Hepatic expression of galectin-3 and receptor for advanced glycation end products in patients with liver disease

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Background: Advanced glycation end products (AGEs) are a heterogeneous group of glycosylated proteins (of which carboxymethyl-lysine (CML) is the most common) which accumulate during ageing processes and play an important role in the pathogenesis of a variety of chronic diseases. Impaired hepatic function might result in elevated levels of AGEs, as the liver represents the major site of AGE metabolism. The actions of AGEs are mediated by various receptors, among which the AGE-receptor complex (including galectin-3 as an essential part) is thought to have a cytotoxic effect, and receptor for advanced glycation end product (RAGE) a cytotoxic effect.

Aim: To assess the relationship between CML and expression of galectin-3 and RAGE in different histological structures in biopsy specimens from patients with varying degrees of liver impairment.

Method: Immunohistochemical staining of 164 biopsies from patients with varying degrees of liver impairment was performed to determine the levels of CML, galectin-3 and RAGE in hepatocytes, Kupffer cells and bile ducts by a semiquantitative score.

Results: Independent of diagnosis, CML and RAGE were detected in hepatocytes, whereas galectin-3 was only present in hepatocytes of cirrhotics. By contrast, CML and galectin-3 were highly expressed in Kupffer cells (well correlating levels, highest scores in cholestasis) whereas expression of RAGEs was not significant. All three assessed biochemical markers showed their highest levels of expression/detection in bile ducts.

Conclusion: These findings indicate an increased susceptibility of hepatocytes to the detrimental effects of AGEs and underline the protective function of Kupffer cells. Furthermore, the biliary system seems to play an important role in the disposition of AGEs.
Assessment of immunostaining

On the basis of the stained percentage of selected histological structures (hepatocytes, Kupffer cells and bile ducts), values of 0–3 were assigned to each tissue section. 0, no stained structures; 1, <33% stained; 2, 33–66% and 3: >66%. Likewise intensity of staining was graded: 0, no staining; 1, weak staining; 2, medium and 3, strong. By multiplying both values, a semiquantitative staining score was obtained.

Statistical analysis

All results were evaluated by Wilcoxon’s signed rank test, Kruskal–Wallis test and Dunn’s multiple comparison test, setting the significance level to p<0.05 (GraphPad Prism Software, San Diego, California, USA).

RESULTS

Figure 1 summarises the semiquantitative staining scores of CML, RAGE and galectin-3 in the examined histological structures (hepatocytes, Kupffer cells and bile ducts) from subjects of different diagnoses (healthy control, steatosis hepatis, hepatitis, cholestasis and cirrhosis). Based on the Kruskal–Wallis test, some significant differences could be observed between the different sites and patient populations.

Immunohistochemical staining patterns in healthy patients

Hepatocytes and bile ducts of controls showed strong signals for CML, which were weaker (p<0.001) in Kupffer cells. Also, staining for RAGE was strongest in hepatocytes and bile ducts, whereas Kupffer cells were almost devoid of a signal (p<0.001). Figure 2 presents a typical example for staining of galectin-3. In the control group, hepatocytes and Kupffer cells were free of staining (p<0.05), but positive bile ducts (with varying degrees of staining intensity) were seen in all examined cases.

Immunohistochemical staining patterns in patients with hepatic dysfunction

Staining for CML was strong in hepatocytes and bile ducts independent of diagnoses. In Kupffer cells, signals were weaker (p<0.001) but continuously present. Marked levels of RAGE were detected in hepatocytes, with no difference between diagnoses. Bile ducts showed even stronger signals, except in steatosis hepatis. Kupffer cells had negative to weak staining for RAGE, independent of the kind of hepatic impairment.

In steatosis, hepatitis and hepatitis, hepatocytes showed little or no expression of galectin-3. In cholestasis, positive signals occurred more often, although they were limited both in extent and intensity. Big nodules of strong positive signals were seen only in cases of cirrhosis (fig 3). Kupffer cells were shown to express galectin-3 throughout all diagnoses, although signals were strongest in cases of cholestasis (fig 4). Independent of the diagnosis, the highest scores were detected in bile ducts.
DISCUSSION

The underlying rationale of this study is an association between impaired liver function and consecutively rising AGE levels triggering an overexpression of galectin-3 in the liver as part of an AGE–receptor complex. Binding of AGEs to this complex is thought to lead to their internalisation and degradation, and thereby to tissue protection.

Following this concept, a trend towards an increased presence of CML in subjects with hepatic impairment as opposed to controls should be expected. Surprisingly, our results indicate no difference between the tested groups, independent of the histological structure examined. This finding could indicate that higher levels of circulating AGES (as expected in patients with hepatic dysfunction) do not cause a stronger internalisation in hepatocytes, Kupffer cells and/or bile ducts. On the other hand, stronger internalisation could also trigger a higher rate of AGE degradation, keeping internalised levels equal.

Nonetheless, the observed high staining scores of hepatocytes of patients with liver cirrhosis could indicate a relationship between hepatic dysfunction, subsequent rising of AGE levels and galectin-3 upregulation. In agreement with our data, Hsu et al. showed 1999 that galectin-3 is absent in normal hepatocytes, but is abundantly expressed in cirrhotic liver and frequently in hepatocellular carcinoma. The authors speculated that upregulated galectin-3 expression is due to the high mitotic index of proliferating hepatocytes or part of a malignant transformation process. On the other hand, Iacobini et al. recently showed the protective effect of galectin-3 against AGE-induced organ damage, using a galectin-3 knockout mouse model.

Independent of hepatic impairment, the absence or low presence of RAGEs, which transmit the detrimental effects of AGEs versus the marked levels of cytoprotective galectin-3 in Kupffer cells, is supportive for the protective nature of this cell type.

By contrast, hepatocytes were more or less devoid of galectin-3 (except for subjects with cirrhosis), but showed strong stainings for RAGEs. This finding could indicate a susceptibility of hepatocytes regarding the toxic potential of AGEs.

Compared with the above-discussed cell types, bile ducts showed the highest levels for CML, RAGEs and galectin-3. This indicates that the biliary system is an important site for the disposition of AGEs. It could be speculated whether AGEs are, apart from intracellular degradation, also subject to biliary excretion. Similar to our findings, Shimonishi et al. showed a constitutive (but weak) galectin-3 expression in bile ducts, with a marked rise in staining intensity in case of biliary obstruction. Even though we were unable to reproduce this mechanism (staining scores of bile ducts in cholestasis did not significantly exceed scores in other diagnoses), their data support the idea of a biliary excretion of AGEs. The evident galectin-3 upregulation in cholestasis shown by Shimonishi could be due to elevated reabsorption of AGEs by the AGE-receptor complex as a reaction to obstructed bile ducts.

In support of this hypothesis, we found a strong expression of galectin-3 in Kupffer cells during cholestasis. Smedsrod et al. have reported that after intravenous administration of AGE-bovine serum albumin in rats, uptake of AGE-bovine serum albumin in liver endothelial, Kupffer and parenchymal cells accounted for approximately 60%, 25% and 10–15%, respectively, of hepatic elimination. This further supports the contention that AGEs are excreted in the bile, which could trigger the Kupffer cells to upregulate galectin-3 due to rising AGE concentration in cholestasis.

Our findings show a complex pattern in expression of RAGE and galectin-3 in the examined histological structures of the liver. Furthermore, we showed some associations between
Take-home messages

- The biliary system seems to play an important role in the disposition of AGEs.
- While galectin-3 shows a complex expression pattern in the examined histological structures of the liver, some associations exist between different forms of functional liver impairment and galectin-3 expression.
- Kupffer cells seem to play a protective role against the detrimental effects of AGEs, while hepatocytes seem to show an increased susceptibility.

different forms of functional liver impairment and galectin-3 expression. These phenomena may be linked to the function of galectin-3 in the hepatic disposition of AGEs. Further studies are needed to define more clearly the role of the biliary system in this context.

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