Post-Transcriptional Regulation of Breast Cancer Resistance Protein after Intestinal Ischemia-Reperfusion

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Breast cancer resistance protein (BCRP), the product of the ABCG2 gene, is a recently identified ATP binding cassette half-transporter. BCRP protein is expressed in a variety of tumor cells and many normal human tissues. In the small intestine, BCRP can limit the influx and facilitate the efflux to prevent intracellular accumulation of BCRP substrates. Ischemia-reperfusion (I/R) induces the release of reactive oxygen species, and organs are severely damaged by I/R. It has been shown that the expression of transporters was altered in the organ after I/R. The present study was undertaken to clarify the expression of BCRP after intestinal I/R. We showed that the expression level of Bcrp was significantly decreased at 1 h after I/R. Bcrp mRNA level was not altered at 1 h after I/R. These results suggest that Bcrp expression was regulated by a post-transcriptional regulation mechanism after intestinal I/R. Bcrp mRNA level was increased at 24 h after I/R, and the expression level of Bcrp protein was of the same level or slightly increased compared with sham operated-rats. Bcrp was slightly located at the intestinal membrane at 24 h after intestinal I/R. These results suggested that Bcrp was not translocated to the intestinal membrane after intestinal I/R. There is little information on post-transcriptional regulation compared with information on transcriptional regulation. In this study, it was shown that Bcrp expression is regulated by post-transcriptional regulation after intestinal I/R. These results of this study may provide important information for further studies aimed at revealing the biological function of Bcrp.

Key words breast cancer resistance protein; intestinal ischemia-reperfusion; regulation

Materials and Methods

Animals Male Wistar rats, aged 7 to 9 weeks (250—350 g in weight), were obtained from Jla (Tokyo, Japan). The housing conditions were the same as those described previously. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals”.

Intestinal I/R Model Surgical procedures were carried out as described in a previous report with some modification. The animals were anesthetized with sodium pentobarbital (30 mg/kg body weight, i.p. injection). Through a midline laparotomy, each rat was subjected to 30 min of ischemia by ligating small anastomosing vessels and occluding the superior mesenteric artery (SMA), and reperfusion was induced by removing the clamp. The abdomen was then covered with a sterile plastic wrap.

Quantitative Real-Time PCR Total RNA was prepared from tissue homogenate using an ISOGEN (Nippon Gene, Tokyo) and an RNase-Free DNase Set (QIAGEN). Single-strand cDNA was made from 2 μg total RNA by reverse transcription (RT) using a ReverTra Ace (TOYOBO). Quantita-
tive real-time PCR was performed using an ABI PRISM 7700 sequence Detector (Applied Biosystems) with Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen) as per the manufacturer’s protocol. PCR was performed using Bcrp-specific primers through 40 cycles of 95°C for 15s, 50°C for 30s and 72°C for 30s after pre-incubation at 50°C for 2 min and 95°C for 15 min or using GAPDH-specific primers. The specific primers to rat Bcrp and GAPDH were designed on the basis of sequences in the GenBankTM database (accession no.: AB094089 and AF106860, respectively). The sequences of the specific primers were as follows: the sense sequence (641–660) was 5'-GTG TGG ACT CAA GCA CAG CA-3' and the antisense sequence (771–790) was 5'-TGA GTT TCC CAG AAG CCA GT-3' for rat BCRP, and the sense sequence (1034–1053) was 5'-ATG GGA AGC TGG TCA TCA AC-3' and the antisense sequence (1235–1254) was 5'-GTG GTG TGT GCC ACC CAT CAC AA-3' for rat GAPDH.

Western Blot Analysis The samples, intestinal brush-border-membrane vesicles (BBMVs), were prepared as described previously.14 The protein concentrations of these samples in clear supernatant were determined by the method of Lowry et al.15 Each sample was denatured at 100°C for 3 min in a loading buffer containing 50 mM Tris–HCl, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.002% BPB and 3.6 M urea and separated on 4.5% stacking and 10% SDS polyacrylamide gels. Proteins were transferred electrophoretically onto nitrocellulose membranes (Trans-Blot; BIO-RAD) at 15 V for 90 min. The membranes were blocked with PBS containing 0.05% Tween 20 (PBS/T) and 10% non-fat dry milk for 1 h at room temperature. After being washed with PBS/T, the membranes were incubated overnight at room temperature with monoclonal anti-breast cancer resistance protein (BXP-21) (Sigma) (dilution of 1 : 250) or mouse anti-actin monoclonal antibody (Chemicon) (dilution of 1 : 500) and washed three times with PBS/T for 10 min each time. The membranes were subsequently incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Santa Cruz Biotechnology) at a dilution of 1 : 20. The specimens were subsequently incubated for 1 h at room temperature with FITC-conjugated donkey anti-mouse secondary antibody (Santa Cruz Biotechnology) at a dilution of 1 : 100. Nuclei were stained with DAPI. The localization of Bcrp protein was visualized by using a confocal microscope (Zeiss LSM-510; Carl Zeiss Inc., Thornwood, NY, U.S.A.).

Immunohistochemistry Slicing and staining the ileums of rats were performed by Sapporo General Pathology Laboratory Co., Ltd. Briefly, the frozen ileums of rats were sectioned at 5 μm in thickness and fixed in 4% paraformaldehyde. After washing with PBS, the specimens were incubated overnight at 4°C with monoclonal anti-breast cancer resistance protein (BXP-21) (Sigma) (dilution of 1 : 250) or mouse anti-actin monoclonal antibody (Chemicon) (dilution of 1 : 500) and washed three times with PBS/T for 10 min each time. The bands were visualized by enhanced chemiluminescence according to the instructions of the manufacturer (Amersham).

RESULTS AND DISCUSSION

Intestinal I/R promotes inflammation and multi-organ failure (MOF).31 The liver and kidney can be affected by MOF after intestinal I/R.16,17 In the first part of this study, we investigated the expression level of Bcrp mRNA in the jejunum, ileum, kidney, and liver. In the ileum, Bcrp mRNA expression level was increased at 24 h after intestinal I/R (Fig. 1), but the expression level of Bcrp mRNA was not altered at any time in the jejunum. Bcrp expression level in the ileum is higher than that in the jejunum. Therefore, Bcrp mRNA in the ileum might affect more severely after intestinal I/R than mRNA in the jejunum. Bcrp mRNA expression level was not altered in the liver and kidney after intestinal I/R. Although both mechanisms of I/R injury and remote organ injury after I/R are not identified, remote organ injury may be different from ischemic organ injury. Tanaka et al. has been reported that Mrp2 mRNA level was decreased in the liver after hepatic I/R, but was increased in the kidney.18 These findings suggest that effects of I/R on ischemic organ were different from that on remote organs. Therefore, alteration of Bcrp mRNA in the liver and kidney after I/R is different from that in the ileum.

We investigated whether Bcrp protein expression correlated with mRNA expression. The result showed that the expression level of Bcrp was significantly decreased at 1 h after I/R in the ileum (Fig. 2). Bcrp expression level was increased as time dependent and same or slightly high level at 24 h after intestinal I/R compared with the level in sham operated-rats. The expression level of Bcrp mRNA was not altered at 1 h after intestinal I/R. These results suggest that Bcrp expression was regulated by a post-transcriptional regulation mechanism after intestinal I/R.

Bcrp is expressed on the intestinal apical membrane and prevents the intracellular accumulation of Bcrp substrates. The result of Western blot analysis using BBMVs suggested that Bcrp expression in the brush border membrane was significantly decreased at 1 h after intestinal I/R compared with that on remote organs. Therefore, alteration of Bcrp mRNA in the liver and kidney after I/R is different from that in the ileum.
tinal membrane at 1 h after intestinal I/R but was located near
the nuclei. Bcrp was slightly located at the intestinal mem-
brane at 24 h after intestinal I/R. The result of immunohisto-
chemistry was correlated with the results of Western blotting
analysis. These results suggested that Bcrp was, at least in
part, translated but not was translocated to the intestinal
membrane after intestinal I/R.

Recently, steroid/hormone receptors have been shown to
be involved in transcription of transporters. Bcrp mRNA has
been shown to be induced by peroxisome proliferators-acti-
vated receptor γ (PPARγ), estrogen receptor (ER) and prog-
esterone receptor (PR). 19—21) Mdr1, encodes P-gp, has been
shown to be induced by pregnane X receptor (PXR) and con-
stitutive androstane receptor (CAR). 22,23) Multidrug resist-
ance-associated protein (MRP) 2 mRNA has been shown to
be induced by farnesoid X receptor (FXR), PXR and CAR. 24)
However, there is little information on post-transcriptional
regulation compared with information on transcriptional reg-
ulation. In this study, it was shown that Bcrp expression is
regulated by post-transcriptional regulation after intestinal
I/R. However, the mechanism of this regulation is not clear.
Further detailed investigation is needed to elucidate post-
transcriptional regulation of Bcrp after intestinal I/R. These
results of this study may provide important information for
further studies aimed at revealing the biological function of
Bcrp.

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Fig. 2. Time Course of Bcrp Protein Expression in the Ileum after I/R
Western blotting using ileal BBMVs (15 µg protein/lane) at 1, 6, 24 h after I/R. (A)
Representative results of Western blotting analysis for Bcrp and β-actin. (B) The ratio
of Bcrp/β-actin after intestinal I/R. Each column represents the mean with S.D. of 3—4
measurements. * p<0.05, significantly different from sham.

Fig. 3. Immunohistochemistry of Bcrp in the Ileum after I/R
The expression of Bcrp was determined at 1 h, 6 h and 24 h after I/R. Sham operated-rats were used as controls. Localization of Bcrp was determined using an antibody against
Bcrp (green). Nuclei were stained with DAPI (blue).


