Attenuating effect of Myakuryu on mesenteric microcirculatory disorders induced by ischemia and reperfusion

Jing-Yan Han a,b,* Yoshinori Horie a, Dan Li b, Yasutada Akiba a, Hiroshi Nagata a, Soichiro Miura c, Masaya Oda d, Hiromasa Ishii a and Toshifumi Hibi a

Abstract. Myakuryu (MR) is a newly developed herbal medicine composing Crataegue oinnatifida bge (COB), Panax notoginseng (PN) and Ginkyo biloba (GB). To examine the effectiveness of MR, we investigated its effects on rat mesenteric microcirculatory injury induced by ischemia/reperfusion (I/R). The mesenteric microcirculation of ileocecal portion of a male Wistar rat was observed through an inverted-type intravital microscope assisted with a charge-coupled devise (CCD) camera. Mesenteric I/R was conducted by a ligation of the mesenteric artery and vein (10 min) and subsequent release of the occlusion. We measured venular diameter, the number of adherent leukocytes, dihydrorhodamine 123 (DHR) fluorescence as an indicator of oxidative stress and mast cell degranulation, with or without MR extract (0.4 g/kg b.w.) via an orogastric tube 1hr before I/R. The diameters of the observed mesenteric venules were not changed after the mesenteric I/R. MR had no effect on venular diameter. The leukocytes adhering to the post-capillary venular walls started just after reperfusion, and increased thereafter. The increased number of adherent leukocytes was significantly reduced by treatment with MR. DHR fluorescence ratio was significantly increased along the venular wall. MR attenuated the increased oxidation. The mesenteric I/R induced mast cell degranulation. The increase in mast cell degranulation was inhibited by MR. In conclusion, oral administration of MR attenuates I/R-induced microvascular damages in rat mesentery. MR has a therapeutic potential for prevention of I/R-related microvascular injury.

Keywords: Chinese medicine, oxygen radicals, leukocyte adherence, mast cell degranulation, ischemia–reperfusion

1. Introduction

Myakuryu (MR) is a new developed herbal medicine composing Crataegue oinnatifida bge (COB), Panax notoginseng (PN) and Ginkyo biloba (GB) [1]. Each of these components has individual effects. COB has been clinically used in China for hypercholesteremia. It has been reported to protect oxidative stress [2], but there is no report about effects of COB on the microcirculation. PN is the roots of the Chinese traditional herb Panax notoginseng (Burk) F.H. Chen. In our previous study [3], cardiotonic pills (CP), including PN as a major ingredient, could attenuate the I/R-induced adhesion of leukocyte to

*Corresponding author. E-mail: kan@chuigaku.co.jp.
mesenteric venular wall in rats without inhibition of oxidative stress. There are several studies to indicate that GB protects oxidative stress [4], and attenuate the reduction of RBC velocity, adhesion of leukocyte to venular wall caused by I/R [5–7]. However, little is known about whether GB has protective effects on production of oxygen radicals from microvascular endothelial cell and mast cell degranulation. This study was aimed to investigate the effect of MR on microcirculatory injury induced by ischemia–reperfusion (I/R). As it is well known, I/R leads to an microcirculatory damage frequently accompanied by endothelial cell injury, enhancing the increased adherence of leukocytes, oxygen free radicals and mast cell degranulation [8–11]. In the present experiment, we evaluated microcirculatory changes induced by I/R in rat mesentery with and without effect of MR.

2. Materials and methods

2.1. Preparation of MR

Myakuryu (MR) was prepared by Tianjin Tasly group (Tianjin, China). The components are as follows: Panax notoginseng extract is 10% (including Panax notoginseng saponins = 30%), Crataegue oinnatifida bge extract is 85% (including flavonoid = 3–5%) and Gingo biloba extract is 5% (including flavonoids = 24%, terpenoid = 6%, ginkgolic acid < 5 ppm).

2.2. Mesentery microcirculation observation

All animals were handled according to the guidelines of the Keio University Animal Research Committee. Male Wistar rats (200–250 g; Saitama Experimental Animal Center, Saitama, Japan) were fasted for 12 hr before the experiment, but were allowed free access to water. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital, 30 mg/kg body wt. The abdomen was opened via a midline incision 20–30 mm long. The ileocecal portion of the mesentery 20–30 cm caudal was gently drawn out, exteriorized and mounted on a transparent plastic stage specially designed for the rat. The mesentery was kept warm and moist by continuous superfusion with Krebs–Ringer-bicarbonate buffered solution at 37°C. Microcirculatory hemodynamics in the mesentery was observed by a transillumination method by using an inverted microscope (Diaphot TMD-2S; Nikon, Tokyo, Japan). The mesentery was transilluminated with a 12-V, 100-W, direct current-stabilized light source. A video camera mounted on the microscope projected the image onto a color monitor, and the images were recorded with a videocassette recorder. A video time-date generator projected the time and stopwatch function onto the monitor. Single, unbranched venules with diameter ranging between 25 and 40 µm and length > 200 µm were selected for study.

2.3. Experimental protocol for I/R injury

After 10 min of basal observation of the hemodynamics in the rat mesenteric microvasculature, the I/R was performed by ligation of the mesenteric artery and the corresponding vein simultaneously with a snare created from a polyethylene tube for 10 min and subsequent release of the blood flow occlusion (n = 6). Sham-operated rats without I/R were used as control (n = 6).

In I/R + MR group (n = 6), MR (0.4 g/kg b.w.) was administered via an orogastric tube 1 hr before I/R.
2.4. Measurement of microvascular parameters

The images of venules were obtained through a charge-coupled devise (CCD) color video camera system (CC-090; Flovel, Tokyo, Japan). The diameters of microvessels were determined time-dependently using a video measuring gauge (IV-560; Hoei, Tokyo, Japan). The leukocytes that had adhered to the venular walls were identified by reviewing the dynamic images of leukocytes recorded through this system. Adherent leukocytes were defined as cells that attached to the same site for more than 30 s judging from the replayed video images. The number of adherent leukocytes was counted along venules (25–40 µm in diameter, 200 µm in length) randomly selected from the videotape images recorded.

The oxidant-sensitive fluorescent probe dihydrorhodamine 123 (DHR; Molecular Probes) was added to the mesenteric superfusate (10 µmol/l) to monitor oxidant stress in venular walls as described previously [11]. DHR fluorescence intensity on the venular wall was monitored and calculated with an image processor.

Mast cells were identified by vital staining with topical application of 0.1% toluidine blue to the mesentery 30 min after the onset of I/R. The numbers of both nondegranulated mast cells and degranulated mast cells were counted from the CCD video images, and the ratio of the number of degranulated mast cells to the total number of mast cells is expressed as the degranulated mast cell ratio.

2.5. Statistical analysis

Data were analyzed by standard statistical methods, i.e., one-way ANOVA and Fisher’s post hoc test. All values reported as mean ± SD of values from 6 rats, and statistical significance was set at \( P < 0.05 \).

3. Results

The diameters of mesenteric venules under baseline conditions were 35.5 ± 2.6, 37.3 ± 1.1 and 38.7 ± 2.5 µm in control groups, I/R groups and MR+ groups, respectively. The diameters of the observed mesenteric venules were not changed after I/R. MR has no effect on venular diameter during I/R.

At 30 min after I/R, the number of leukocytes adherent to mesenteric venule wall in the control group was only (1.8 ± 0.6/100 µm). The number of leukocytes adhering to the post-capillary venular walls increased at 30 min after reperfusion (18.3 ± 1.6/100 µm). The increased number of adherent leukocytes was significantly reduced by treatment with MR (Fig. 1).

![Fig. 1. Effects of treatment with MR on the changes in the number of leukocytes adhering to mesenteric venules induced by I/R. Effects were assessed 30 min after reperfusion. ∗p < 0.05 vs control group, ∗p < 0.05 vs I/R group.](image-url)
As shown in Fig. 2, the intensity of DHR fluorescence was significantly increased along the venular wall at 30 min after reperfusion in the I/R group. MR was attenuated the increased oxidative.

Figure 3 shows the ratio of degranulated mast cell in the area along the microvesseles 30 min after the reperfusion. In the control groups, the mast cell degranulation ratio was 22 ± 2.1%. It was significantly increased in I/R animals (55.5 ± 2.3%). MR treatment significantly attenuated the I/R-induced increase of mast cell degranulation.

4. Discussion

In the present study, we demonstrated that MR attenuated the number of adherent leukocyte to venular wall, DHR fluorescence intensity on the venular wall and mast cell degranulation in rat mesentery induced by I/R.
The diameters of the observed mesenteric venules were not changed after I/R. MR has no effect on venular diameter during I/R. These results suggest that MR does not affect vascular tone (constriction and dilation). Therefore, MR has protective effects on I/R-induced injury other than vasodilation.

A likely mechanism is scavenging of oxygen radicals. It is reported that I/R produces oxygen radicals in endothelial cells [12]. The I/R-induced oxygen radicals could result in expression of adhesion molecules on endothelial cells and leukocytes [13–15]. Leukocyte adhesion to endothelial cells causes microvascular dysfunction. DHR was oxidized to fluorescent rhodamine 123 by intracellular and extracellular secondary H$_2$O$_2$-dependent reactions [16–18]. We used the oxidant-sensitive fluorescent probe DHR, whose oxidation was reported to occur by reactions of not only H$_2$O$_2$ but reactive oxygen species. The intensity of DHR fluorescence was significantly increased along the venular wall after I/R. The number of leukocytes adhering to the post-capillary venular walls started just after reperfusion, and further increased at 30 min after reperfusion. MR reduced the increased number of adherent leukocytes accompanied with attenuation of the increased oxidative stress elicited by I/R. These results in the present study suggest that MR prevents the I/R-induced microvascular dysfunction via its ability as an anti-oxidant.

However, there is a possibility that MR prevents the I/R-induced microvascular dysfunction through other mechanisms than anti-oxidation, because one of the components of MR, PN, could also attenuate the I/R-induced adhesion of leukocyte to mesenteric venular wall in rats without inhibition of oxidative stress [3]. Since MR reduced the increased number of adherent leukocytes elicited by I/R, MR might be able to induce expression of adhesion molecules either on the endothelial cells or leukocytes.

Mast cell degranulation was products induced by factors such as tumor necrosis factor, platelet activating factor, histamine [19–22], and so on, released in response to superoxide exposure promote leukocyte adhesion in postcapillary venules [23]. Thus, mast cell degranulation may contribute to oxidant stress elicited by I/R. Treatment with MR significantly attenuated the I/R-induced increase mast cell degranulation. This result indicates that MR can prevent cytokine production outside vessels, which lead to aggravation of I/R-induced microvascular dysfunction.

References

[1] J.Y. Han, Medicine or health food for prevention and treatment of microcirculatory disorders and life-style related disease, Patent proposal: 2004-140288 (Japan); 200510069450.7 (China).


Copyright of Clinical Hemorheology & Microcirculation is the property of IOS Press and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.