Assessment of transporters of Gd-EOB-DTPA in various hepatocellular nodules during hepatocarcinogenesis induced in rat livers

N. Tsuda[1], and O. Matsui[2]

[1]Medical Affairs, Bayer Yakuhin, Ltd., Osaka, Osaka, Japan, [2]Department of Radiology, Kanazawa University Graduate School of Medical Science, Kanazawa, Ishikawa, Japan

Introduction

Gd-EOB-DTPA is a hepatobiliary contrast agent for MRI, which partially accumulates in hepatocytes and bile via some transporters after injection. It is well known that the organic anion transporting polypeptide 1 (oatp1) mediates the uptake of Gd-EOB-DTPA by hepatocytes, and that the multidrug resistance protein 2 (mrp2) mediates the biliary excretion of Gd-EOB-DTPA in rats; however, it is unclear how the activity of these transporters change according to the progress of hepatic carcinogenesis. We investigated the difference in the oatp1 and mrp2 activity among various hepatocellular nodules during hepatocarcinogenesis by laser capture microdissection (LCM) coupled with the reverse transcription polymerase chain reaction (RT-PCR) in order to predict the efficacy of Gd-EOB-DTPA enhanced MRI in the differential diagnosis of these nodules.

Methods

Animal model

Male wistar rats received 0.01 wt/vol% of N-nitrosomorpholine solution for 18 to 22 weeks to induce hepatic tumors.

Tissue preparation

After the carcinogenesis induction period, livers were removed, frozen and stored at -80 °C. Whole liver was divided into15 blocks, and 15 slides of 15 micrometer for each block were prepared. Three slides (the slide number: 1, 8, 15) were stained with hematoxylin and eosin to differentiate well-differentiated HCC (HCCwell), moderately-differentiated HCC (HCCmod), poorly differentiated HCC (HCCpoor) and hyperplastic nodules (HPN). Five tumors for each group were prepared. As a control, the slides prepared from normal rats were used.

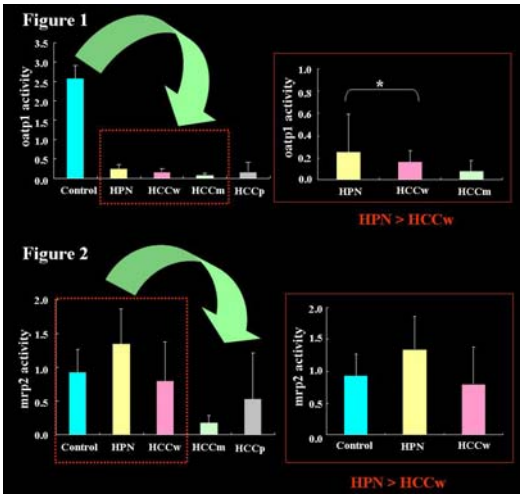
Laser capture microdissection (LCM), RNA extraction, and RT-PCR

The LCM was performed to collect mRNA of OATP1 and MRP2 transporters in each tumor using the unstained slides (except for the slide number: 1, 8, 15). The extracted mRNA was reverse-transcribed to generate the cDNA, which was subjected to the RT-PCR with a SYBR(r) PrimeScript (r) RT PCR Kit (Takara Bio Inc). By this technique, we compared the transporter (oatp1 and mrp2) activity in each tumor.

Results and Discussion

HCCwell, HCCmode, HCCpoor, and HPN were induced in model rats, and five tumors for each group were subjected to the RT-PCR. Three out of five HPNs were typical benign HPNs (i.e. low grade HPN), whereas two HPNs showed the tissue structure like a trabecular type that seemed to be a borderline lesion (i.e. high grade HPN). In addition, three out of five HCCwells were classical HCCwell; however, two HCCwell showed the poor cellular atypia and tissue structure like a solid type that seemed to be a borderline lesion (i.e. early HCC).

We succeeded in the extraction of RNA from each tumor (n=5), and compared the oatp1 and mrp2 activities in each group. As a result, it was found that the oatp1 activity of HCCwell, HCCmod, HCCpoor, and HPN groups intensively decreased in comparison with that of control group (Figure 1). In the comparison among the tumors, the oatp1 activity of HPN group was significantly higher than that of HCCwell group (Figure 1). On the other hand, the mrp2 activity of HPN group tended to be higher than that of the control group; however, the mrp2 activity of HCCwell group seemed to be lower than that of the control group (Figure 2). For precious assessment of the transporter activity, each data (from HPN, HCCwell, and control groups) was plotted in the Figure 3. The plots of HPN and HCCwell groups shifted to the left-hand on the graph, because the oatp1 activity intensively decreased in the HPN and HCCwell groups. The plots of HPN group were over the area of the plots of HCCwell group, because the mrp2 activity increased in the HPN group. The HPN, HCCwell, and control groups were plotted in the different area; however, two plots of HPNs (high grade HPN) closed by the area of HCwell and two plots of HCCwell (early HCC) were included in the area of HPN group. Therefore, it was suggested that the Gd-EOB-DTPA-enhanced MRI would be useful for the differential diagnosis among various hepatocellular nodules during carcinogenesis, whereas some of HPNs might share the same findings as those of some of HCCwell lesions on the Gd-EOB-DTPA-enhanced MRI.



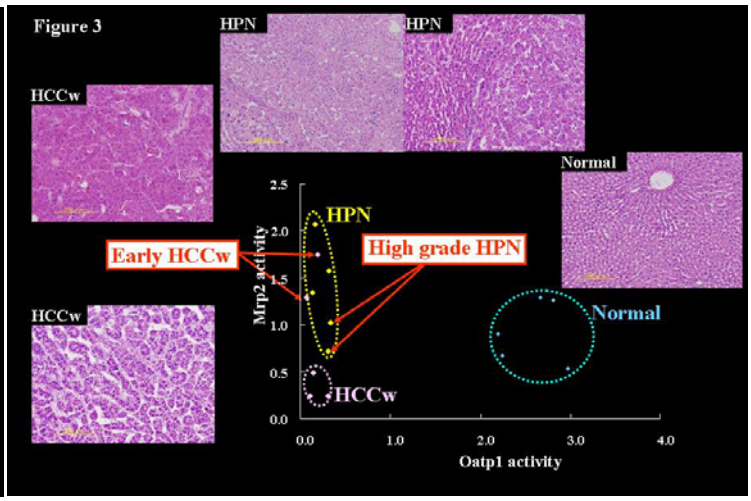


Fig.1 Comparison of the oatp 1 activity among HPN and HCCs groups. *: p < 0.05 versus HCCwell group (n=5).
Fig.2 Comparison of the mrp2 activity among HPN and HCCs groups.
Fig.3 Correlation between the oatp1 and mrp2 activities in HPN, HCCwell and control groups.