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Protective Effect of Rutin on Splanchnic Injury Following Ischemia and Reperfusion in Rats

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A splanchic artery occlusion for 90 min followed by reperfusion of the mesenteric circulation resulted in a severe form of circulatory shock characterized by endothelial dysfunction, severe hypotension, marked intestinal tissue injury, and a high mortality rate. The effect of rutin, a flavonoid having antiprostanoid, anti-inflammatory, antithrombotic, antioxidant effect, were investigated in a model of splanchnic artery occlusion (SAO) shock in urethane anesthetized rats. Occlusion of the superior mesenteric artery for 90 min produced a severe shock state resulted in a fatal outcome within 120 min of reperfusion in many rats. Rutin was given as a bolus (1.28 mg/kg) 10 min prior to reperfusion. Administration of rutin significantly improved mean arterial blood pressure in comparison to vehicle treated rats ($p<0.05$). Rutin treatment also resulted in a significant attenuation in the increase in plasma amino nitrogen concentration, intestinal myeloperoxidase activity, intestinal lipid peroxidation, infiltration of neutrophils in intestine and thrombin induced adherence of neutrophils to superior mesentric artery segments. These results suggest that rutin provides beneficial effects in part by preserving endothelial function and attenuating neutrophil accumulation in the ischemic reperfused splanchnic circulation.

Key Words: Rutin, Ischemia, Reperfusion

INTRODUCTION

Reperfusion of a vascular bed which had previously been ischemic, results in a cascade of deleterious cellular events which eventually lead to tissue injury. More specifically, occlusion of the splanchnic circulation followed by reperfusion (SAO) produces alterations in several cardiovascular parameters, as well as the formation of toxic substances which promote the development and progression of circulatory shock. The process of reperfusion injury is characterized by an inflammatory response in which polymorphonuclear leukocytes (PMNs) are believed to play an important role (Tsao et al, 1990; Entman et al, 1991).

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Upon reperfusion, many activated PMNs accumulate in the microvasculature, resulting in microvascular plugging (Engler et al, 1983; Lefer et al, 1991). The activated PMNs induce tissue injury by the release of a variety of cytotoxic substances, including oxygen-derived free radicals, inflammatory cytokines and proteolytic enzymes (Weiss, 1989). Many of these substances may mediate vascular endothelial dysfunction as well as contribute to tissue injury (Buerke et al, 1994). As is the case with other organs, ischemia and reperfusion of the mesenteric circulation resulted in the accumulation of leukocytes in the mesentery and intestinal wall (Kurtel et al, 1992). Of those leukocytes accumulating in the intestinal mucosa following ischemia and reperfusion, more than 80% have been identified as neutrophils (Oliver et al, 1991). This is also consistent with evidence that either decreasing the number of circulating PMNs or the administration of monoclonal antibodies directed against cell adhesion molecules

can lead to significant tissue protection against reperfusion injury (Romson et al, 1983; Ma et al, 1992; Weyrich et al, 1993; Lefer et al, 1994).

Flavonoids including rutin comprise a large group of plant-derived compounds with multiple biological effects. Flavonoids have been shown to modify eicosanoid biosynthesis (anti-prostanoid and anti-inflammatory response), protect low density lipoprotein from oxidation (prevent atherosclerotic plaque formation), prevents platelet aggregation (antithrombotic effects), promote relaxation of cardiovascular smooth muscle (antihypertensive and antiarrhythmic effects), and have antiviral and carcinostatic properties (Formica & Regelson, 1995). They appear to possess both antioxidant and chelating abilities (Havsteen, 1986). They have (been) reported to decrease lipid peroxidation of fatty acids, liposomes, mitochondria and microsomes (Chung et al, 1991), and to inhibit degradation of protein by Cu^{2+} and H_2O_2 (Park et al, 1991) and oxidant generation by the neutrophil NADPH oxidase system (Tauber et al, 1984). However, several flavonoids sometimes are suggested to have prooxidant properties under some reaction conditions (Ochiai et al, 1984). It is reported that phenolic compounds gossypol, quercetin and myricetin remarkably accelerate production of hydroxyl radical from H_2O_2 in the presence of Fe^{3+}-EDTA at neutral pH (Laughton et al, 1989). Thus, the biological function of flavonoids in the oxidative tissue injury has not been clarified. In addition, their actions as an antioxidant in vivo are uncertain.

Therefore the objectives of this investigation were to determine if rutin provided beneficial effects in a well established rat model of splanchnic ischemia and reperfusion, and to elucidate any mechanisms which may be involved.

METHODS

Experimental protocol

Male Sprague-Dawley rats, body weight 250~300 g were anesthetized with urethane (1.5 g/kg) intraperitoneal injection. The trachea was cannulated to maintain a patent airway throughout the experiment. A polyethylene catheter was inserted in one carotid artery to monitor mean arterial blood pressure (MABP), and a second catheter was placed in the contralateral external jugular vein for infusion of rutin or its vehicle. MABP was recorded on a Grass model 7 oscillographic recorder using Statham P23 AC pressure transducers (Gould, Cleveland, OH). The abdominal cavity was opened via a midline laparotomy, and the superior mesenteric arteries (SMA) were isolated near their aortic origin.

After stabilization, SMA was completely occluded for 90 minutes using nontraumatic arterial clamps. At the end of the ischemic period, the clamp was removed, and the splanchnic circulation was reperfused. Rats were observed for 2 hours after reperfusion or until their MABP declined to 50 mmHg, at which time the experiment was terminated. Rats experiencing massive acute circulatory collapse (i.e., MABP <50 mmHg) within the first 30 minutes post- reperfusion were excluded from the study since this is usually associated with a significant degree of hemorrhage. Rats were randomly assigned to one of four groups: a) sham-operated control rats or b) sham-operated control plus rutin rat (1.28 mg/kg bolus i.v.) in which all surgical procedures were performed except that the mesenteric arteries were not occluded, c) SAO plus vehicle (saline 0.3 ml bolus i.v.), or d) SAO plus rutin (1.28 mg/kg bolus). Bolus administration of either vehicle or rutin were given 10 minutes before reperfusion. At the time of circulatory collapse or at the end of the 2 hour reperfusion period, the SMA was removed and studied using isolated vascular ring experiments, and a section of ileal tissue was removed and used for analysis of myeloperoxidase (MPO) activity, lipid peroxidation and histologic examination.

Determination of tissue myeloperoxidase (MPO)

Ileal MPO activity, an enzyme occurring virtually exclusively in polymorphonuclear leukocytes (PMN), was determined using the method of Bradley et al (1982) as modified by Mullane et al (1985). A hemorrhage-free area of the ileum approximately 8 to 10 cm in length, at least 30 cm distal to the stomach, was dissected and carefully rinsed in 0.9% NaCl. The sample was then homogenized in 0.5% HTAB (hexadecyltrimethyl ammonium bromide, Sigma Chemical Co., St. Louis, MO, which was dissolved in 50 mM potassium phosphate buffer at pH 6.0) using a Polytron (PCU-2) homogenizer (Kinematica GMBH, Luzern, Switzerland). Homogenates were centrifuged at 12,500 g at 4°C for 30 minutes. The supernatant were then collected and reacted with 0.167 mg/ml of

o-dianisidine dihydrochloride (Sigma Chemical Co.) and 0.0005% H_2O_2 in 50 mM phosphate buffer at pH 6.0. The resultant change in absorbance was determined spectrophometrically at 460 nm. One unit of MPO is defined as that quantity of enzyme hydrolysis 1 mM of peroxide/min at 25°C.

Isolation and labeling of autologous rat PMNs

Peripheral blood (15 ml) was drawn from the carotid artery cannulated at the beginning the surgical procedure and anticoagulated with citrate-phosphate-dextrose solution (1.5 : 10 vol/vol, anticoagulant to whole blood). PMNs were isolated by a procedure modified with Lafrado & Olsen (1986). After centrifugation of whole blood, the pellet was mixed with 8 ml of 6% dextran (MW 60,000~90,000; Sigma Chemical Co., St. Louis, MO) and phosphate buffer saline (PBS) to allow the red blood cells to settle. The leukocyte-rich upper fraction was layered onto a percoll/platelet-poor plasma (PPP) gradient (density gradients of 80%, 62%, and 50%). Following centrifugation at 3,000 rpm for 40 min, PMNs were collected from the 62% and 80% interface and washed in PBS. PMNs obtained by this method were generally >95% pure and >95% viable. Isolated PMNs were then labeled with a Zynaxis PKH-2 cell linker (Zynaxis Cell Science Inc., prepared for Sigma Immunochemicals, Malvern, PA) based on the procedure of Yuan & Fleming (1990). Two ml of diluant and 10 μl of dye were added to a loose cell pellet containing approximately 10 million cells. Following a seven min incubation period, 200 μl of PPP were added to stop the reaction and 2 ml of PBS were added to underlay the suspension. The mixture was then centrifuged for 10 min at 1,800 rpm. The cells were resuspended in PBS, counted and utilized in the adherence assay.

In vitro adherence of PMN to thrombin stimulated superior mesenteric artery endothelium

PMNs were isolated and fluorescently labeled as previously described. The SMA was dissected out, placed in warm K-H buffer (Krebs Henseleit buffer) consisting of (in mmol/l): NaCl 118, KCl 4.75. $CaCl_2$ 2.54, KH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 12.5, and glucose 10, and cleaned of fat and connective tissue. The SMA was sectioned into 2~3 mm rings, opened, and then placed into wells containing 2 ml K-H solution. The segments were incubated with 2 U/ml thrombin (Sigma Chemical Co., St. Louis, MO) in a shaker bath at 37°C for 10 min. After a 10 minute incubation, the segments were transferred to a fresh K-H buffer. Approximately one million PMNs were incubated with thrombin-stimulated endothelium alone or in combination with 50 or 100 μM rutin. Following a 20 min incubation period at 37°C, the segment was washed with K-H buffer and placed endothelial side up on a slide glass. PMNs adhering to the endothelium were counted using epifluorescence microscopy (Laborlux 12, Leitz, Germany). PMNs were counted in five different fields of endothelial surface and expressed as adherent PMNs/mm^2 of endothelial surface.

Ex vivo PMN adherence to superior mesenteric artery endothelium

PMNs were isolated and fluorescently labeled as previously described. Segments from superior mesenteric artery were isolated from each rat and placed into warmed K-H buffer. Arteries were cut into rings of 2 to 3 mm length. The rings were then opened, and placed with the endothelial surface up into a cell culture dish filled with 3 ml of oxygenated K-H solution and incubated in culture dishes with autologous labeled PMNs (1.2×10^6 cells) for 20 min at 37°C in a shaker bath which stimulates shear forces. Following the 20 min incubation period, segments were washed with K-H buffer and placed on slide glass. PMNs adhering to the endothelium were counted by using epifluorescence microscope (Laborlux 12, Leitz, Germany). Five different fields of each endothelial surface were counted and the results were expressed as adherent PMNs/mm^2 of endothelial surface.

Plasma free amino nitrogen

Blood samples were kept on ice and centrifuged at 2,500 g for 20 minutes at 4°C, with the supernatant employed for biochemical assay. Plasma free amino-nitrogen concentration were determined by the method using plasma samples deproteinized with 5% trichloroacetic acid. The free amino-nitrogen concentration was used as a index of total plasma proteolysis and expressed in units per milliliter (U/ml). One unit is equivalent to 10 nmol of serine.

Measurement of lipid peroxidation

A hemorrhage-free area of the ileum 8 to 10 cm in length, at least 30 cm. distal to the stomach, was dissected and carefully rinsed in 0.9% NaCl. The sample was then homogenized in 10 volume K-H buffer by using a Polytron (PCU-2) homogenizer (Kinematica GMBH, Luzern, Switzerland). Homogenates were centrifuged at 12,500 g at 4°C for 30 minutes. The supernatant were then collected. Lipid peroxidation of the ileum was estimated from malondialdehyde concentration measured by thiobarbituric acid method. The supernatant of ileum (1 mg protein/ml) was added to the reaction mixture consisting of 150 mM KCl and 50 mM NaH_2PO_4, pH 7.4. The reaction was started by adding ileal tissue to the mixture, final volume 1.0 ml. After 30 min of incubation, the reaction was stopped by adding 1.0 ml of 1% 2-thiobarbituric acid (TBA) in 50 mM NaOH and 1.0 ml of 2.8% trichloroacetic acid. The chromophore was developed by boiling in a water bath for 10 min. After cooling to the room temperature, the absorbance was measured at 532 nm (Gutteridge, 1981). The concentration of malondialdehyde was expressed as nmol/mg protein using the molar extinction coefficient of 1.52×10^6/M/cm (Placer et al, 1966).

Statistical analysis

All values in the text and figures are presented as mean±standard errors of the mean (SEM) of n independent experiments. Statistical analysis was performed using Student's *t*-test for paired data. Probabilities of 0.05 or less were considered to be significant in all cases.

RESULTS

Effects of rutin on mean arterial blood pressure (MABP)

Occlusion of the superior mesenteric artery resulted in an increase in MABP of 50~70 mmHg for all SAO groups (Fig. 1) with mean arterial blood pressures reaching 160~168 mmHg for all SAO groups. During the 90 minute occlusion period there was a slow decline in MABP to levels comparable to pre-occlusion values. However, upon reperfusion, all

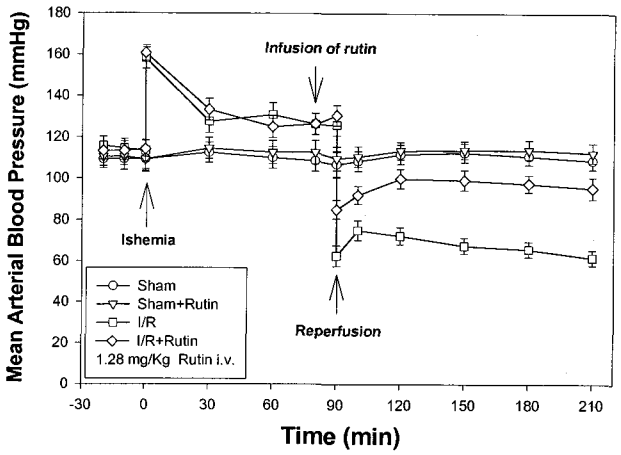

Fig. 1. Time course of mean arterial blood pressure in rats subjected to sham, sham+rutin, I/R, and I/R+rutin. All values are means±standard error of surviving animals at each time point, n=5~6.

SAO rats exhibited a precipitous decline of MABP to approximately 60~70 mmHg. The administration of rutin (1.28 mg/kg) had no significant effect on MABP over the 2 hour observation period in sham trauma rats. This finding indicates that neither the surgical procedures nor the administration of rutin contributed to the alterations in MABP observed in rats subjected SAO shock. SAO rats given with vehicle demonstrated slight increase (p=NS) in MABP following the initial decline of MABP which was followed by a steady secondary decline in MABP during the remainder of the reperfusion period. In contrast, those SAO rats treated with rutin (1.28 mg/kg) showed an immediate and sustained increase in MABP following the initial decline which persisted over the entire 120 minute reperfusion period. In fact, at the end of the observation period no significant differences were observed in MABP between sham-operated control and receiving rutin SAO rats.

Effects of rutin on the PMNs adherence to the SMA vascular endothelium

One of the early events in PMNs-mediated reperfusion injury is an increase in the adherence of PMNs to the reperfused endothelium. In order to determine whether rutin inhibits the adherence of rat PMNs to rat SMA endothelial cells, the effects of rutin were determined on unactivated PMN adherence to stimulated SMA endothelium in vitro (Fig. 2). Few unactivated PMN adhered to unstimulated SMA end-

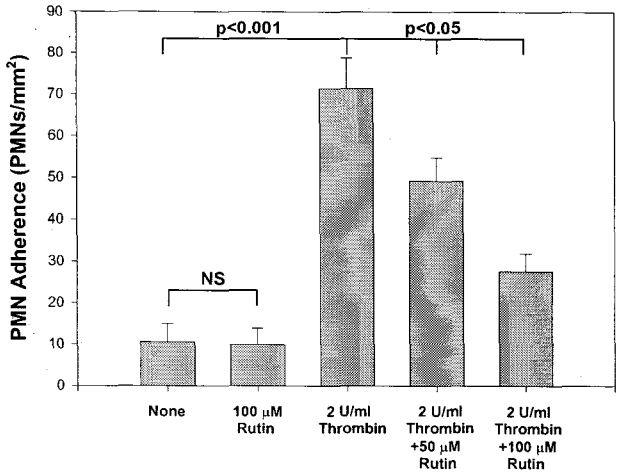

Fig. 2. In vitro effect of rutin on PMN adherence to thrombin-stimulated (2 U/ml) rat superior mesenteric artery. Data are expressed as number of PMNs/mm^2. Bar heights represent means and brackets indicate ± SEM, n=5.

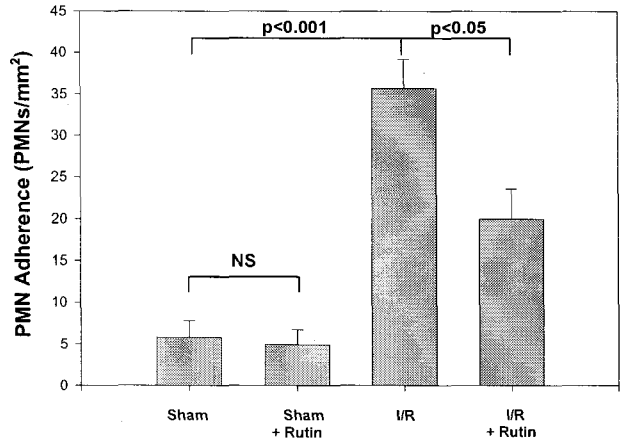

Fig. 3. Effects of in vivo administration of rutin on the in vitro adherence of unstimulated PMN to non ischemic-reperfused superior mesenteric artery endothelium and ischemic-reperfused superior mesenteric artery endothelium. Data are expressed as number of PMNs/mm^2. Bar heights represent means and brackets indicate ± SEM.

othelium. However, stimulation of SMA endothelium with thrombin (2 U/ml) for 10 min resulted in a significant ($p<0.001$) 7-fold increase in adherent PMNs to the endothelium in comparison to unstimulated endothelium. Addition of rutin inhibited PMN adherence to the endothelium in a concentration-dependent manner. Incubation of SMA segments with 50 μM rutin resulted in a 31.1% inhibition of PMN adherence and 100 μM rutin inhibited adherence by

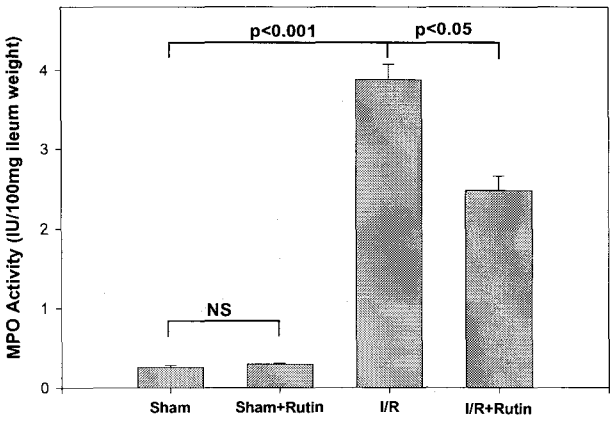

Fig. 4. Effects of rutin on ileal myeloperoxidase (MPO) activity in sham, sham + rutin, I/R, and I/R + rutin. Data are expressed as Unit/100 mg ileum weight. Bar heights represent means and brackets indicate ± SEM, n=5.

61.3%. We measured the adherence of PMNs following the in vivo administration of rutin (1.28 mg/kg) (Fig. 3). When the unstimulated autologous PMNs were incubated with nonischemic SMA segments for 20 min, relatively few adhered to the endothelium regardless of the group of origin. In SAO rats receiving rutin, unstimulated PMNs incubated with the ischemic SMA segments resulted in a significant decrease in PMN adherence ($p<0.05$). These results suggest that a protective effect of rutin is contributed to in part an inhibiting of the interaction between neutrophils and mesenteric vascular endothelium.

Effects of rutin on ileal myeloperoxidase activity

Accumulation of PMNs in the ischemic-reperfused intestinal tissue is considered one of the primary contributory mechanisms to reperfusion injury. The MPO activity in the intestinal tissue was measured as a marker for PMN accumulation (Fig. 4). The MPO activity was low in all sham-operated control rats (0.26 ± 0.02 U/100 mg tissue weight). However, ischemia of the mesenteric circulation followed by reperfusion in untreated rats resulted in high increase in ileal MPO activity (3.87 ± 0.20 U/100 mg tissue weight). Treatment of SAO rats with 1.28 mg/kg rutin significantly ($p<0.05$) attenuated ileal MPO activity in comparison to untreated rats, indicating that rutin retarded the accumulation of PMNs in the postischemic mesentery.

Effects of rutin on plasma free amino nitrogen

We also measured plasma levels of free amino nitrogen as a index of plasma proteolyis, which results from the liberation of proteolytic enzymes into the circulation following circulatory shock. Only moderate levels of proteolysis occurred in sham operated control rats (Fig. 5). However, ischemia of the mesenteric circulation followed by reperfusion in untreated rats resulted in significant ($p<0.001$) increases in plasma free amino nitrogen. Treatment of SAO rats with rutin significantly ($p<0.05$) inhibited level of plasma free amino nitrogen in comparison to untreated rats.

Effects of rutin on lipid peroxidation of intestine

We measured lipid peroxidation of intestine as an index of oxidant-induced in injured tissue, which results from the production of reactive oxygen species (i.e. superoxide radical, hydroxyl radical, hydrogen peroxide). The lipid peroxidation was low in all sham operated control rats. However, ischemia of the mesenteric circulation followed by reperfusion in untreated rats resulted in a 2-fold increase in lipid peroxidation. Treatment of SAO rats with rutin inhibited significantly ($p<0.05$) lipid peroxidation of intestine in comparison to untreated rats (Fig. 6).

DISCUSSION

The celiac, superior mesenteric, and inferior mesenteric arteries supply blood to the liver, pancreas, and intestine with up to 90% of the blood flow. An occlusion of superior mesenteric artery results in an abrupt increase in systemic arterial blood pressure by approximately 25 to 50 mmHg, which is due primarily to a decrease in the baroreceptor input to the central medullary vasomotor center. The large increase in MABP diminishes during the ischemia and returns to near pre-occlusion levels by the end of the ischemic period. Upon reperfusion of splanchnic circulation a precipitous fall in MABP ensues with values ranging from 50 to 70 mmHg. This is followed by a moderate increase in pressure within the first 30 minutes of reperfusion and a steady decline throughout the remainder of the reperfusion period to values incompatible with life (i.e., approximately 45 mmHg).

In this investigation we observed a response similar to this indicating that our rats had been subjected to severe circulatory shock as a result of SAO/reperfusion. This type of circulatory shock is characterized by the formation and accumulation of toxic factors produced during the ischemic period that are released into the systemic circulation upon reperfusion of splanchnic organ (Lefer & Barenholz, 1972). These toxic mediators induce proteolysis, severe hypotension, hemoconcentration, cardiac depression, and changes in vasoactivity. Occlusion alone of the

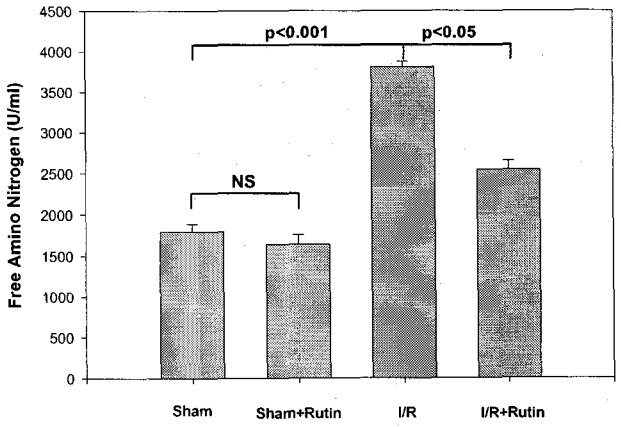

Fig. 5. Effects of rutin on plasma free-amino nitrogen (FAN) in sham, sham+rutin, I/R, and I/R+rutin. Data are expressed as Unit/ml blood. Bar heights represent means and brackets indicate±SEM, n=5.

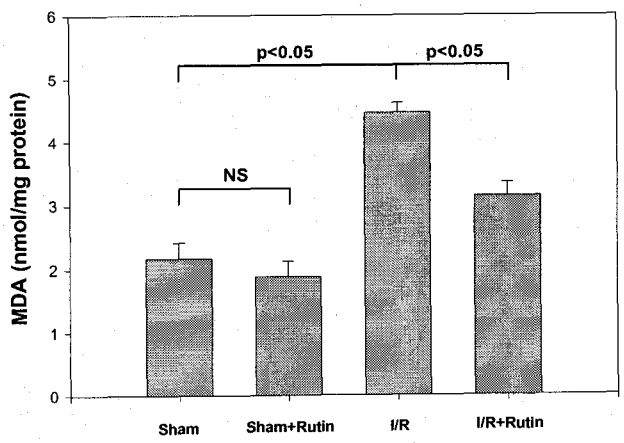

Fig. 6. Effects of rutin on ileal lipid peroxidation in sham, sham+rutin, I/R, and I/R+rutin. Data are expressed as malondialdehyde nmol/mg protein using the molar extinction coefficient of 1.52×10^6/M/cm. Bar heights represent means and brackets indicate±SEM, n=5.

major splanchnic arteries results in disturbances in parenchymal and microvascular permeability, and the production of cytotoxic substances from the pancreas and platelets. Upon reperfusion, these potentially toxic metabolites including a myocardial depressant factor and thromboxane A_2 are introduced into the systemic circulation where they exacerbate the shock state. In addition, the restoration of blood flow in the previously ischemic region also allows for the interaction of leukocytes, particularly PMNs, with the endothelium which has been identified as one of the early phases of the inflammatory process (Butcher, 1991) and these PMNs also release leukotrienes, cytokines, reactive oxygen species and proteases (Grisham et al, 1986; Buerke et al, 1994; Rubin et al, 1994).

During the process of reperfusion injury, PMNs are involved in the injury to splanchnic organs as well as in the development and progression of endothelial dysfunction (Lefer et al, 1991; Rubin et al, 1994). Endothelial dysfunction associated with ischemia and subsequent reperfusion is an early event following reperfusion (Lefer et al, 1991). This dysfunction is due in large part to an increased superoxide and reduced NO generation by the endothelium (Lefer & Lefer, 1993) and is evidenced by a decreased relaxation of the mesenteric arteries to endothelium dependent vasodilators (Karasawa et al, 1991; Carey et al, 1992). Additionally, the dysfunctional endothelium becomes activated and interactions between leukocytes and the endothelium significantly increase 30 min following reperfusion (Weyrich et al, 1993). By 2 hours postreperfusion, numerous PMNs have extravasated through the endothelium and migrated into the interstitium (Weyrich et al, 1993). We observed about fifteen fold increase in ileal MPO activity following SAO and reperfusion, indicative of a large number of PMN residing in the interstitium.

Once PMNs become adherent to the vascular endothelium or migrate to the interstitium, their release of proteases along with oxygen-derived free radicals propagates localized tissue injury. Oxygen radicals released by PMNs are capable of inactivating nitric oxide as well as endogenous protease inhibitors enabling serine proteases such as elastase and cathepsin G to exacerbate tissue injury (Karasawa et al, 1991). This is followed by the release of chemoattractants such as platelet activating factor, leukotriene B_4, interleukin-1β, and tumor necrosis factor-α, by activated PMNs and endothelial cells. The release of these substances results in the recruitment of additional PMNs to the site of injury thereby exacerbating existing endothelial dysfunction and tissue injury.

Inflammation is a process involved in several states and a limitation of the inflammatory process has been the target of numerous therapeutic strategies for these disorders. Administration of monoclonal antibodies directed against adhesion molecules (Weyrich et al, 1993), blockers of adhesion molecule ligands (Buerke et al, 1994; Takada et al, 1997), and substances which inhibit adhesion molecule expression (Lefer et al, 1993) have all proven to be successful in limiting the severity of the inflammatory process. In addition to these approaches, recent studies have demonstrated the beneficial effects of flavonoids as anti-inflammatory agents (Formica & Regelson, 1995). Due to the highly integrated cascade of events surrounding the inflammatory response, several mechanisms may be involved in the anti-inflammatory properties of rutin. Flavonoids have been shown to inhibit eicosanoid synthesis (i.e., leukotriene, thromboxane) by inhibiting both lipoxygenase and cyclooxygenase activities, as well as inhibiting the non-enzymatic peroxidation of polyunsaturated fatty acids required for the activation of these oxygenase. Flavonoids do not only inhibit LDL oxidation, platelet aggregation, calmodulin, and protein kinase C activity, but also scavenge oxygen derived-free radicals (Formica & Regelson, 1995). Data from this study show that rutin, which lacks direct anti-thrombin activity, is capable of attenuating unstimulated PMN adherence to thrombin stimulated endothelium. Thus, we now show that rutin is capable of attenuating adherence of PMNs to endothelial cells when either the endothelium or the PMNs are stimulated.

The production of large amount of superoxide and hydrogen peroxide increases the number of rolling or adherent PMNs (Suzuki et al, 1989; Johnston et al, 1996) primarily by overwhelming endogenous NO. NO is able to inactivate both superoxide and hydrogen peroxide (Rubanyi et al, 1991; Huie & Padmaja, 1993; Johnston et al, 1996) thereby protecting surrounding cells from free radical mediated damage. In addition, nuclear factor-κB (NF-κB), a transcription factor which upregulates P-selectin, E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 gene expression, has been shown to be inhibited by NO (De Caaterina et al, 1995). NO has also been shown to prevent the expression of key

adhesion molecules on the cell surface (Armstead et al, 1997). Oxygen-derived free radical (Huie & Padmaja, 1993) have also been shown to directly activate NF-κB, enhancing the synthesis of key adhesion molecules. Administration of rutin may scavenge superoxide, hydroxyl radical, and hydrogen peroxide release from activated PMNs and ischemic vascular endothelium. Therefore, rutin may provide significant protective effects by inhibiting the action of potentially injurious oxygen radicals thereby attenuating endothelial dysfunction and preventing the stimulation of adhesion molecule transcription factors.

These results are consistent with previous studies demonstrating beneficial effect of flavonoid (quercetin) on brain ischemia-reperfusion (Shutenko et al, 1999). In this study, we have shown that the administration of rutin attenuates hypotension in rats subjected to 90 min of splanchnic ischemia followed by reperfusion. The beneficial effects of rutin are due at least in part to an attenuation of endothelial dysfunction which is an early consequence of ischemia and reperfusion of the splanchnic vasculature. This was evidenced by the fact that rutin treatment significantly preserved endothelial function and inhibited both the in vitro PMN adherence to vascular endothelium as well as in vivo PMN accumulation within the splanchnic organs. It appears as though the primary mechanism of protection involves the scavenge of toxic mediators released by activated neutrophils during reperfusion of the splanchnic circulation, which serves to protect the endothelium, thus preserving endothelial and inhibiting leukocytes-endothelial cell interactions. This study, in conjunction with previous investigations, demonstrates the potential therapeutic benefits of rutin during inflammatory states such as splanchnic artery occlusion and reperfusion.

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