006F after cardiopulmonary arrest in dogs. Stroke, 19(11), 1371-1378.

Okabe, E., Odajima, C., Taga, R., Kukreja, R.T., Hess, M.L., & Ito, H. (1988). The effect of oxygen free radicals on calcium permeability and calcium loading at steady state in cardiac sarcoplasmic reticulum. Molecular Pharmacology, 34, 388-394.

Otani, H., Tanaka, H., Inoue, T., Umemoto, M., Omoto, K., Tanaka, K., Sato, T., Osako, T., Masuda, A., Nonoyama, A., & Kagawa, T. (1984). In vitro study on the contribution of oxidative metabolism of isolated rabbit heart mitochondria to myocardial reperfusion injury. Circulation Research, 55, 168-175.

Oyanagui, Y. (1978). Inhibition of superoxide anion production in nonstimulated guinea pig peritoneal exudate cells by anti-inflammatory drugs. Biochemical Pharmacology, 27, 777-782.

Patole, M., Swaroop, A., & Ramasarma, T. (1986). Generation of H_2O_2 in brain mitochondria. Journal of Neurochemistry, 47, 1-8.

Petrone, W.F., English, D.K., Wong, K., & McCord, J.M. (1980). Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma. Proceedings of the National Academy of Science USA, 77, 1159-1163.

Rao, P.S., & Mueller, H.S. (1983). Lipid peroxidation and acute myocardial ischemia. Advances in Experimental Medicine and Biology, 161, 347-363.

Rossi, F., Della Bianca, V., Grzeskowiak, M., & Zeni, L. (1986). Mechanisms of Free Radicals Production in Granulocytes. In Novelli & F. Ursini (Eds.), Oxygen Free Radicals in Shock. International Workshop, Florence, 1985 (pp.15-28). Basel: Karger.

Rotolio, G. (1986). Biochemical mechanisms of oxy-radical production and the role of the anti-oxidant enzymes in relation to hypoxic and ischemic tissue damage. In Novelli & F. Ursini (Eds.), Oxygen Free Radicals in Shock. International Workshop, Florence, 1985 (pp.1-8). Basel: Karger.

Ruzicka, F.J., Beinert, H., Schilper, K.L., Dunham, W.R., & Sands, R.H. (1975). Interaction of ubisemiquinone with a paramagnetic component in heart tissue. Proceedings of the National Academy of Science, 72, 2886-2891.

Sanan, S., Sharma, G., Balveer, S., Sanan, D.P., & Wadhera, P. (1989). Evaluation of desferrioxamine mesylate on survival and prevention of histo-pathological changes in the liver in haemorrhagic shock: an experimental study in dogs. Resuscitation, 17, 63-75.

Siesjo, B.K. (1981). Cell damage in the brain: A speculative synthesis. Journal of Cerebral Blood Flow and Metabolism, 1, 155-185.

Siesjo, B.K. (1984). Cerebral circulation and metabolism. Journal of Neurosurgery, 60, 883-908.

Simpson, P.J., Fantone, J.C., & Lucchesi, B.R. (1988). Myocardial ischemia and reperfusion injury: Oxygen radicals and the role of the neutrophil. In B. Halliwell (Ed.), Oxygen Radicals and Tissue Injury: Proceedings of an Upjohn Symposium (pp.63-80). Bethesda, MD: Federation of American Societies for Experimental Biology.

Singal, P.K., Kapur, N., Dhillon, K.S., Beamish, R.E., & Dhalla, N.S. (1982). Role of free radicals in catecholamine-induced cardiomyopathy. Canadian Journal of Physiology and Pharmacology, 60, 1390-1397.

Snelling, L.K., Ackerman, A.D., Dean, J.M., North, M.C., & Traystman, R.J. (1987). The effects of superoxide dismutase on neurological injury (cerebral blood flow and evoked potentials) following global cerebral ischemia. Anesthesiology, 67, 1987.

Tappel, A.L., & Zalkin, H. (1959). Inhibition of lipid peroxidation in mitochondria by vitamin E. Archives of Biochemistry and Biophysics, 80, 333-336.

Thomas, C.E., Morehouse, L.A., & Aust, S.D. (1985). Ferritin and superoxide dependent lipid peroxidation. Journal of Biological Chemistry, 260, 3275-3280.

Travis, M.A., & Hall, E.D. (1987). The effects of chronic two-fold dietary vitamin E supplementation on sub-arachnoid hemorrhage induced brain hypoperfusion. Brain Research, 418, 366-370.

Turrens, J.F., Alexandre, A., & Lehninger A.L. (1985). Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. Archives of Biochemistry and Biophysics, 237, 408-414.

Ursini, F. (1986). The multilevel system against lipid peroxidation in living tissues. In Novelli & F. Ursini (Eds.), Oxygen Free Radicals in Shock. International Workshop, Florence, 1985 (pp.9-14). Basel: Karger.

Watson, B.D., Busto, R., Goldberg, W.J., Santiso, M., Yoshida, S., & Ginsberg, M.D. (1984). Lipid peroxidation in vivo induced by reversible global ischemia in rat brain. Journal of Neurochemistry, 42, 268-274.

Watson, B.D. & Ginsberg, M.D. (1988). Mechanisms of lipid peroxidation potentiated by ischemia in the brain. In B. Halliwell (Ed.), Oxygen Radicals and Tissue Injury: Proceedings of an Upjohn Symposium (pp.81-91). Bethesda, MD: Federation of American Societies for Experimental Biology.

Werns, S.W., Shea, M.J., & Lucchesi, B.R. (1985). Free radicals in ischemic myocardial injury. Free Radicals in Biology and Medicine, 1, 103-110.

Wolff, S.P., Garner, A., & Dean, R.T. (1986). Free radicals, lipids, and protein degradation. Trends in Biochemical Sciences, 11, 27-31.

Yoshida, S., Busto, R., & Watson, B.D. (1985). Postischemic cerebral lipid peroxidation in vitro: modification by dietary vitamin E. Journal of Neurochemistry, 44, 1593-1601.

Young, W. (1980). H_2 clearance measurement of blood flow: A review of technique and polarographic principles. Stroke, 11, 552-564.

Zweir, J.L., Flaherty, J.T., & Weisfeldt, M.L. (1987). Direct Measurement of free radical generation following reperfusion of ischemic myocardium. Proceedings of the National Academy of Science, USA, 84, 1404-1407.

Zweir, J.L., Rayburn, B.K., Flaherty, J.T., & Weisfeldt, M.L. (1986). The effect of superoxide dismutase on free radical concentrations in post-ischemic myocardium. Circulation, 74(suppl. II), 371.