Anti-Selectin Therapy Against Hepatic Ischemia-Reperfusion Injury

Kubes P, Payne D, Woodman RC. Molecular mechanisms of leukocyte recruitment in postischemic liver microcirculation. Am J Physiol 2002;283:G139-G147. Reprinted with permission.

Abstract

Evidence shows that leukocyte recruitment into inflamed liver sinusoids does not require selectins, with one notable exception: ischemia-reperfusion (I/R). We used intravital microscopy to directly visualize the liver microcirculation during I/R and localized endotoxemia (liver superfused with lipopolysaccharide). General anti-selectin therapy (fucoidan) or anti-adhesion therapy with an antithrombin inhibitor (hirudin) was also used. Many neutrophils rolled and adhered in postsinusoidal vessels and sequestered in the sinusoids during I/R and local endotoxin superfusion. Although fucoidan blocked rolling in both forms of inflammation, leukocyte recruitment into sinusoids was only blocked in I/R. Adhesion was also inhibited in postischemic sinusoids with a second anti-adhesive agent (hirudin). Because liver I/R inevitably induces ischemia upstream in the intestine, anti-selectin therapy may prevent intestinal injury, which could prevent downstream liver inflammation. To test this hypothesis, we completely removed the intestine and rerouted blood flow from the superior mesenteric artery to the superior mesenteric vein. I/R was induced in the liver microcirculation, and many leukocytes rolled and adhered in postsinusoidal venules and adhered in sinusoids. Although fucoidan significantly reduced the rolling in postsinusoidal vessels, adhesion persisted in the sinusoids. Our data suggest that anti-adhesion therapy is effective in liver I/R in the sinusoids and postsinusoidal venules, perhaps in part due to its beneficial effect on the intestine.

Comments

An acute inflammatory reaction is characterized by the accumulation of leukocytes, in particular neutrophils, at

the site of inflammation. During the last decade, many basic aspects of an inflammatory response were investigated. In general, the process requires first that circulating neutrophils are slowed down within venules (rolling phenomenon) to expose them to proinflammatory mediators for activation. The second step is the firm adherence of neutrophils to vascular endothelial cells. The third step is the extravasation followed by migration towards the site of inflammation, and lastly the adherence to the target. Each of these steps, which take place in postcapillary venules, involve certain families of adhesion molecules. The initial rolling of neutrophils along the vessel wall is mediated by members of the selectin family and their ligands.[1,2]

The selectin family consists of three members, endothelial (E)-, platelet (P)-, and leukocyte (L)-selectin. Despite the name, selectin molecules are expressed on multiple cell types; *e.g.*, P-selectin is expressed on platelets and on endothelial cells. Structural characteristics of members of the selectin family include a lectin domain at the NH2-terminus, which is critical for the binding properties. In addition, all selectins have an EGF-like domain and variable numbers of consensus repeat domains.[1] The protein is anchored in the plasma membrane by a transmembrane domain, which is followed by a cytoplasmic tail. Therefore, selectins do not only function as adhesion molecules but can also act as signal transducer for cell activation.[3] Because of their essential role in the initial step of neutrophil migration to an inflammatory site, blocking selectins results in reduced numbers of rolling neutrophils, which prevents firm adhesion, transmigration, and ultimately drastically reduces the number of neutrophils at the site of inflammation. If neutrophils are involved in aggravating a tissue injury during an inflammatory response, blocking of selectins will result in a protective effect. Reduced organ injury with anti-selectin therapy has been shown in a number of experimental models.[1,2]

Although E- and P-selectin can be transcriptionally up-regulated and expressed on hepatic endothelial cells,[4] the inflammatory process in the liver is more complicated than in other organs because neutrophils can adhere in sinusoids and in postsinusoidal venules.[5-7] Whereas selectins are involved in neutrophil rolling in postsinusoidal venules,[4] neutrophils do not roll in sinusoids[6,8] and, therefore, selectins are not necessary for neutrophil accumulation in this vascular bed.[6,9,10] On the other hand, most neutrophils, which are involved in the injury process, appear to transmigrate from sinusoids and not from postsinusoidal venules.[7] Thus, anti-selectin therapy should have no beneficial effect in models of liver inflammation. In striking contrast to this assumption, beginning with the first report in 1995,[11] there is a growing number of manuscripts from different groups, which report reduced neutrophil accumulation in the liver and hepatoprotection with therapeutic strategies directed against selectins or their ligands. Mechanistally, these results make no sense unless some of the earlier conclusions are wrong (*e.g.,* neutrophils actually transmigrate from postsinusoidal venules) or some facts had been overlooked in the interpretation of previous results. The recent paper by Kubes et al.[12] provides an interesting explanation for the controversy surrounding the role of selectins in liver inflammation. As a basis for the investigation, the investigators recognized that virtually all reported protective effects with anti-selectin therapy involve experimental models of ischemia-reperfusion injury.[12] To investigate this hypothesis in more detail, the investigators occluded the superior mesenteric artery of cats for 1 hour and studied the leukocyte behavior in sinusoids and postsinusoidal venules with intravital microscopy during 2 hours of reperfusion. Mesenteric artery occlusion causes ischemia in both the intestine and the liver. As therapeutic intervention, the investigators used fucoidan, a general selectin inhibitor. In the first experiments, the investigators showed that mesenteric artery occlusion results in leukocyte rolling and adhesion in postsinuoidal venules and causes accumulation of leukocytes in sinusoids. Consistent with previous results in P-selectin–deficient mice,[13] fucoidan substantially reduced rolling and adherence of leukocytes in venules and attenuated their accumulation in sinusoids. In additional experiments, the thrombin inhibitor hirudin had no effect on leukocyte rolling but reduced adhesion in venules and leukocyte accumulation in sinusoids. On the other hand, fucoidan only reduced leukocyte rolling in postsinusoidal venules after endotoxin superfusion. No effect on venular adhesion or sinusoidal leukocytes accumulation was observed. These data are again in agreement with earlier results in liver inflammation models.[5,9] Kubes et al. then proceeded to extirpate the intestine before ischemia. In this model, mesenteric artery occlusion selectively induced ischemia in the liver. Under these conditions, the overall number of rolling leukocytes in postsinusoidal venules was less compared with experiments in animals, which had an intact intestine. Whereas fucoidan again eliminated the rolling in postsinusoidal venules, it did not affect the firm adherence of leukocytes in venules or their accumulation in sinusoids. Together these experiments support several important conclusions. First, in contrast to other vascular beds, selectin-mediated leukocyte rolling does not appear to be important for the firm adhesion in postsinusoidal venules. Recent data from Fox-Robichaud and Kubes suggest that the integrin $\alpha 4\beta 1$ may be responsible for this

process.[14] A second, more important conclusion is that all reported beneficial effects of anti-selectin therapy in models of hepatic ischemia-reperfusion injury are most likely indirect effects due to reduced intestinal injury. These results also indicate that intestinal injury may be a critical factor affecting the extent of liver injury in most experimental models and clinical situations of liver ischemia, *e.g.,* hemorrhagic shock, liver transplantation, and the cross-clamping of portal vein and hepatic artery (Pringle maneuver). Last but not least, these data suggest that therapeutic intervention strategies and mechanistic conclusions obtained in other organs should not be blindly applied to the liver. This organ is unique in many aspects including the microcirculation and the inflammatory response. Significant progress in these areas requires unbiased approaches and sound mechanistic investigations.

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