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Immunodetection of aldose reductase in normal and diseased human liver

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Summary. Aldose reductase is an NADPH-dependent aldo-keto reductase best known as the rate-limiting enzyme of the polyol pathway that is implicated in the complications of diabetes. Aldose reductase appears to be involved in a variety of disease states other than diabetes, presumably due to its ability to catalyze the reduction of a broad spectrum of aldehydes, including some cytotoxic products of lipid peroxidation. Although the data regarding expression of aldose reductase in normal liver are conflicting, prior studies have suggested that the enzyme may be induced in diseased liver. The goal of these studies was to characterize expression of aldose reductase in normal and diseased human liver, using RT-PCR, Western analysis and immunohistochemistry. Aldose reductase transcripts and protein were detected at low levels in control human livers. In contrast, levels of aldose reductase mRNA and protein were increased in chronically diseased human livers. Immunohistochemistry demonstrated localization of aldose reductase in sinusoidal lining cells; dual immunofluorescence confocal microscopy with the macrophage marker, CD68, confirmed that the aldose reductase-positive sinusoidal lining cells were Kupffer cells. Abundant aldose reductase-positive, CD68-positive cells were present in the fibrous septa of cirrhotic livers, accounting for the increase in immunoreactive aldose reductase in diseased livers. Immunostaining of human lung, spleen and lymph node revealed that macrophages in those tissues also express aldose reductase. These data are the first to demonstrate that aldose reductase is expressed by human macrophages in various tissues and suggest that this enzyme may play a role in immune or inflammatory processes.

Key words: Aldo-keto reductase, 4-hydroxy-2-nonenal, Kupffer cells, Macrophages, Polyol pathway

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Introduction

Aldose reductase (alditol:NAD(P)$^+$1-oxidoreductase, EC 1.1.1.21) is an NADPH-dependent aldo-keto reductase that has been the subject of longstanding interest because of its putative role in the pathogenesis of diabetic complications. This enzyme catalyzes the first and rate-limiting step in the polyol pathway, in which the reduction of glucose to sorbitol is coupled to the oxidation of NADPH. Sorbitol is subsequently oxidized to fructose in a reaction that generates NADH from NAD$^+$. Biochemical consequences of increased flux of glucose through this pathway include alterations in the ratios of the redox couples NADP$^+$/NADPH and NAD$^+$/NADH, increased levels of sorbitol and decreases in myo-inositol. These alterations are believed to contribute to the pathogenesis of diabetic complications by a variety of mechanisms including oxidative stress resulting from decreased availability of reduced glutathione due to competition between aldose reductase and glutathione reductase for NADPH, "pseudohypoxia" related to the accumulation of NADH, osmotic stress from the accumulation of sorbitol and disturbances in signal transduction caused by alterations in phosphoinositide metabolism (Brownlee, 2001). In support of this concept, pharmacologic inhibitors of aldose reductase have shown benefit in animal models of diabetes.

In addition to its well-known role in the polyol pathway, recent studies indicate that aldose reductase may play a role in a broad spectrum of pathological

processes. One potential mechanism to account for the widespread involvement of aldose reductase in disease is the ability of the enzyme to catalyze the reduction of aldehydes such as 4-hydroxy-2-nonenal (4HNE) (Srivastava et al., 1995; Vander Jagt et al., 1995). 4HNE is a cytotoxic byproduct of lipid peroxidation that is believed to participate in the pathogenesis of a variety of pathological conditions (Esterbauer et al., 1991). In vitro studies have shown that aldose reductase expression is induced by 4HNE in some cell types and that inhibition of aldose reductase activity sensitizes cells to the cytotoxicity of 4HNE (Spycher et al., 1996, 1997). In support of the concept that rapid detoxification of aldehydes by aldose reductase serves a cytoprotective function, pharmacologic inhibition of aldose reductase was associated with increased numbers of apoptotic cells as well as 4HNE content in inflamed arteries in a murine model of giant cell arteritis (Rittner et al., 1999).

In contrast to the exacerbation of injury observed with aldose reductase inhibitors in the arteritis model, inhibitors of the enzyme have been reported to exert beneficial effects in a variety of other rodent models, including ischemic myocardial injury, arterial balloon injury, and cachexia of cancer (Ramasamy et al., 1997; Kawamura et al., 1999; Tracey et al., 2000; Ruef et al., 2000; Bhatnagar et al., 2001). The extent to which the effects of aldose reductase inhibition in these diverse conditions is attributable to modulation of aldehyde metabolism is unclear, particularly in light of recent data demonstrating the participation of aldose reductase in inflammatory and apoptotic signaling in response to cytokines and growth factors (Ramana et al., 2002, 2003). Thus, while the mechanism/s by which aldose reductase participates in the injury process in these circumstance has not been clearly defined, these studies indicate a broader role for this enzyme than has been previously recognized.

Conflicting data have been presented regarding the presence of aldose reductase in the liver. Several studies employing enzymatic methods concluded that aldose reductase is absent from human and rodent liver (Hers, 1960; Tulsiani and Touster, 1977; Srivastava et al., 1984; Vander Jagt et al., 1990). Consistent with those results, very low levels of aldose reductase mRNA were reported in rat liver based on Northern blot hybridization (Nishimura et al., 1988). In contrast, Wirth and Wermuth, using immunohistochemical techniques, reported that aldose reductase is present in hepatocytes, Kupffer cells and bile ducts of human liver (Wirth and Wermuth, 1984). More recently, the distribution of aldo-keto reductases in human tissue was investigated using immunoblot analysis performed with highly specific antibodies generated from recombinant peptides (O'Connor et al., 1999). Using this sensitive method, these authors found that aldose reductase is undetectable in normal human livers (O'Connor et al., 1999). Although the reasons for the disparities among these studies are not clear, the data overall appear to suggest that aldose reductase is expressed at a low level in liver, if at all.

Although the presence of aldose reductase in normal liver remains a subject of controversy, limited data suggest that aldose reductase may be induced in diseased liver. For example, O'Connor et al. reported that aldose reductase was detectable in the livers of two human subjects with alcoholic liver disease (O'Connor et al., 1999). These findings are strikingly similar to observations made in the Long-Evans cinnamon rat, a mutant strain that is a model for Wilson's disease (Takahashi et al., 1996). Neither aldose reductase mRNA nor protein is detectable in the livers of cinnamon rats prior to the development of hepatitis or in the livers of a closely related rat strain that does not develop hepatitis, while expression of aldose reductase in the livers of the cinnamon rats shows a temporal relationship with the onset of liver injury (Takahashi et al., 1996). It has been suggested that these findings may indicate de novo induction of hepatic aldose reductase expression in response to the generation of cytotoxic aldehydes resulting from the underlying disease process. Hepatocytes are known to have a robust capacity to metabolize 4HNE and related aldehydes via mechanisms that do not appear to involve aldose reductase (Esterbauer et al., 1991; Siems et al., 1997). Given that lipid peroxidation-derived aldehydes such as 4-HNE are believed to play a major role in hepatic fibrogenesis via their effects on nonparenchymal cells (Brown, 2000), the question arises as to whether hepatocytes or nonparenchymal liver cells are the cellular source of aldose reductase in the diseased liver. Likewise, it is unclear whether the induction of aldose reductase is a specific response to certain types of liver injury. Thus, the aims of this study were to investigate the effects of acute and chronic liver disease on expression of aldose reductase in human livers and to determine the cellular localization of the enzyme in these tissues.

Materials and methods

Liver tissues

Over 60 independent human liver samples were used in these studies (9 for RT-PCR, 42 for Western blot and 15 for immunohistochemistry). Samples of cryopreserved (-80 °C) human livers were obtained from patients undergoing liver transplantation for fulminant (acute) liver failure or for end-stage liver disease from cirrhosis due to various chronic liver diseases, including chronic hepatitis B and C, alcoholic liver disease, primary biliary cirrhosis, autoimmune hepatitis and hepatocellular carcinoma. Control samples were derived from normal donor livers. The sources of the samples have been described previously (Kladney et al., 2002). Samples were derived from adults of both sexes and were coded to maintain patient anonymity. Archived blocks of formalin-fixed paraffin-embedded livers and various nonliver tissues were obtained from the Department of Pathology, Saint Louis University Health

Sciences Center. The study was approved by the Saint Louis University Human Studies Committee.

RT-PCR detection of aldose reductase mRNA

Total RNA was isolated from frozen liver samples using the Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions and quantitated spectrophotometrically. Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen Life Technologies) was used to synthesize cDNA from 3 mg total RNA in a reaction containing 500 µM each dATP, dTTP, dCTP and dGTP, 40 Units of RnaseIN (Promega, Madison, WI), 250 ng random hexamer oligonucleotide primer, 10 mM DTT, 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$ in a total volume of 20 ml. The reaction was incubated at 37 °C for 60 mins, then 70 °C for 15 mins. The cDNA product was amplified by PCR using 800 nM oligonucleotide primers for human aldose reductase (sense: 5'-TGA-GTG-CCA-CCC-ATA-TCT-CA-3'; antisense: 5'-TGT-CAC-AGA-CTT-GGG-GAT-CA-3'), and in a separate tube, for G3PDH5' and G3PDH3' (Clontech, Palo Alto, CA). Amplification was carried out for 30 cycles of 94 °C for 30 sec, 56 °C for 30 sec, 72 °C for 30 sec. PCR-amplified DNA was electrophoresed on 1.8% agarose gels containing 1 mg/ml ethidium bromide, which were photographed with UV transillumination. The 244-bp aldose reductase PCR product was purified using Qiaquick PCR Purification Kit (Qiagen, Chattsworth, CA), and sequenced on the PE-Applied Biosystems Model 3700 Fluorescent Automated DNA Sequencer (the University of Iowa College of Medicine DNA Core Facility) using the same oligonucleotide primers that were used for PCR. The nucleotide sequence of the aldose reductase PCR product was aligned in both the sense and antisense orientations with published nucleotide sequence (GenBank accession number BC010391) using BLASTN (National Center for Biotechnology Information, NIH, Bethesda, MD), and was found to be identical (expect value = $2x10^{-95}$ and $3x10^{-97}$, respectively).

SDS-PAGE and immunoblotting

Portions of frozen liver tissue (approximately 100 mg/sample) were homogenized for 1 min in 20 volumes of lysis buffer (50 mmol/L Tris-HCl, pH 6.8, 5% (v/v) b-mercaptoethanol and 1% (w/v) sodium dodecyl sulfate) using an SDT Tissumizer (Tekmar Company, Cincinnati, OH). Homogenates were boiled for 5 min, sonicated (3x10 sec bursts) and centrifuged at 14,000 rpm at 4 °C for 10 min. The protein content of the supernatant was quantitated using a modification of the Lowry assay and the indicated quantities were loaded into the wells. Proteins were subjected to electrophoresis in 10% (w/v) polyacrylamide gels (Mini Protean II system; BioRad Laboratories, Richmond, CA). The resolved proteins were electrophoretically transferred to nitrocellulose membranes. Nonspecific binding sites were blocked with 20% nonfat dried milk/0.5% Tween-Tris-buffered saline. After thorough washing, the membrane was first incubated with rabbit anti-aldose reductase antibody diluted 1:1,000, then with peroxidase-conjugated goat anti-rabbit IgG diluted 1:5,000 (Santa Cruz, Santa Cruz, CA). After extensive washing, bound antibody was detected using chemiluminescence according to the manufacturer's instructions (ECL; Amersham International, Little Chalfont, Bucks, UK). Western analysis was also performed using two other polyclonal anti-aldose reductase antibodies (generously provided by Dr. Robert Sorenson, University of Minnesota, and Dr. David Vander Jagt, University of New Mexico.)

Immunohistochemistry

Sections (4- to 5-µm thick) were cut from formalin-fixed, paraffin-embedded tissues. Sections were deparaffinized and rehydrated by passage through a graded series of ethanol and distilled water. For aldose reductase immunohistochemistry, the antigen was retrieved by heating the slides in a pressure cooker in 3 mol/L urea in distilled water for 10 min. Endogenous peroxidase activity was quenched by incubation in 0.3% (v/v) H_2O_2 in methanol for 20 min at room temperature. Sections were incubated at room temperature for 60 min with rabbit anti-aldose reductase antibody diluted 1:50. Biotinylated anti-rabbit antibody was used at a dilution of 1:200. Primary antibody was omitted on sections serving as negative controls. Immunostaining was performed using an avidin-biotin-horseradish peroxidase system (Vector Laboratories, Burlingame, CA) with 3-amino-9-ethylcarbazole as the chromogen.

For immunofluorescence, liver sections were deparaffinized, rehydrated and antigen-retrieved as described above before incubation with a mixture of the aldose reductase antibody and murine monoclonal antibody against CD68, a macrophage/monocyte marker (M814, DAKO, Carpinteria, CA) or the aldose reductase antibody and murine monoclonal antibody to α-smooth muscle actin, a marker for hepatic stellate cells (IA4, Sigma, St. Louis, MO). The sections were subsequently incubated with fluorescein isothiocyanate-labeled anti-mouse IgG and Texas Red-labeled anti-rabbit IgG antibodies (Vector Laboratories) at a dilution of 1:50 and examined using confocal microscopy.

Results

To determine whether transcripts for aldose reductase are present in human liver, RT-PCR was performed on RNA isolated from normal and diseased human livers. RT-PCR analysis of human liver RNA revealed the presence of a 244-bp PCR product, consistent with the expected size of the aldose reductase amplicon (Fig. 1). The abundance of the aldose reductase PCR product after 30 cycles of amplification was relatively modest in both normal and diseased liver,

suggesting that the transcript is not abundant in human liver; however, the abundance of the aldose reductase transcript tended to be somewhat greater in the samples from cirrhotic livers from patients with chronic hepatitis C and alcoholic liver disease (Fig. 1).

To assess for the presence of aldose reductase protein in normal and diseased human livers, Western analysis was performed. Consistent with the result of the RT-PCR analysis, the anti-aldose reductase antibody recognized a protein of approximately 36 kD in extracts of human liver on Western blotting, consistent with the molecular weight of aldose reductase. Immunoreactive aldose reductase was observed in all nondiseased human livers examined, but its abundance was uniformly low in these specimens. In contrast, greater amounts of aldose reductase were found in liver samples obtained from patients with a variety of chronic diseases, including alcoholic liver disease, hepatitis B and C, and autoimmune hepatitis (Fig. 2). Hepatocellular carcinomas also demonstrated robust aldose reductase expression (Fig. 2). Immunoreactive aldose reductase was increased in acute liver damage as well, as judged by several cases of fulminant hepatic failure (data not shown). Western blots performed using polyclonal anti-aldose reductase antibodies developed in two other laboratories yielded comparable results (data not shown).

To determine the localization of aldose reductase, immunohistochemistry was performed on sections of human livers. The predominant cells demonstrating aldose reductase immunoreactivity in nondiseased livers were sinusoidal lining cells with a morphologic appearance suggestive of Kupffer cells (Fig. 3A). Additional aldose reductase-positive cells were observed within the portal tracts. These included the epithelium of the bile ducts, the endothelium of the hepatic arterioles, occasional cells in the stroma and nerves (Fig. 3A). Sinusoidal lining cell reactivity similar to that of control livers was present in an hepatocellular carcinoma and within the nodules of cirrhotic livers (Fig. 3B, C). In addition, numerous aldose reductase-positive cells were found in fibrotic septa (Fig. 3D). No immunoreactivity was present in sections in which primary antibody had been omitted.

To identify the aldose reductase-positive sinusoidal lining cells, dual immunofluorescence confocal microscopy was performed. Colocalization of aldose reductase and CD68, a macrophage/ monocyte marker, was observed in the sinusoidal lining cells (Fig. 4A). Some, but not all, of the aldose reductase-positive cells in the fibrotic septa were also CD68-positive (Fig. 4B), but no colocalization of aldose reductase was observed with α-smooth muscle actin, a marker for activated hepatic stellate cells (not shown). These results indicate that the aldose reductase-positive cells lining the sinusoids are Kupffer cells and that some of those in the septa are infiltrating monocytes or macrophages. Because expression of aldose reductase by macrophages has not been generally appreciated, other tissues containing large numbers of macrophages were examined by immunohistochemistry. This revealed numerous aldose reductase-positive macrophages in human lymph node, lung and spleen (Fig. 5A-C).

Discussion

Conflicting data have been presented regarding the

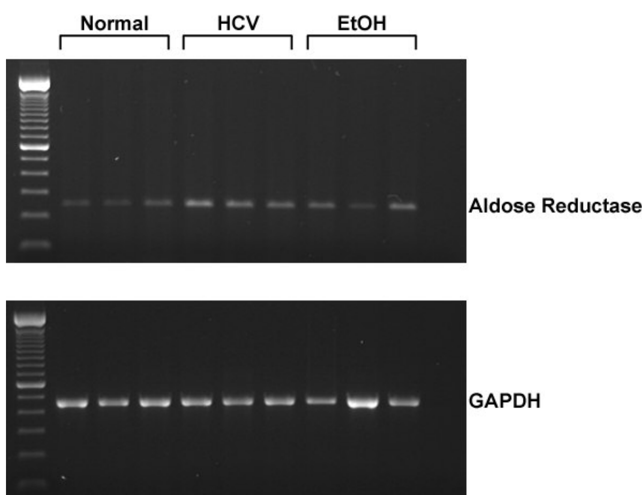

Fig. 1. RT-PCT detection of aldose reductase mRNA in human livers. Total RNA was isolated from human livers and reverse transcribed. The cDNA products were amplified using specific primers for aldose reductase and GAPDH as described in Methods. PCR-amplified DNA was electrophoresed and photographed. Normal: normal donor liver; HCV, cirrhosis due to chronic hepatitis C; EtOH: cirrhosis due to alcoholic liver disease.

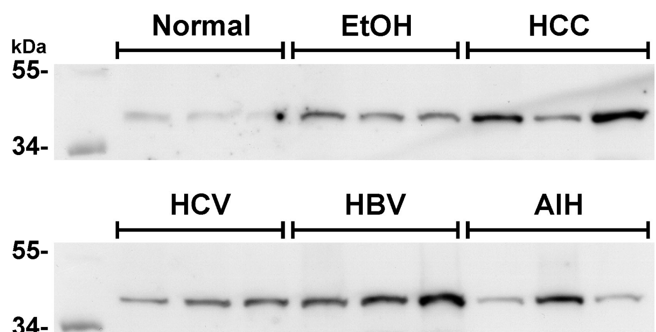

Fig. 2. Immunodetection of aldose reductase in human livers. Detergent extracts of liver homogenates (30 mg protein) were separated in 1% SDS on a 10% polyacrylamide gel and transblotted onto a nitrocellulose membrane. Immunoreactive material was detected with a polyclonal rabbit anti-human aldose reductase antibody as described in Methods. The first lane in both panels contains molecular weight markers (Cruz markers, Santa Cruz Biotechnology). EtOH: alcoholic liver disease; HCC: hepatocellular carcinoma; HCV: chronic hepatitis C infection; HBV: chronic hepatitis B infection; AIH: autoimmune hepatitis.

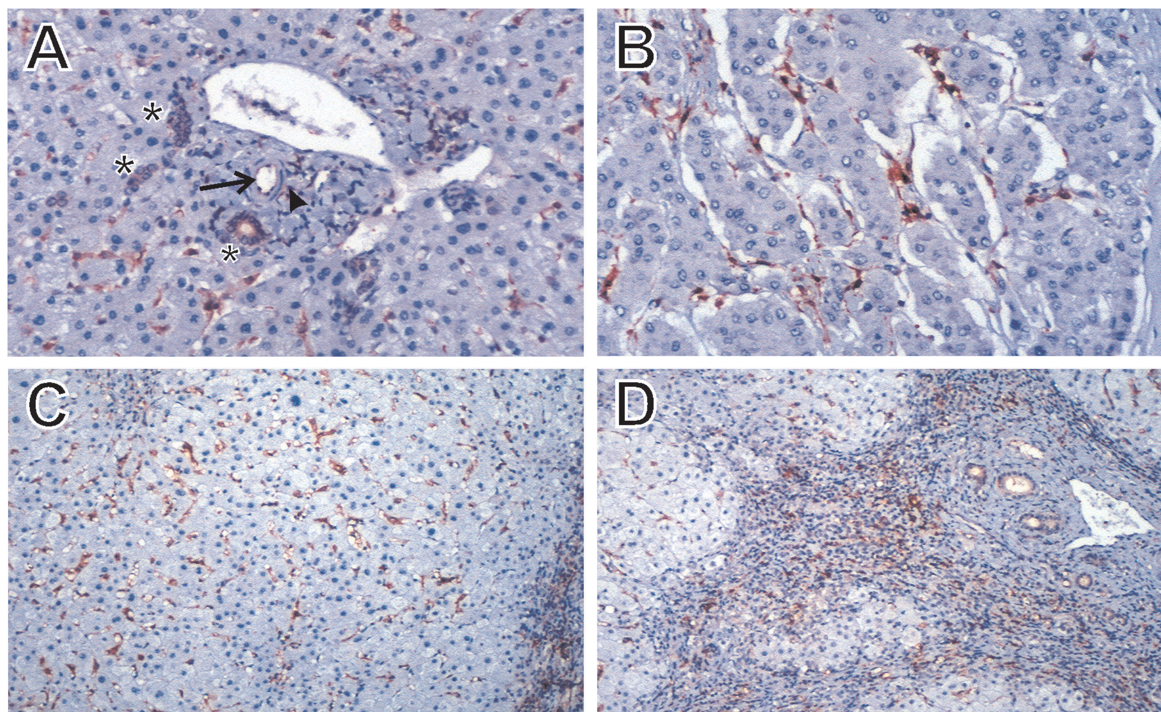

Fig. 3. Immunoreactivity for aldose reductase is localized to sinusoidal lining cells and portal tract structures in histologically normal livers. In cirrhotic livers, abundant aldose reductase-positive cells are also present in the fibrotic septa. **A**. Section from histologically normal liver incubated with rabbit polyclonal antibody to aldose reductase. Primary antibody was detected as red reaction product using an avidin-biotin-peroxidase system as described in Methods. In addition to the immunoreactive sinusoidal lining cells, the epithelium of bile ductules (indicated by asterisks), endothelium of the hepatic arteriole (long arrow), and occasional stromal cells (arrowhead) within the portal tract are also aldose-reductase positive. **B.** Section from an hepatocellular carcinoma stained as described above demonstrates immunoreactivity of sinusoidal lining cells for aldose reductase. **C.** Lower-power view of a nodule within a cirrhotic liver demonstrating sinusoidal lining cell immunoreactivity. **D.** Lower-power view of fibrotic septa in a cirrhotic liver illustrating abundant aldose reductase-positive cells in fibrotic septa. Sections were counterstained with hematoxylin. Original magnification 20x (A, B); 10x (C,D).

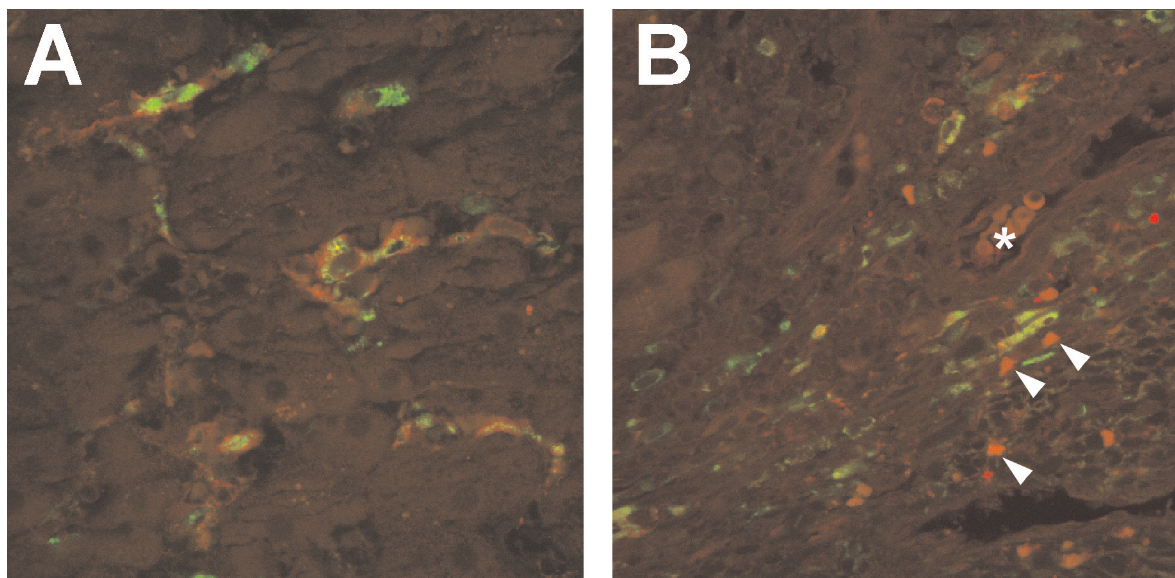

Fig. 4. Double immuno-fluorescence confocal microscopy demonstrates colocalization of aldose reductase and CD68 in human Kupffer cells. **A.** Numerous cells expressing both CD68+ (green staining) and aldose reductase (red staining) are seen along the sinusoids of human liver. **B.** In fibrotic septa, most of the aldose-reductase-positive cells are also CD68-positive, with a smaller number of CD68-negative, aldose reductase-positive cells (arrowheads). In the upper right-hand corner, aldose reductase-positive erythrocytes are present within a vascular channel (asterisk). Original magnification, x 60

presence of aldose reductase in human liver. In this report, we provide evidence that aldose reductase transcripts and protein are present at low levels in histologically normal human liver and that their abundance is increased in acute and chronic liver disease. Our observations regarding diseased liver are in agreement with those of O'Connor et al. who reported detection of immunoreactive aldose reductase in liver specimens from two individuals with alcoholic liver disease. However, in this previous study, Western blot analysis failed to detect aldose reductase in histologically normal human livers (O'Connor et al., 1999). The disparity between those results and our finding of detectable, albeit low, levels of aldose reductase in nondiseased livers is probably due to the greater quantity of protein used on our Western blots. Indeed, our observation that aldose reductase expression is restricted to quantitatively minor cell populations in normal liver, which collectively comprise only a small proportion of total hepatic RNA, protein, and enzyme activity, likely accounts for the negative or ambiguous results of previous studies.

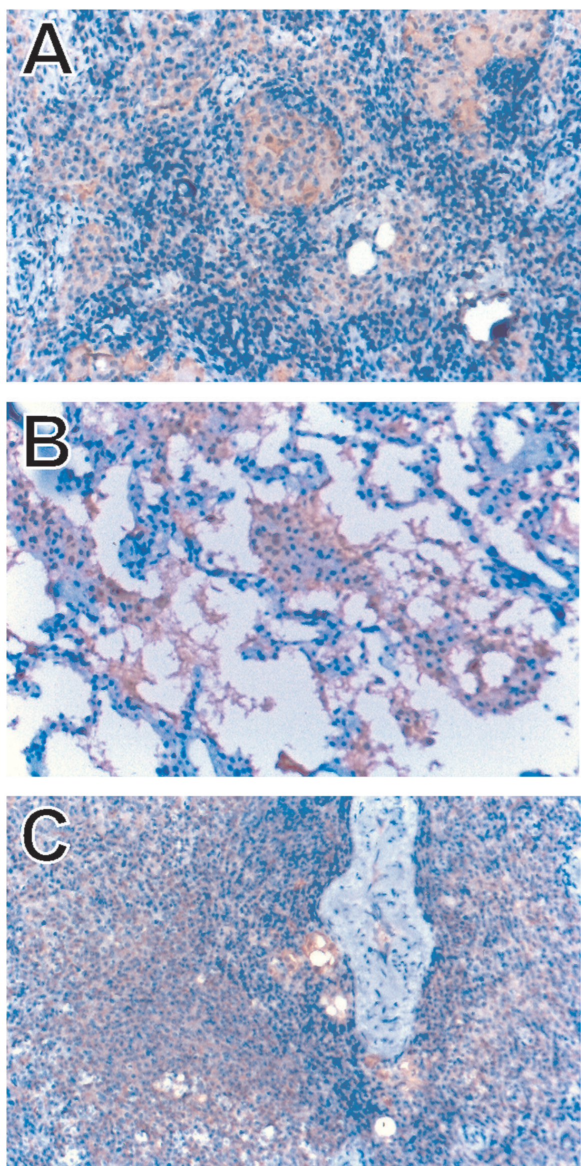

Fig. 5. Aldose reductase-positive macrophages are observed in extrahepatic human tissues. **A.** Lymph node. Macrophages in germinal centers and in sinuses demonstrate immunoreactivity with the aldose reductase antibody, while the surrounding lymphocytes are negative. **B.** Lung. In contrast to the non-reactive alveolar parenchymal cells, clusters of intra-alveolar macrophages shows immunoreactivity for aldose reductase. **C.** Spleen. This low-power photomicrograph highlights a large group of aldose reductase-positive cells, presumably macrophages, in the white pulp of the spleen. Sections were incubated with polyclonal rabbit antibody to aldose reductase. Primary antibody was detected as red reaction product using an avidin-biotin-peroxidase system as described in Methods. Original magnification: A, B, x 40; C, x 20

Our immunohistochemical studies demonstrate unequivocally that Kupffer cells are the major cell type expressing aldose reductase in normal human liver. In addition, aldose reductase is also present in biliary epithelium, nerves and the endothelium of hepatic arterioles. The increase in the immunoreactive enzyme in chronically diseased human livers appears to be due, at least in part, to the presence of large numbers of aldose reductase-positive cells in fibrotic septa, many of which are CD68+, and thus likely represent infiltrating monocytes/macrophages. However, given that *in vitro* studies have demonstrated induction of aldose reductase expression in some cell types exposed to 4HNE or prooxidants such as H_2O_2 (Spycher et al., 1996, 1997; Rittner et al., 1999), the possibility that the abundance of aldose reductase protein is increased on a per cell basis must also be considered.

Induction of aldose reductase by toxic metabolites generated by rapidly proliferating cells has also been postulated to account for overexpression of aldose reductase in hepatocellular carcinomas, a phenomenon that may contribute to resistance of these tumors to chemotherapy (Cao et al., 1998; Lee et al., 2001). In this context, the finding that aldose reductase immunoreactivity was not observed in the malignant hepatocytes, but rather in the sinusoidal lining cells of the tumor (Fig. 3B) is intriguing. Further studies are needed to determine the relationship between aldose reductase expression in hepatocellular carcinoma, the cellular localization of the enzyme and responsiveness to chemotherapy.

Like human liver, rat liver has been thought to contain little or no aldose reductase. Recently, however, using a proteomics approach, aldose reductase was identified in rat hepatic stellate cells, an important nonparenchymal cell type involved in hepatic fibrogenesis (Kristensen et al., 2000). In contrast, our immunohistochemical studies failed to identify aldose reductase in either quiescent or activated hepatic stellate cells in human liver. This may indicate species

differences in the cellular localization of aldose reductase in the liver. It is nonetheless interesting to note the parallel between our observations in human livers affected by a variety of inflammatory conditions and the finding that hepatic aldose reductase levels increase with the onset of hepatitis in Long-Evans cinnamon rats, a mutant strain that develops copper overload and is a model for Wilson's disease (Takahashi et al., 1996). More detailed evaluation of the cellular source/s of aldose reductase in rat liver is needed to determine whether rat liver is a relevant model for assessing the involvement of aldose reductase in hepatic injury.

To our knowledge, this is the first report demonstrating the presence of aldose reductase in macrophages in a variety of human tissues. Aldose reductase was previously found in macrophages in inflamed temporal arteries where it was proposed that de novo expression of the enzyme was induced by exposure to aldehydes generated in the inflammatory milieu (Rittner et al., 1999). Our findings extend those observations by demonstrating that aldose reductase is present in the resident macrophages of human liver even in the absence of inflammation or injury. Furthermore, we show that macrophages in several extrahepatic tissues are also aldose reductase-positive. Taken together, these observations suggest that aldose reductase expression is a common feature of human macrophages and support the proposal of Rittner et al. that the aldose reductase may be involved in immune and/or inflammatory processes.

Further studies will be needed to determine the role played by aldose reductase in macrophage function. In this context, it is interesting to consider our recent results indicating that aldose reductase activity is involved in signaling in response to cytokines and growth factors. For example, the proliferative response of vascular smooth muscle cells exposed to mitogens is reduced by inhibition of aldose reductase by pharmacologic means or by treatment with antisense oligonucleotides. This effect is associated with decreased activation of protein kinase C and diminished activation and nuclear translocation of NF-κB (Ramana et al., 2002). Similar effects on NF-κB signaling are observed with aldose reductase inhibition in human lens epithelial cells exposed to hyperglycemia or tumor necrosis factor-a, which results in prevention of apoptosis in response to these treatments (Ramana et al., 2003). It is tempting to speculate that aldose reductase may similarly influence activation of NF-κB in macrophages, based on the observation that IL-1 production by LPS-stimulated human monocytes was significantly reduced by ponalrestat, an aldose reductase inhibitor (Kawamura et al., 1999). If this speculation is correct, it is reasonable to propose that inhibition of aldose reductase might be a useful therapeutic strategy in some inflammatory conditions, including those affecting the liver where Kupffer cells are an important source of NF-κB-regulated inflammatory mediators (Gaweco et al., 2000; Nanji, 2002).

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References

Bhatnagar A., Ruef J., Liu S.Q., Srivastava S. and Srivastava S.K. (2001). Regulation of vascular smooth muscle cell growth by aldose reductase. Chemico-Biological Interactions 130-132, 627-636.

Brown K.E. (2000. An overview of hepatic fibrogenesis. Viral Hep. Rev. 6, 5-27.

Brownlee M. (2001). Biochemistry and molecular cell biology of diabetic complications. Nature 414, 8813-8820.

Cao D., Fan S.T. and Chung S.S.M. (1998). Identification and characterization of a novel human aldose reductase-like gene. J. Biol. Chem. 273, 11429-11435.

Esterbauer H., Schaur R.J. and Zollner H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical. Biol. Med. 11, 81-128.

Gaweco A.S., Wiesner R.H., Porayko M., Rustgi V.K., Yong S., Hamdani R., Harig J., Chejfec G., McClatchey K.D. and Van Thiel D.H. (2000). Intragraft localization of activated nuclear factor κB in recurrent hepatitis C virus disease following liver transplantation. Hepatology 31, 1183-1191.

Hers H.G. (1960). L'aldose reductase. Biochim. Biophys. Acta 37, 120-126.

Kawamura I., Lacey E., Yamamoto N., Sakai F., Takeshita S., Inami M., Nishigaki F., Naoe Y., Tsujimoto S., Manda T., Shimomura K. and Goto T. (1999). Ponalrestat, an aldose reductase inhibitor, inhibits cachexia syndrome induced by colon26 adenocarcinoma in mice. Anticancer Res. 19, 4105-4112.

Kladney R.D., Cui X., Bulla G.A., Brunt E.M. and Fimmel C.J. (2002). Expression of GP73, a resident Golgi membrane protein, in viral and nonviral liver disease. Hepatology 35, 1431-1440.

Kristensen D.B., Kawada N., Imamura K., Miyamoto Y., Tateno C., Seki S., Kuroki T. and Yoshizato K. (2000). Proteome analysis of rat hepatic stellate cells. Hepatology 32, 268-277.

Lee K.W.Y., Ko B.C.B., Jiang Z., Cao D. and Chung S.S.M. (2001). Overexpression of aldose reductase in liver cancers may contribute to drug resistance. Anti-Cancer Drugs 12, 129-132.

Nanji A. (2002). Role of Kupffer cells in alcoholic hepatitis. Alcohol 27, 13-15.

Nishimura C., Graham C., Hohman T.C., Nagata M., Robinson W.G. and Carper D. (1988). Characterization of mRNA and genes for aldose reductase in rat. Biochem. Biophys. Res. Commun. 153, 1057-1059.

O'Connor T., Ireland L.S., Harrison D.J. and Hayes J.D. (1999). Major differences exist in the function and tissue-specific expression of human aflatoxin B_1 aldehyde reductase and the principal human aldo-keto reductase AKR1 family members. Biochem. J. 343, 487-504.

Ramana K.V., Chandra D., Srivastava S., Bhatnagar A., Aggarwal B.B. and Srivastava S.K. (2002). Aldose reductase mediates mitogenic signaling in vascular smooth muscle cells. J. Biol. Chem. 277, 32063-32070.

Ramana K.V., Friedrich B., Bhatnagar A. and Srivastava S.K. (2003). Aldose reductase mediates cytotoxic signals of hyperglycemia and TNF-α in human lens epithelial cells. FASEB J. 17, 315-317.

Ramasamy R., Oates P.J. and Schaefer S. (1997). Aldose reductase inhibition protects diabetic and nondiabetic rat hearts from ischemic injury. Diabetes 46, 292-300.

Rittner H.L., Hafner V., Klimiuk P.A., Szweda L.I., Goronzy J.J. and Weyand C.M. (1999). Aldose reductase functions as a detoxification system for lipid peroxidation products in vasculitis. J. Clin. Invest. 103, 1007-1013.

Ruef J., Liu S.Q., Bode C., Tocchi M., Srivastava S., Runge M.S. and Bhatnagar A. (2000). Involvement of aldose reductase in vascular smooth muscle cell growth and lesion formation after arterial injury. Arterioscler. Thromb. Vasc. Biol. 20, 1745-1752.

Siems W.G., Zollner H., Grune T. and Esterbauer H. (1997). Metabolic fate of 4-hydroxynonenal in hepatocytes: 1,4-dyhydroxynonene is not the main product. J. Lipid Res. 38, 612-622.

Spycher S.E., Tabataba-Vakili S., O'Donnell V.B., Palomba L. and Azzi A. (1996). 4-Hydroxy-2-nonenal induces transcription and expression of aldose reductase. Biochem. Biophys. Res. Commun. 226, 512-516.

Spycher S.E., Tabataba-Vakili S., O'Donnell V. B., Palomba L. and Azzi A. (1997). Aldose reductase induction: a novel response to oxidative stress of smooth muscle cells. FASEB J. 11, 181-188.

Srivastava S.K., Ansari N.H., Nair G.A. and Das B. (1984). Aldose and aldehyde reductases in human tissues. Biochim. Biophys. Acta 800, 220-227.

Srivastava S., Chandra A., Bhatnagar A., Srivastava S. K. and Ansari N. H. (1995). Lipid peroxidation product, 4-hydroxynonenal and its conjugate with GSH are excellent substrates of bovine lens aldose reductase. Biochem. Biophys. Res. Comm. 217, 741-746.

Takahashi M., Hoshi A., Fujii J., Miyoshi E., Kasahara T., Suzuki D., Aozasa K. and Taniguchi N. (1996). Induction of aldose reductase gene expression in LEC rats during the development of hereditary hepatitis and hepatoma. Jpn. J. Cancer Res. 87, 337-341.

Tracey W.R., Magee W.P., Ellery C.A., MacAndrew J.T., Smith A.H., Knight D.R. and Oates P.J. (2000). Aldose reductase inhibition alone or combined with an adenosine A_3 agonist reduces ischemic myocardial injury. Am. J. Physiol. Heart. Circ. Physiol. 279, H1447-H1452.

Tulsiani D.R.P. and Touster O. (1977). Resolution and partial characterization of two aldehyde reductases of mammalian liver. J. Biol. Chem. 252, 2545-2550.

Vander Jagt D.L., Hunsaker L.A., Robinson B., Stangebye L.A. and Deck L.M. (1990). Aldehyde and aldose reductase from human placenta. J. Biol. Chem. 265, 10912-10918.

Vander Jagt D.L., Kolb N.S., Vander Jagt T.J., Chino J., Martinez F.J., Hunsaker LA. and Royer R.E. (1995). Substrate specificity of human aldose reductase: identification of 4-hydroxynonenal as an endogenous substrate. Biochim. Biophys. Acta 1249, 117-126.

Wirth H-P. and Wermuth B. (1984). Immunohistochemical localisation of aldehyde and aldose reductase in human tissues. Progr. Clin. Biol. Res. 74, 231-239.

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