FUCOIDAN IMPROVES THE RENAL BLOOD FLOW IN THE EARLY STAGE OF RENAL ISCHEMIA/REPERFUSION INJURY IN THE RAT

It has been shown that monoclonal anti-P-selectin antibody administration protects renal function in an ischemic model of acute renal failure. This study was designed to evaluate the effect of administration of fucoidan, P-selectin inhibitor, on reduction in renal blood flow induced by ischemia/reperfusion injury in the rat. Experiments were performed on male Wistar rats weighing 350—400 g. The systemic blood pressure (mm Hg) (BP) and renal blood flow (RBF) were monitored continuously and renal vascular resistance (RVR) was calculated. After 20 min period of stabilization animals (6 rats in each group) received one of the following agents administered by continuous i.v. infusion during 165 min: 1 mg/kg of body weight of fucoidan (F1), 10 mg/kg of fucoidan (F10), 100 mg/kg of fucoidan (F100), 10 mg/kg of heparin (H), or 0.9% NaCl solution (control). After 15 min of drug administration the renal vessels of the both kidney were occluded with vascular clamps for 60 min. There were no significant changes in the initial values of RBF, RVR and BP between groups. None procedure affected significantly BP during all experiments. In F10 RBF returned to the initial values in 70th min of reperfusion and did not change up to 90th min. This value was significantly higher than respective value in the control group. In F1 group RBF in 90th min was also higher than in the control group, but it was not statistically significant. The dose of heparine and fucoidan used in the H and F100 groups failed to preserve RBF during reperfusion. In the present study we found that administration of fucoidan — P-selectin inhibitor, increases significantly postischemic renal blood flow and may have renoprotective activity.

Key words: ischemia/reperfusion injury, p-selectin, fucoidan, rat, kidney.

INTRODUCTION

There is increasing evidence that neutrophils play a key role in the development of organ injury induced by ischemia and reperfusion (I/R). Mechanisms of neutrophil-mediated tissue damage include release of oxygen-derived free radicals, activation of proteases and increased synthesis of cyclooxygenase products (1, 2). Neutrophil recruitment from blood to extravascular sites of inflammation depends on interaction between adhesion molecules — integrins, immunoglobulin superfamily members, selectins and selectin-ligands (3). It has been shown that blocking of some adhesion molecules like ICAM-1 (4), β2-integrins — (5) or selectins (6, 7, 8)
significantly reduce tissue damage after ischemia/reperfusion. Inhibition of interaction of P-selectin reduces I/R-induced injury of the heart (6, 7), brain (9), and the liver (10). In an ischemic model of acute renal failure monoclonal anti-P-selectin antibody administration also improves renal function (11).

Selectins are single-chain transmembrane glycoproteins containing an N-terminal lectin domain, an epidermal growth factor domain, complement regulatory repeats and short cytoplasmatic domains. Three kinds of them have been identified and characterized: L-, P-, and E-selectins (3). P-selectins are expressed on activated platelets and also by endothelium within seconds of stimulation by proinflammatory mediators like oxygen radicals, thrombin, complement components, histamine, (1, 3) as well as during ischemia/reperfusion injury (12). Selectins are involved in an initial step of neutrophils transmigration — tethering and rolling of free-flowing leukocytes to endothelial cells. During rolling along the endothelium, neutrophils become activated, and their firm adhesion and transmigration depends on interaction with integrins (13).

One of the mechanisms involved in the postischemic acute renal failure (ARF) is an increase in renal vascular resistance during reperfusion. Renal vascular vasoconstriction results in decrease in renal blood flow (RBF), glomerular filtration rate and rate of sodium excretion followed by a loss of RBF autoregulation during later period. It was found that neutrophil adherence to the endothelium induce vasoconstriction (14) and fucoidan, P-selectin inhibitor, reduces vasoconstrictive response at the site of arterial injury in pigs (15).

This study was design to evaluate the effect of administration of fucoidan, P-selectin inhibitor, on reduction in renal blood flow induced by ischemia/reperfusion injury in the rat.

MATERIALS AND METHODS

The study protocols were approved by the Local Ethical Committee. Experiments were performed on 30 male Wistar rats weighing 350—400 g obtained from Animal House of the Medical University of Warsaw (Poland). Animals were divided into 5 groups assigned as: F_1, F_10, F_100. C. H. Anaesthesia was induced by an intraperitoneal injection of 1 ml per 100 g body wt of chloralum hydratum (3.6%) and maintained by repeated injections of 0.25 ml per 100 g body wt of the same solution every 40 min. The arterial blood pressure (mm Hg) (BP) was monitored continuously via a catheter inserted into the femoral artery. All intravenous infusions were given via catheter placed in the femoral vein. Both kidneys were exposed via midline incision and renal arteries and veins were stripped. Probe of ultrasonic Doppler flowmeter (Transonic, Itaca, USA) was placed on the left renal artery for renal blood flow measurement. After 20-min period of stabilisation the following total dose of fucoidan (Sigma GmbH) was administered by continuous i.v. infusion during 165 min: F_1 — 1 mg/kg, F_10 — 10 mg/kg, F_100 — 100 mg/kg. In the heparin group (H) the total dose of heparin 10 mg/kg was infused over a period of 165 min. In the control group (C) 0.9% NaCl solution was administered. The rate of drug administration was 0.5 ml solution per 100 g per body wt per hour. After 15 min of drug administration the renal vessels of the both kidney were occluded with little vascular clamps for 60 min. The whole time of experiment (excluding the stabilisation period) lasted 165 min (including 15 min of preconditioning, 60 min of
ischemia and 90 min of reperfusion). At the conclusion of the experiment kidneys were weighted and RBF values were expressed per 1g of the kidney mass (ml/min g). Renal vascular resistance (RVR) was calculated from BP and RBF values (RVR = BP/RBF) and expressed as renal vascular resistance units (RRU).

Data are presented as mean ± SE. The initial values and the values in 90th min of reperfusion of RBF, RVR and BP were evaluated by one-way analysis of variance and Dunnett's test was used for comparison between all experimental groups and the control. The Student t-test was used to compare data between the initial values and the values in 90th min of reperfusion in each of groups. The significance level was set at P < 0.05.

RESULTS

There were no significant changes in the initial values of RBF, RVR and BP between groups. Blood pressure was not affected thorough the all experimental period. During first 3 minutes after releasing the clamp a drop in RBF was observed in some of groups studied (C, H and F100). Then RBF started to increase steadily, reaching a plateau after 15 minutes in all of groups except group F10. In F10 RBF returned to the initial values in 70th min of reperfusion and did not change up to 90th min. Its value was significantly higher than respective value in the control group. In F1 group RBF in 90th min was also higher than in the control group, but it was not statistically significant (Fig. 1). The dose of heparin and fucoidan used in the H and F100 groups respectively failed to preserve RBF during the reperfusion period. Data in details are presented in the Table 1.

![Image of Renal blood flow (RBF) after 15-minutes of ischemia in rats treated with fucoidan 1 mg/kg b.w. (F1 group) (■), fucoidan 10 mg/kg b.w. (F10 group) (▲), fucoidan 100 mg/kg b.w. (F100 group) (×), heparin 10 mg/kg b.w. (H group) (•) and 0.9% NaCl (C group) (○).](image-url)
Table 1. The values of renal blood flow (RBF), renal vascular resistance (RVR) and blood pressure (BP) in the initial period and in the 90th min of reperfusion in all groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Renal Blood Flow (ml/min/g)</th>
<th>Renal Vascular Resistance (RRU)</th>
<th>Mean Blood Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.047 ± 0.658</td>
<td>38.838 ± 9.056</td>
<td>72.6 ± 11.73</td>
</tr>
<tr>
<td>Initial values</td>
<td>1.099 ± 0.526(^{b})</td>
<td>79.583 ± 24.144(^{b})</td>
<td>73.8 ± 13.75</td>
</tr>
<tr>
<td>90th min of reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F(_{1})</td>
<td>1.619 ± 0.400</td>
<td>50.081 ± 10.966</td>
<td>77.6 ± 3.36</td>
</tr>
<tr>
<td>Initial values</td>
<td>1.118 ± 0.156(^{b})</td>
<td>67.019 ± 12.461(^{b})</td>
<td>77.8 ± 6.31</td>
</tr>
<tr>
<td>90th min of reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F(_{10})</td>
<td>1.787 ± 0.439</td>
<td>36.481 ± 18.825</td>
<td>78.1 ± 8.03</td>
</tr>
<tr>
<td>Initial values</td>
<td>1.695 ± 0.575(^{a})</td>
<td>42.645 ± 25.929(^{a})</td>
<td>84.8 ± 7.43</td>
</tr>
<tr>
<td>90th min of reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F(_{100})</td>
<td>1.580 ± 0.211</td>
<td>56.729 ± 13.036</td>
<td>76.2 ± 6.76</td>
</tr>
<tr>
<td>Initial values</td>
<td>0.763 ± 0.132(^{b})</td>
<td>99.976 ± 14.333(^{b})</td>
<td>75.1 ± 8.48</td>
</tr>
<tr>
<td>90th min of reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>1.735 ± 0.327</td>
<td>48.609 ± 3.286</td>
<td>83.2 ± 11.88</td>
</tr>
<tr>
<td>Initial values</td>
<td>0.926 ± 0.208(^{b})</td>
<td>80.346 ± 15.436(^{a})</td>
<td>71.4 ± 6.46</td>
</tr>
<tr>
<td>90th min of reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.

\(^{a}\)p < 0.05 as compared to the control (C) group, \(^{b}\)p < 0.05 as compared to the initial values of the same group.

DISCUSSION

The main finding of the present study is that preischemic administration of P-selectin inhibitor, fucoidan, increase the blood flow in the first hour of reperfusion as compared to the control receiving saline. Interestingly, high dose of fucoidan failed to preserve RBF during reperfusion.

The protective effect on renal blood flow occurs in the first minutes after onset of the reperfusion period.

It has been shown that fucoidan, a sulphated polysaccharide obtained from marine algi, selectively blocks P-selectin of the surface of different cell types. P-selectin is only one of the endothelial selectins which is also constitutively expressed. In the quiescent state, P-selectin molecules are found in the alpha granules of platelets and in Weibel-Palade bodies of endothelial cells. Upon stimulation, these structures fuse with the cell membrane causing release of their contents and expose P-selectin to the luminal surface (16). P-selectin appears on the surface of EC between seconds and 30 min after stimulation (3, 12, 17). The late expression of P-selectin (11) is probably transcriptionally induced by cytokines such as TNF-alpha or interleukin-1 beta, derived from activated neutrophils. In the present study, administration of fucoidan
preceded ischemic period and was continued during ischemia and reperfusion thus it may affect all mechanism inducing P-selectin expression on cell surface, and protect them from neutrophil activation.

After ischemia-reperfusion P-selectin molecules were detected in glomeruli, both on vessels endothelial cells and platelets (11). At least three mechanisms are involved in the posts ischemic increased in renal vascular resistance. First, activated neutrophils are more adherent and, along with aggregated platelets and erythrocytes can impair or obstruct blood flow in small vessels (so called no-reflow phenomenon). Second, activated neutrophils are the source of vasoconstrictive mediators like phospholipase products (18, 19) which potentiate vascular obstruction.

Third, activated neutrophils release agents (oxygen reactive species, proteinases) which aggravate injury to endothelial cells already subjected to anoxia and reoxygenation. Endothelial damage results in a decreased vasodilatory response to hypoxia and acetylcholine, which may result in further decrease in blood flow (20). It was found that after reperfusion neutrophils and platelets act in cooperative manner in tissue damage (21). It was speculated that this intercellular interactions are caused by P-selectin up-regulation which is observed on platelets and endothelial cells after reperfusion. Blockade of the selectin family diminishes this synergism between platelet and neutrophils (21).

Fibrin deposition in the peritubular capillaries and vasa recta may play a role in I/R — induced ARF, and that heparinization prevented the morphologically demonstrable deposition (22). The protective effect of heparin is a result of its anticoagulant properties (23). One can speculate that protective effect of fucoidan is concerned only with its anticoagulation properties (24). In the present study, to elucidate this hypothesis, heparin in the standard anticoagulant dose was used in one of the groups (H). In the H group RBF in the posts ischemic period was greater (though not significantly) than in the control group, but still lower than in the fucoidan groups. As anticoagulant properties of fucoidan, at the doses used, are not significant, the above data suggest that these properties are not responsible for protective effect of fucoidan administration.

Moreover, anticoagulant and fibrinolytic properties of fucoidan are limited only to low concentration range (24). At higher plasma concentration fucoidan promotes clot formation. In addition, Durig et al. (25) found that high molecular weight fraction of low sulphated fucoidan induced irreversible platelet aggregation in dose dependent manner. These effects may be responsible for fail in protection of renal blood flow in the F_{100} group as compared to the F_{10}.

One can hypothesised that increased blood flow in the reperfusion may result in tissue damage due to rapid reoxygenation, changes in pH or
intracellular ion transport. However this process named reperfusion injury—or re-flow paradox is associated with activation of neutrophils (1) and action of theirs degranulation products and generation of oxygen free-radicals. Inhibition of P-selectin may protect against neutrophil activation and risk of increased blood flow through ischemic tissue.

Singbartl et al. (11) showed a clear role for P-selectin in ischemia/reperfusion — induced ARF. Blocking P-selectin by specific antibody reduces neutrophil infiltration into postischemic kidneys and protects from the development of renal failure. In the present study we found that administration of fucoidan, P-selectin inhibitor increased significantly postischemic renal blood flow. We hypothesised, that this is the first stage of protective effect of blocking P-selectin in I/R renal injury.

REFERENCES


Received: November 11, 2000
Accepted: January 10, 2001

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