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Effects of a BLT receptor antagonist on local and remote reperfusion injuries after transient ischemia of the superior mesenteric artery in rats

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Abstract

Reperfusion of ischemic vascular beds may lead to recruitment and activation of leukocytes, release of mediators of the inflammatory process and further injury to the affected vascular bed and to remote sites. Neutrophils appear to play a major role in the pathophysiology of reperfusion injury. Amongst inflammatory mediators shown to activate neutrophils and induce their recruitment in vivo, much interest has been placed on the role of leukotriene (LT)B_4. Here, we have assessed the effects of the BLT receptor antagonist (+)-1-(3*S*,4*R*)-[3-(4-phenyl-benzyl)-4-hydroxy-chroman-7-yl]-cyclopentane carboxylic acid (CP 105,696) in a model of neutrophil-dependent ischemia and reperfusion injury in the rat. The superior mesenteric artery was isolated and ischemia was induced by its total occlusion for 30 min. After 30 min of reperfusion, injury was assessed by evaluating the extravasation of Evans blue, an index of vascular permeability, and the levels of myeloperoxidase, an index of neutrophil accumulation, in the intestine, mesentery and lung. The neutrophil-dependence of the local (intestine and mesentery) and remote (lung) injury was confirmed by using fucoidin, a selectin blocker, and WT-3, an anti-CD18 monoclonal antibody. Post-ischemic treatment with CP 105,696 dose-dependently inhibited vascular permeability and neutrophil accumulation in the intestine and mesentery. CP 105,696 also blocked the vascular permeability changes, but not neutrophil accumulation, in the lungs after reperfusion injury. Virtually identical results were obtained with another BLT receptor antagonist, 1-(5-ethyl-2-hydroxy-4-(6-methyl-6-(1*H*-tetrazol-5-yl)-heptoxy)-phenyl)ethanone (LY255283). Our results suggest that post-ischemic treatment with BLT receptor antagonists may inhibit local and remote ischemia and reperfusion injury by blocking both the accumulation and/or activation of neutrophils.

Keywords: Ischemia–reperfusion; Neutrophil; Oedema; Mesenteric artery; Inflammation

1. Introduction

The reduction in blood flow, ischemia, to an organ or a vascular bed can lead to significant tissue injury and cell death if it is prolonged. Thus, one of the major goals in the treatment of ischemia is to restore blood flow to normal values, i.e. to "reperfuse" the ischemic vascular bed (Lefer and Lefer, 1996; Willerson, 1997). However, reperfusion of ischemic vascular beds may lead to recruitment and activation of leukocytes, release of mediators of the inflammatory process and further injury to the affected vascular bed (Lefer and Lefer, 1996; Willerson, 1997). Thus, strategies which limit the damage induced by the reperfusion process may be useful in the treatment of ischemic disorders in various organs (Willerson, 1997).

Among the cell types involved in the injury following reperfusion of an ischemic tissue, neutrophils are of major importance (Cornejo et al., 1997; Willerson, 1997). Thus, there has been much interest in the development of strategies which block neutrophil recruitment and/or activation into sites of ischemia and reperfusion injury. The current paradigm for neutrophil recruitment into sites of inflammation predicts there are three steps for the migration of these cells into tissue (reviewed by Springer, 1994). Initially, neutrophils roll on endothelial cells, a process mediated by selectins and their carbohydrate ligands. The rolling neutrophil can then be activated by mediators which act on seven-transmembrane receptors leading to calcium-depen-

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dent activation of integrins on the neutrophil surface. Blockade of selectin- or integrin-dependent interactions inhibits neutrophil recruitment and neutrophil-dependent injury following ischemia and reperfusion of various tissues (Cornejo et al., 1997). Alternatively, neutrophil recruitment and neutrophil-dependent injury can be modulated by antagonists at serpentine receptors (Willerson, 1997). Amongst inflammatory mediators shown to activate neutrophils and induce their recruitment in vivo, much interest has been placed on the role of leukotriene (LT)B_4. This lipid mediator acts on a seven-transmembrane receptor and has been shown to mediate neutrophil recruitment in several models of inflammation (Crooks and Stockley, 1998).

In the present study, we describe a model of reperfusion injury following ischemia of the superior mesenteric artery (SMA). Local injury to the intestine, mesentery, and remote injury to the lung were evaluated. Initial experiments using the selectin blocker fucoidin and the anti-CD18 monoclonal antibody WT-3 were performed to confirm the neutrophil dependence of the injuries observed. We then evaluated the effects of the treatment with leukotriene B_4 receptor antagonist (+)-1-(3*S*,4*R*)-[3-(4-phenyl-benzyl)-4-hydroxy-chroman-7-yl]-cyclopentane carboxylic acid (CP 105,696). Of interest, CP 105,696 was administered after ischemia had initiated and prior to reperfusion. This is an important consideration because it bears the closest resemblance to what happens in the real clinical situation when patients are usually seen during the period of ischemia.

2. Material and methods

2.1. Animals

Male Wistar rats (200–220 g) obtained from the Bioscience unit of our Institution were housed in standard conditions and had free access to commercial chow and water.

2.2. Ischemia and reperfusion injury

Rats were anaesthetized with urethane (140 mg/kg, i.p.) and laparotomy was performed. The SMA was isolated and ischemia was induced by totally occluding the SMA for 30 min. After ischemia, reperfusion was initiated by removal of the occlusion. Initial kinetic experiments were performed to evaluate the optimal time for reperfusion-induced injury after ischemia. In these experiments, animals were reperfused for 7.5, 15, 30, 60 or 120 min. Sham-operated animals or animals only made ischemic were used as controls for the reperfusion-induced injury.

Following these initial experiments, the reperfusion time of 30 min was chosen to evaluate the effects of the various pharmacological treatments. The following pharmacological treatments were used at doses previously shown to be effective in vivo: fucoidin (10 mg/kg in saline, Ley et al., 1993; Kubes et al., 1995); WT3 (1 mg/kg, in 10 mM phosphate buffered-saline, Matsuo et al., 1994); CP 105,696 (0.03–3 mg/kg, in a 3% dimethylsulphoxide (DMSO) solution, Showell et al., 1995); 1-(5-ethyl-2-hydroxy-4-(6-methyl-6-(1*H*-tetrazol-5-yl)-heptoxy)-phenyl)ethanone (LY255283) (3 mg/kg, in 10 mM phosphate buffered-saline, Karasawa et al., 1991). Control animals received drug vehicles or mouse Immunoglobulin (Ig) G antibody (WT-3 control). Drugs or WT-3 were administered i.v. 5 min prior to reperfusion. Although in the published studies with CP 105,696 the drug is administered orally, we reasoned this route was not justified in anesthetized animals. Therefore, the drug was given s.c. 20 min prior to reperfusion, a time of administration shown to be effective in preliminary studies and similar to that used in other studies (Showell et al., 1995).

2.3. Evaluation of changes in vascular permeability

The extravasation of Evans blue dye into the tissue was used as an index of increased vascular permeability (De Matos et al., 1999). Evans blue (20 mg/kg) was administered i.v. (1 ml/kg) via a femoral vein 2 min prior to reperfusion of the ischemic artery. At various times (see above) after reperfusion, fragments of the duodenum (10 cm) were cut open and allowed to dry in a Petri dish for 24 h at 37°C. The dry weight of the tissue was calculated and Evans blue was extracted using 3 ml of formamide (24 h at room temperature). The amount of Evans blue in the tissue was obtained by comparing the extracted absorbance with that of a standard Evans blue curve read at 620 nm in an automatic plate reader. Results are presented as the amount of Evans blue in μg per 100 mg of tissue. The mesentery was also extracted en bloc, halved and a similar extraction procedure was performed. The right ventricle was flushed with 20 ml of phosphate buffered saline to wash the intravascular Evans blue in the lungs. The left lung was then excised and used for Evans blue extraction. The right lung was used for the determination of myeloperoxidase as described below.

2.4. Myeloperoxidase levels

The extent of neutrophil accumulation in the intestine, mesentery and right lung tissue was measured by assaying myeloperoxidase activity as previously described (Ivey et al., 1995; De Matos et al., 1999). Briefly, a fragment of duodenum, half the mesentery and the flushed right lung of the animals that had undergone ischemia and reperfusion injury were removed and snap frozen in liquid nitrogen. Upon thawing, the tissue (1 g of tissue per 19 ml of buffer) was homogenized in pH 4.7 buffer (0.1 M NaCl, 0.02 M $NaPO_4$, 0.015 M NaEDTA), centrifuged at $260 \times g$ for 10 min and the pellet underwent hypotonic lysis (15 ml of 0.2% NaCl solution followed 30 s later by addition of an

equal volume of a solution containing NaCl 1.6% and glucose 5%). After a further centrifugation, the pellet was then resuspended in 0.05 M $NaPO_4$ buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide and re-homogenized. One ml aliquots of the suspension were transferred into 1.5-ml Eppendorf tubes followed by three freeze–thaw cycles using liquid nitrogen. These were then centrifuged for 15 min at $10{,}000 \times g$, the pellet was resuspended to 1 ml and samples of intestine, mesentery and lung were diluted 1:30, 1:10 and 1:50, respectively, prior to the assay. These dilutions were determined to be optimal in preliminary experiments. Myeloperoxidase activity in the resuspended pellet was assayed by measuring the change in optical density (O.D.) at 450 nm using tetramethylbenzidine (1.6 mM) and H_2O_2 (0.5 mM). Results were expressed as changes in absorbance (O.D.) of the diluted sample per 100 mg of lung tissue.

2.5. Drugs and antibody

The following drugs were obtained from Sigma (USA): urethane, DMSO, Evans blue, fucoidin, hexadecyltrimethylammonium bromide. The BLT receptor antagonist CP 105,696 was a kind gift of Pfizer (Groton, USA), LY255283 was a gift from Eli Lilly (IN, USA) and WT3 was kindly provided by Dr Paul Hellewell (University of Sheffield, UK).

2.6. Statistical analysis

Results are shown as the mean ± S.E.M. Percent inhibition was calculated by subtracting the background levels of Evans blue extravasation or myeloperoxidase (obtained in sham-operated animals) from control and treated animals. Difference was compared by using analysis of variance (ANOVA) followed by Student–Newman–Keuls post-hoc analysis. Results with a $P < 0.05$ were considered significant.

3. Results

3.1. Model of ischemia–reperfusion injury

The initial experiments were designed to establish the kinetics of the intestinal injury following ischemia of the SMA. An ischemia time of 30 min was used in all experiments and the time of reperfusion varied from 0 to 120 min. As seen in Fig. 1(A), an increase in vascular permeability, as assessed by the extravasation of Evans blue dye, was first significant and maximal at 30 min after reperfusion (Fig. 1(A)). In separate experiments, the number of neutrophils in the small intestine was estimated by assessing the levels of myeloperoxidase following various times of reperfusion. A significant level of myeloperoxidase was first detected at 30 min following reperfusion, but there were marked increases in myeloperoxidase levels from 30 to 120 min of reperfusion (Fig. 1(B)). Because the increase of vascular permeability was maximal at 30 min and significant myeloperoxidase levels were also measurable at this time point, all further experiments were carried out using this period of reperfusion.

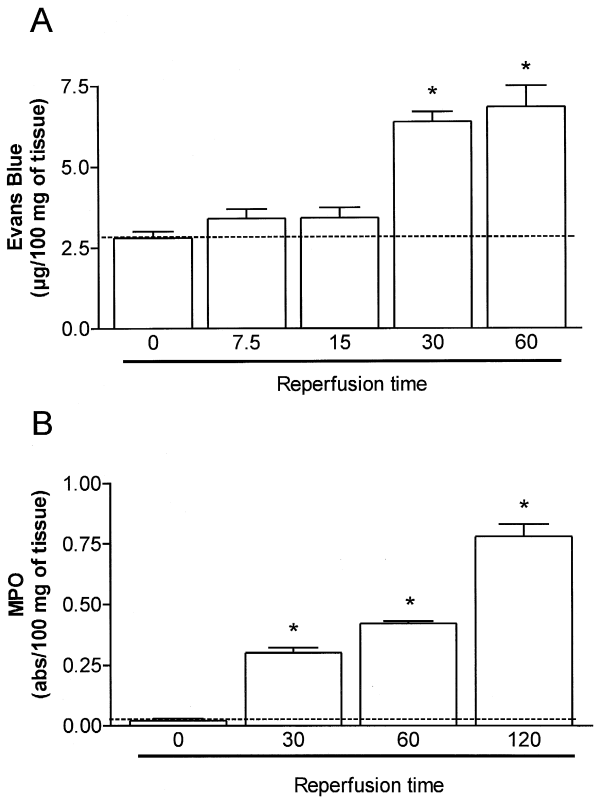

Fig. 1. Kinetics of the (A) changes in vascular permeability and (B) neutrophil accumulation in the intestine following 30 min ischemia and 30 min reperfusion of the superior mesenteric artery. Changes in vascular permeability were assessed by evaluating the extravasation of Evans blue dye. Neutrophil accumulation was assessed by evaluating the tissue levels of myeloperoxidase. The dotted lines across the graphs represent levels of Evans blue or myeloperoxidase in sham-operated animals. Results are the mean ± S.E.M. of four to five animals in each group. $^{*}P < 0.01$ when compared to animals only made ischemic (time 0).

Next, we assessed whether injury to tissues other than the small intestine could be observed after 30 min of ischemia and 30 min of reperfusion. There was a significant increase of vascular permeability (Fig. 2) and in the levels of myeloperoxidase (Fig. 3) in the intestine and mesentery, tissues perfused by the SMA, and in the lung, a remote reperfusion-induced injury. Histological analysis of the small intestine revealed local hyperemia, mild oedema of the submucosal region, scattered epithelial cell loss of villi tip and significant neutrophil accumulation in the submucosal region and in the areas of epithelial cell loss (data not shown). Lung histology showed marked neutrophilia and interstitial oedema. Of note, the neutrophils

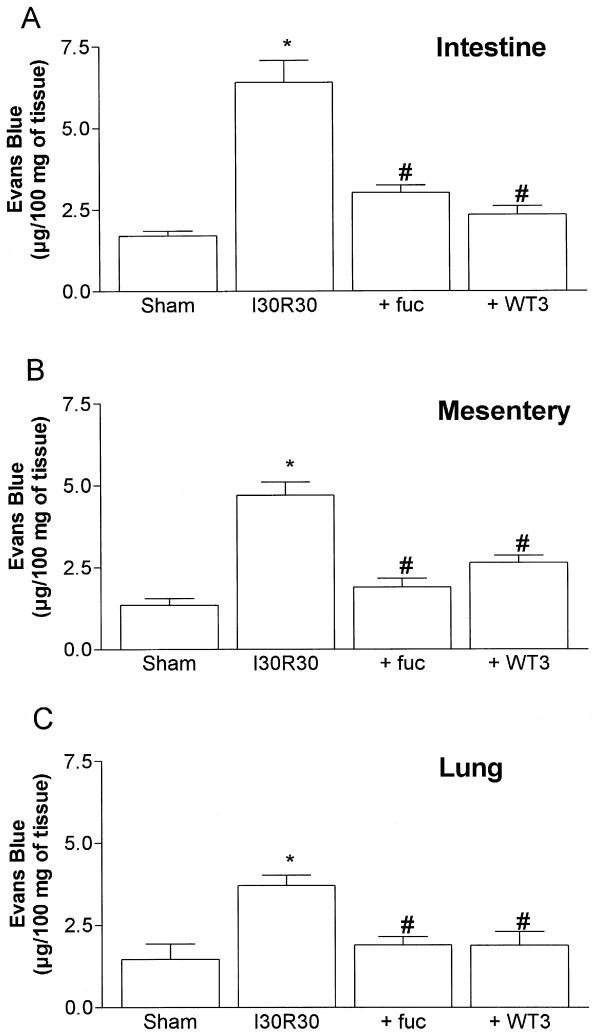

Fig. 2. Effects of the post-ischemic treatment with the selectin blocker, fucoidin, or the anti-CD18 monoclonal antibody, WT3, on the changes in vascular permeability in the (A) intestine, (B) mesentery and (C) lung following 30 min ischemia and 30 min reperfusion of the superior mesenteric artery. Changes in vascular permeability were assessed by evaluating the extravasation of Evans blue dye. Fucoidin (fuc, 10 mg/kg) or WT3 (1 mg/kg) were given i.v. 5 min prior to reperfusion. Control animals received phosphate buffered saline or mouse immunoglobulin. Results of control animals are pooled as these were not different. Results are the mean ± S.E.M. of six to seven animals in each group. $^{*}P < 0.01$ when compared to sham-operated animals and $\#P < 0.01$ when compared to reperfused animals.

were seen in the interstitial space and appeared to be intravascular, with only rare cells found in the alveolar spaces (data not shown).

3.2. Effects of strategies which block neutrophil recruitment

The effects of the post-ischemic treatment with the E- and P-selectin blocker (see Teixeira and Hellewell, 1997 and references therein), fucoidin (10 mg/kg), is shown in Fig. 2. Fucoidin inhibited the increases in vascular permeability in the intestine, mesentery and lung by 72%, 84% and 81%, respectively (Fig. 2). Similarly, fucoidin virtually reversed the accumulation of neutrophils in all tissues examined, as assessed by myeloperoxidase levels (Fig. 3).

Post-ischemic treatment with the anti-CD18 monoclonal antibody, WT3 (1 mg/kg) inhibited by 87%, 62% and 81% the increase in the extravasation of Evans blue in the intestine, mesentery and lung, respectively, following 30 min of reperfusion (Fig. 2). Similarly, myeloperoxidase levels in the intestine, mesentery and lung returned to background levels in WT3-treated animals (Fig. 3). Histo-

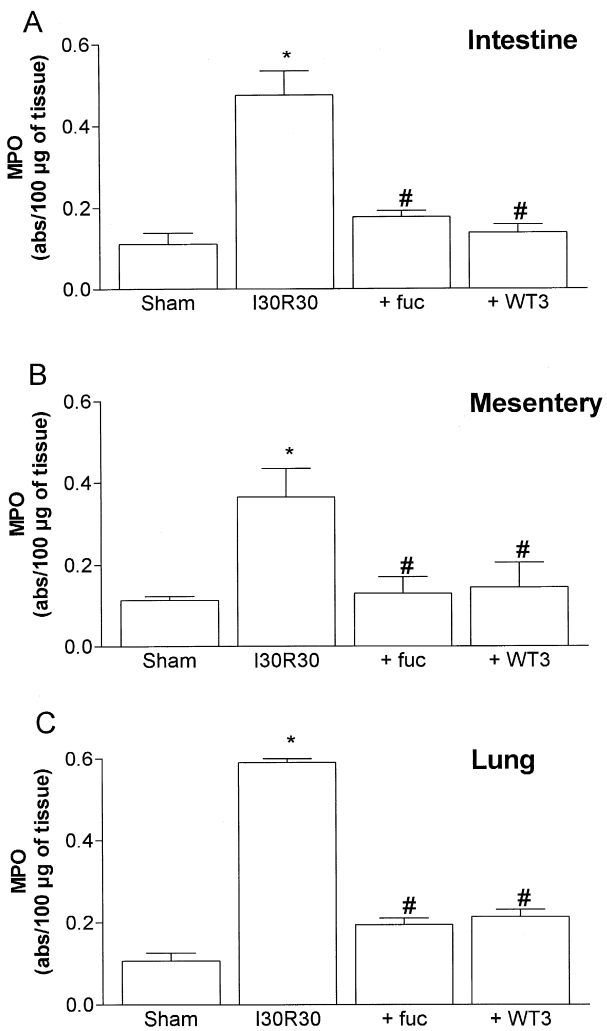

Fig. 3. Effects of the post-ischemic treatment with the selectin blocker, fucoidin, or the anti-CD18 monoclonal antibody, WT3, on the accumulation of neutrophils in the (A) intestine, (B) mesentery and (C) lung following 30 min ischemia and 30 min reperfusion of the superior mesenteric artery. Neutrophil accumulation was assessed by evaluating the tissue levels of myeloperoxidase. Fucoidin (fuc, 10 mg/kg) or WT3 (1 mg/kg) were given i.v. 5 min prior to reperfusion. Control animals received phosphate buffered saline or mouse immunoglobulin. Results of control animals are pooled, as these were not different. Results are the mean ± S.E.M. of six to seven animals in each group. $^{*}P < 0.01$ when compared to sham-operated animals and $\#P < 0.01$ when compared to reperfused animals.

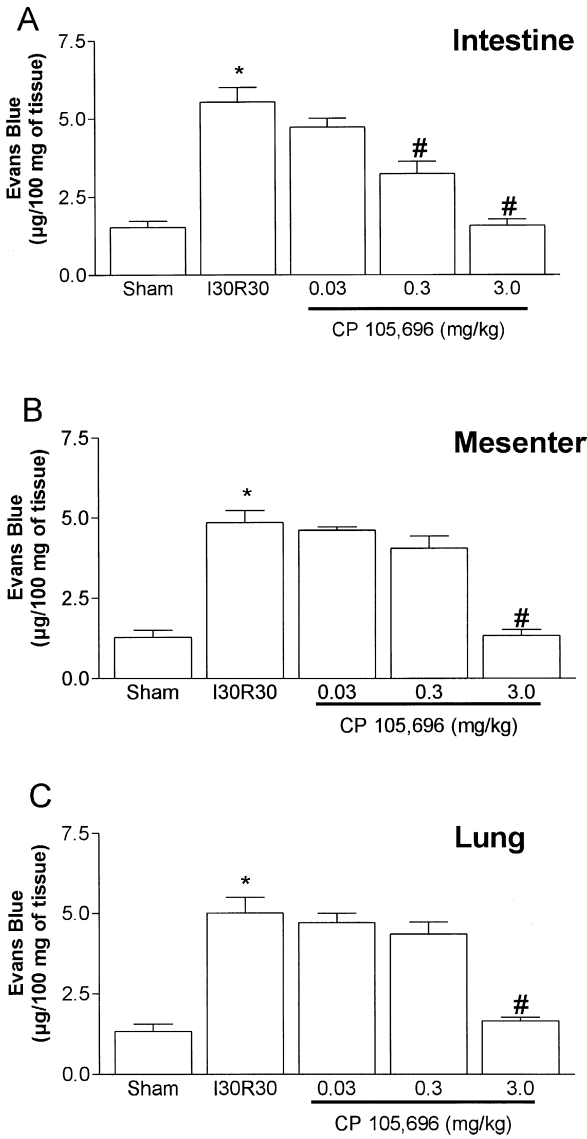

Fig. 4. Dose-dependent effects of treatment with the BLT receptor antagonist CP 105,696, on the changes in vascular permeability in the (A) intestine, (B) mesentery and (C) lung following 30 min ischemia and 30 min reperfusion of the superior mesenteric artery. Changes in vascular permeability were assessed by evaluating the extravasation of Evans blue dye. CP 105,696 (0.03–3.0 mg/kg) or vehicle (DMSO 3%) were given s.c. 20 min prior to reperfusion. Results are the mean ± S.E.M. of five to six animals in each group. $^{*}P < 0.01$ when compared to sham-operated animals and $\#P < 0.01$ when compared to reperfused animals.

logical assessment of intestine and lungs showed a virtually complete inhibition of oedema and leukocyte recruitment in WT3- and fucoidin-treated animals (data not shown).

3.3. Effects of the BLT receptor antagonist CP 105,696 on ischemia and reperfusion injury

The dose-dependent inhibitory effects of the BLT receptor antagonist CP 105,696 (0.03–3.0 mg/kg, s.c.) on the increase in vascular permeability of the intestine, mesentery and lung following ischemia and reperfusion injury of the superior mesenteric is shown in Fig. 4. Maximal inhibition occurred at the dose of 3 mg/kg (Fig. 4). In a similar manner, CP 105,696 effectively and dose-dependently inhibited neutrophil accumulation, as assessed by the levels of myeloperoxidase, in the intestine and mesentery following ischemia and reperfusion injury (Fig. 5(A) and (B). In agreement with these findings, the histological picture of the small intestine in treated animals was virtually identical to that of sham-operated animals (data not

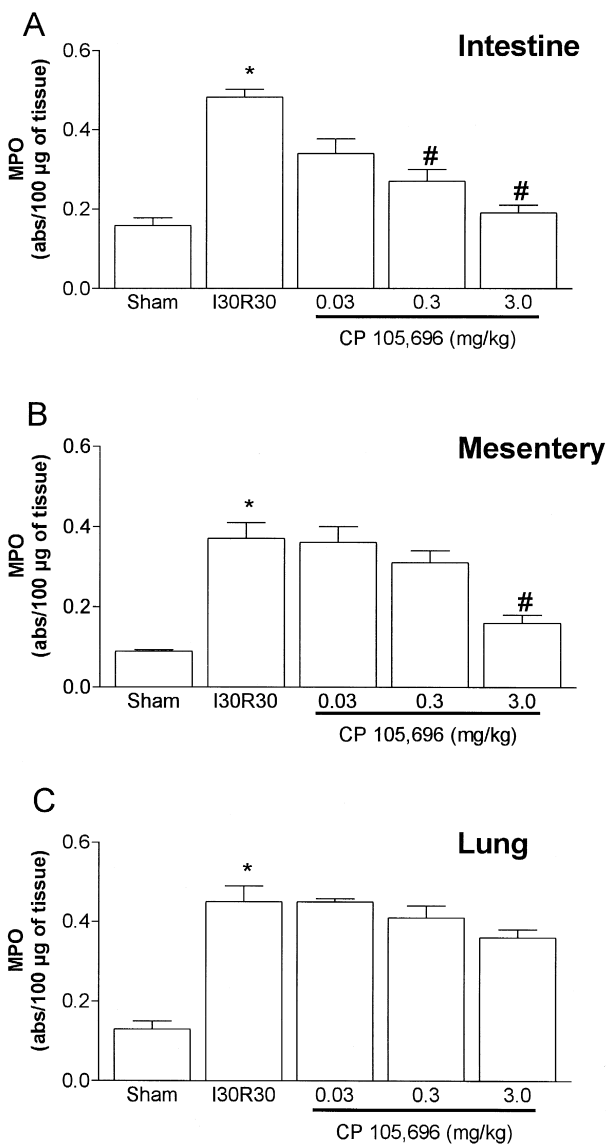

Fig. 5. Dose-dependent effects of treatment with the BLT receptor antagonist CP 105,696, on the accumulation of neutrophils in the (A) intestine, (B) mesentery and (C) lung following 30 min ischemia and 30 min reperfusion of the superior mesenteric artery. Neutrophil accumulation was assessed by evaluating the tissue levels of myeloperoxidase. CP 105,696 (0.03–3.0 mg/kg) or vehicle (DMSO 3%) were given s.c. 20 min prior to reperfusion. Results are the mean ± S.E.M. of five to six animals in each group. $^{*}P < 0.01$ when compared to sham-operated animals and $\#P < 0.01$ when compared to reperfused animals.

Table 1
Effects of the BLT receptor antagonist LY255283 on the changes in vascular permeability and neutrophil accumulation in the intestine, mesentery and lung following 30 min ischemia and 30 min reperfusion of the superior mesenteric artery

	Evans blue (μg/100 mg tissue)			Myeloperoxidase (abs/100 mg tissue)		
	Sham	Ischemia/reperfusion		Sham	Ischemia/reperfusion	
		Vehicle	LY255283		Vehicle	LY255283
Intestine	1.82 ± 0.15	5.40 ± 0.47 *	1.81 ± 0.04 * *	0.12 ± 0.02	0.49 ± 0.07 *	0.19 ± 0.01 * *
Mesentery	1.16 ± 0.11	4.24 ± 0.30 *	1.65 ± 0.23 * *	0.18 ± 0.02	0.45 ± 0.03 *	0.21 ± 0.03 * *
Lung	1.16 ± 0.18	4.28 ± 0.53 *	2.7 ± 0.16 * *	0.19 ± 0.03	0.50 ± 0.09 *	0.43 ± 0.07

Neutrophil accumulation was assessed by evaluating the tissue levels of myeloperoxidase. LY255283 (3 mg/kg) or vehicle (phosphate buffered saline) were given i.v. 5 min prior to reperfusion. Results are the mean ± S.E.M. of five animals in each group.
* $P < 0.01$ when compared to sham-operated animals.
* * $P < 0.01$ when compared to reperfused animals.

shown). In contrast, CP 105,696 had little effect on the myeloperoxidase levels in the lung of animals submitted to ischemia and reperfusion of the SMA (Fig. 5(C)). In agreement with the myeloperoxidase data, the histological assessment of lung tissue demonstrated that, in CP 105,696-treated animals, there appeared to be a small reduction of lung neutrophilia when compared to reperfused animals, but the number of neutrophils in the lung was still much greater than that seen in sham-operated animals (data not shown).

For comparison, the effects of the BLT antagonist LY255283 (3 mg/kg, i.v.) are shown in Table 1. Similarly to the effects of CP 105,696, LY255283 significantly inhibited the increase in vascular permeability in the intestine, mesentery and lung following ischemia and reperfusion of the SMA by 100%, 84% and 51%, respectively (Table 1). LY255283 also inhibited *myeloperoxidase* levels in the intestine and mesentery but not in the lung (Table 1).

4. Discussion

Reperfusion of an ischemic vascular bed is accompanied by a local inflammatory response which may cause significant injury to the affected tissue or organ. In addition, reperfusion may also induce remote organ or tissue inflammation and injury. Here, we describe a model of neutrophil-dependent ischemia and reperfusion injury and evaluate the effects of the BLT receptor antagonist CP 105,696. Injury was assessed by evaluating the increase in vascular permeability to Evans blue dye and by measuring the levels of tissue myeloperoxidase, an index of neutrophil accumulation in tissues.

Initial experiments established the kinetics of both increase in vascular permeability and neutrophil accumulation following reperfusion of the SMA made ischemic for 30 min. In agreement with the ability of neutrophils to roll and migrate into reperfused intestine (Milazzo et al., 1996; Hayward and Lefer, 1998), our results clearly demonstrate that 30 min after reperfusion there is a marked neutrophil accumulation and increased vascular permeability in the tissues perfused by the SMA. Of interest, in addition to causing local injury, 30 min reperfusion of the SMA was accompanied by accumulation of neutrophils and increased vascular permeability in the lungs, an organ remote from the site of ischemia.

Two strategies — fucoidin, a selectin blocker, and WT3, an anti-C18 monoclonal antibody — were then used to evaluate whether the accumulation of neutrophils was relevant for the increases in vascular permeability observed. Pretreatment with fucoidin, at a dose (10 mg/kg) similar to that previously shown to inhibit leukocyte rolling in the mesentery (Ley et al., 1993; Kubes et al., 1995), resulted in greatly diminished neutrophil accumulation and Evans blue extravasation. These are the first experiments to demonstrate the beneficial effects of fucoidin on mesenteric ischemia and reperfusion injury and are in agreement with the effects of fucoidin in ischemia and reperfusion in other vascular beds (e.g., Omata et al., 1997; Ritter et al., 1998; Cornejo et al., 1997). Similarly, pretreatment with the anti-CD18 monoclonal antibody WT-3 resulted in diminished reperfusion-induced neutrophil accumulation and increase in vascular permeability in the intestine and mesentery. These results are in agreement with the beneficial role of anti-CD18 in the ischemia and reperfusion injury to other vascular beds (e.g., Matsuo et al., 1994; Zhang et al., 1995; Tjara et al., 1999; Cornejo et al., 1997). Pretreatment with fucoidin or WT-3 also effectively inhibited the accumulation of neutrophils and increased vascular permeability in the lungs of reperfused animals. This is an interesting finding as other studies have reported that lung injury and migration of neutrophils to alveoli can occur despite deficiency or inhibition of all of the known selectins (Mizgerd et al., 1996). One possibility is that by inhibiting the reperfusion injury to the gut, fucoidin would inhibit the release of mediators, which would then activate circulating neutrophils and induce lung injury. We are assessing this possibility at present. Together, the data presented above argue for an essential role of selectins and CD18 integrins in mediating neutrophil accumulation in the intestine, mesentery and lung of animals undergoing ischemia and reperfusion of the SMA. In addition, our results argue for an essential role of neutrophils in mediat-

ing the increase in vascular permeability following reperfusion of the ischemic SMA.

The activation of the BLT receptor in neutrophils by leukotriene B_4 has been shown to induce the recruitment of neutrophils in vivo or their activation in vitro (e.g., Ford-Hutchinson et al., 1980; Yokomizo et al., 1997; Marleau et al., 1999). With the development of potent and effective BLT receptor antagonists, it has been possible to evaluate the role of this lipid mediator in models of various human diseases in vivo (Salmon and Garland, 1991). For example, the BLT receptor antagonist CP 105,696 has been shown to suppress disease pathology or progression in models of asthma (Turner et al., 1996), allergic encephalomyelitis (Gladue et al., 1996) and arthritis (Griffiths et al., 1995). Here, we have investigated the effects of the administration of CP 105,696 in our model of neutrophil-dependent reperfusion injury to the intestine, mesentery and lung. Treatment with CP 105,696 after the onset of ischemia dose-dependently inhibited the increase in vascular permeability in the intestine, mesentery and lung. These results clearly suggest an important role for leukotriene B_4 in mediating neutrophil-dependent local and remote ischemia and reperfusion injury. These results are in line with the ex-vivo production of leukotriene B_4 by intestinal explants of animals submitted to ischemia and reperfusion of the SMA (Stojadinovic et al., 1999). CP 105,696 also effectively inhibited the accumulation of neutrophils in the intestine and mesentery of reperfused animals suggesting that inhibition of Evans blue extravasation might be secondary to the inhibition of neutrophil influx. The ability of CP 105,696 to inhibit neutrophil influx to sites of ischemia and reperfusion is in agreement with previous experiments with BLT receptor-transfected Chinese hamster ovary cells which demonstrated a dominant role for leukotriene B_4 in mediating migration of transfected cells in reperfusion injury of the kidney (Noiri et al., 2000). However, CP 105,696 had no significant effect on the accumulation of neutrophils in the lungs of reperfused animals. This is in contrast to a possible role for a 5-lipoxygenase-derived product in mediating neutrophil recruitment in the lung of mice following hind limb ischemia and reperfusion (Chiang et al., 1999). Thus, at least in the lung, CP 105,696 blocks neutrophil-dependent injury without blocking neutrophil accumulation suggesting that activation of BLT receptors may be more important for the activation of neutrophils in the lungs. We are presently investigating whether other mediators (e.g., platelet-activating factor (PAF) or C5a) may then be responsible for the accumulation of neutrophils in this organ.

Using a more severe model of ischemia and reperfusion of the gut (90 min ischemia and up to 120 min of reperfusion of the splanchnic artery), Karasawa et al. (1991) demonstrated that pre-treatment with the BLT receptor antagonist LY255283 significantly inhibited mortality, ileal accumulation of neutrophils and mesenteric artery endothelial dysfunction. However, these authors did not evaluate changes in vascular permeability, nor did they evaluate injury to a remote site, the lung. For comparison, we performed a series of studies with LY255283, a BLT receptor antagonist structurally distinct from CP 105,696. The results were virtually identical to those obtained with CP 105,696. Thus, LY255283 inhibited neutrophil-dependent ischemia and reperfusion injury but failed to block neutrophil accumulation in the lungs of reperfused animals.

Here, we describe the inhibitory effects of the novel BLT receptor antagonist, CP 105,696, on the neutrophil-dependent reperfusion injuries following transient ischemia of the SMA. Overall, our results suggest that BLT receptor antagonists may inhibit local and remote ischemia and reperfusion injury by blocking both the accumulation and/or activation of neutrophils.

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References

Cornejo, C.J., Winn, R.K., Marlan, J.M., 1997. Anti-adhesion therapy. Adv. Pharmacol 39, 99–142.

Chiang, N., Gronert, K., Clish, C.B., O'Brien, J.A., Freeman, M.W., Serhan, C.N., 1999. Leukotriene B_4 receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. J. Clin. Invest. 104, 309–316.

Crooks, S.W., Stockley, R.A., 1998. Leukotriene B_4. Int. J. Biochem. Cell Biol. 30, 173–178.

de Matos, I.M., Souza, D.G., Seabra, D.G., Freire-Maia, L., Teixeira, M.M., 1999. Effects of tachykinin NK1- or PAF-receptor blockade on the lung injury induced by scorpion venom. Eur. J. Pharmacol. 376, 293–300.

Ford-Hutchinson, A.W., Bray, M.A., Doig, M.V., Shipley, M.E., Smith, M.J., 1980. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 286, 264–265.

Gladue, R.P., Carroll, L.A., Milici, A.J., Scampoli, D.N., Stukenbrok, H.A., Pettipher, E.R., Salter, E.D., Contillo, L., Showell, H.J., 1996. Inhibition of leukotriene B_4-receptor interaction suppresses eosinophil infiltration and disease pathology in a murine model of experimental allergic encehalomyelitis. J. Exp. Med. 183, 1893–1898.

Griffiths, R.J., Pettipher, E.R., Koch, K., Farrell, C.A., Breslow, R., Conklyn, M.J., Smith, M.A., Hackman, B.C., Wimberly, D.J., Milici, A.J., Scampoli, D.N., Cheng, J.B., Pillar, J.S., Pazoles, C.J., Doherty, N.S., Melvin, L.S., Reiter, L.A., Biggars, M.S., Falkner, F.C., Mitchell, D.Y., Liston, T.E., Showell, H.J., 1995. Leukotriene B_4 plays a critical role in the progression of collagen-induced arthritis. Proc. Natl. Acad. Sci. U. S. A. 92, 517–521.

Hayward, R., Lefer, A.M., 1998. Time course of endothelial–neutrophil interaction in splanchinic artery ischemia–reperfusion. Am. J. Physiol. 275, H2080–H2086.

Ivey, C.L., Williams, F.M., Collins, P.D., Jose, P.J., Williams, T.J., 1995. Neutrophil chemoattractants generated in two phases during reperfusion of ischemic myocardium in the rabbit: evidence for a role for C5a and interleukin-8. J. Clin. Invest. 95, 2720–2728.

Karasawa, A., Guo, J.P., Ma, X.L., Tsao, P.S., Lefer, A.M., 1991. Protective actions of a leukotriene B_4 antagonist in splanchnic ischemia and reperfusion in rats. Am. J. Physiol. 261, G191–G198.

Kubes, P., Jutila, M., Payne, D., 1995. Therapeutic potential of inhibiting leukocyte rolling in ischemia/reperfusion. J. Clin. Invest. 95, 2510–2519.

Lefer, A.M., Lefer, D.J., 1996. The role of nitric oxide and cell adhesion molecules on the microcirculation in ischaemia–reperfusion. Cardiovasc. Res. 32, 743–751.

Ley, K., Linnemann, G., Meinen, M., Stoolman, L.M., Gaehtgens, P., 1993. Fucoidin, but not yeast polyphosphomannan PPME, inhibits leukocyte rolling in venules of the rat mesentery. Blood 81, 177–185.

Marleau, S., Laclos, B.F., Sanchez, A.B., Poubelle, P.E., Borgeat, P., 1999. Role of 5-lipoxygenase products in the local accumulation of neutrophils in dermal inflammation in the rat. J. Immunol. 163, 3349–3458.

Matsuo, Y., Onodela, H., Shiga, Y., Shozuhara, H., Ninomiya, M., Kihara, T., Tamatani, T., Miyasaki, M., Kogure, K., 1994. Role of cell adhesion molecules in brain injury after transient middle cerebral artery occlusion in the rat. Brain Res. 656, 344–352.

Milazzo, V.J., Ferrante, R.J., Sabido, F., Silva, M.B. Jr, Hobson, R.W. II, Duran, W.N., 1996. Time course of leukocyte adhesion to endothelium in ischemia–reperfusion. J. Surg. Res. 61, 139–142.

Mizgerd, J.P., Meek, B.B., Kutkoski, G.J., Bullard, D.C., Beaudet, A.L., Doerschuk, C.M., 1996. Selectins and neutrophil traffic: margination and *Streptococcus pneumoniae*-induced emigration in murine lungs. J. Exp. Med. 184, 639–645.

Noiri, E., Yokomizo, T., Nakao, A., Izumi, T., Fujita, T., Kimura, S., Shimizu, T., 2000. An in vivo approach showing the chemotactic activity of leukotriene B_4 in acute renal ischemic-reperfusion injury. Proc. Natl. Acad. Sci. U. S. A. 97, 823–828.

Omata, M., Matsui, N., Inomata, N., Ohno, T., 1997. Protective effects of polysaccharide fucoidan on myocardial ischemia–reperfusion injury in rats. J. Cardiovasc. Pharmacol. 30, 717–724.

Ritter, L.S., Copeland, J.S., McDonagh, P.F., 1998. Fucoidin reduces coronary microvascular leukocyte accumulation early in reperfusion. Ann. Thorac. Surg. 66, 2063–2071.

Salmon, J.A., Garland, L.G., 1991. Leukotriene antagonists and inhibitors of leukotriene biosynthesis as potent therapeutic agents. Prog. Drug Res. 37, 9–90.

Showell, H.J., Pettipher, E.R., Cheng, J.B., Breslow, R., Conklyn, M.J., Farrell, C.A., Hingorani, G.P., Salter, E.D., Hackman, B.C., Wimberly, D.J., Doherty, N.S., Melvin, L.S., Reiter, L.A., Biggers, M.S., Koch, K., 1995. The in vitro and in vivo pharmacologic activity of the potent and selective leukotriene B_4 receptor antagonist CP-105696. J. Pharmacol. Exp. Ther. 273, 176–184.

Springer, T.A., 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76, 301–314.

Stojadinovic, A., Smallridge, R., Nath, J., Ding, X., Shea-Donohue, T., 1999. Anti-inflammatory effects of U74389F in a rat model of intestinal ischemia/reperfusion injury. Crit. Care Med. 27, 764–770.

Teixeira, M.M., Hellewell, P.G., 1997. The selectin binding polysaccharide fucoidin inhibits eosinophil recruitment in vivo. Br. J. Pharmacol. 120, 1059–1066.

Tjara, L.C., Martin, X., Margonari, J., Blanc-Blunat, N., Ishibashi, M., Vivier, G., Panaye, G., Steghens, J.P., Kawashima, M., Treille-Ritouet, D., Dubernard, J.M., Revillard, J.P., 1999. In vivo effects of monoclonal antibodies against rat beta(2) integrins on kidney ischemia–reperfusion injury. J. Surg. Res. 87, 32–38.

Yokomizo, T., Izumi, T., Chang, K., Takuwa, Y., Shimizu, T., 1997. A G-protein-coupled receptor for leukotriene B_4 that mediates chemotaxis. Nature 387, 620–624.

Willerson, J.T., 1997. Pharmacologic approaches to reperfusion injury. Adv. Pharmacol. 39, 291–312.

Zhang, Z.G., Chopp, M., Tang, W.X., Jiang, N., Zhanag, R.L., 1995. Postichemic treatment (2–4 h) with anti-CD11b and anti CD18 monoclonal antibodies are neuroprotective after transient (2 h) focal cerebral ischemia in the rat. Brain Res. 698, 79–85.