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Induction of Aldose Reductase Gene Expression in LEC Rats during the Development of the Hereditary Hepatitis and Hepatoma

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We examined age-related changes in the protein and the mRNA expression of aldose reductase in livers of Long-Evans with a cinnamon-like color (LEC) rats, which develop hereditary hepatitis and hepatoma with aging, using Long-Evans with an agouti color rats as controls. The levels of the protein and mRNA of aldose reductase increased after 20 weeks, at the stage of acute hepatitis, and were maintained at 60 weeks of age, while those of aldehyde reductase seemed to be constant at all ages. The expression of aldose reductase was marked in cancerous lesions in hepatoma-bearing LEC rat liver compared to uninvolved surrounding tissues. These results indicated that elevation of aldose reductase accompanied hepatocarcinogenesis and may be related to the acquisition of immortality of the cancer cells through detoxifying cytotoxic aldehyde compounds.

Key words: Aldose reductase — Aldehyde reductase — LEC rat — Hepatocarcinogenesis

Tumor cells are known to have defense mechanisms against toxic agents, and changes in the activity of some enzymes metabolizing toxic agents have been reported. Increases in aldehyde dehydrogenase activity relative to normal liver have been a consistent finding in both hepatocellular carcinomas and hepatoma cell lines.[1,2] NADPH-dependent aldehyde-reducing activities are also elevated.[3,4] In previous studies from our group[5] and by others[6] it was found that aldose reductase gene expression is increased in rat hepatoma induced by 3′-methyl-4-dimethyl-aminoazobenzene, and other hepatoma cell lines. Several enzymes in the aldo-keto reductase superfamily,[7] including aldose reductase, were found to be important for cell resistance to the cytotoxicity of compounds with an aldehyde moiety.[8–10] Induction of aldose reductase gene expression during hepatocarcinogenesis may render cancer cells resistant to various toxic carbonyl compounds produced during metabolism or to administered anti-cancer drugs.

The LEC[5] rat is a mutant strain that was established from a closed colony of the Long-Evans strain. The hepatic changes in LEC rats are closely associated with copper accumulation in the liver.[11] Deletion of the coding region at the 3′ end of the copper transporting ATPase gene (Atp7b), which is the rat homologue of the Wilson's disease gene (ATP7B), is thought to be a cause of copper accumulation in the LEC rat liver.[12] The mutant rats spontaneously develop acute hepatitis at 12–16 weeks of age, followed by chronic hepatitis, and eventually spontaneous hepatocellular carcinoma.[13] Thus, the LEC rat is regarded as both a model for Wilson's disease and a good experimental model for hepatocarcinogenesis.

Changes in hepatic drug-metabolizing enzymes in the LEC rat during hepatitis and hepatoma are similar to those seen during chemical carcinogenesis.[14–16] It is therefore of interest to determine how levels of aldose reductase gene expression are linked to the development of hereditary hepatitis and hepatoma. In this study we investigated the expression of aldose reductase protein and mRNA during the hepatocarcinogenic process in LEC rats.

MATERIALS AND METHODS

Animals Animals were maintained at the Institute of Experimental Animal Science, Osaka University Medical School. All research procedures complied with the ethical standards of the Helsinki Declaration in 1975. Male rats were anesthetized with diethylether and then dissected. Samples were immediately immersed in liquid nitrogen and preserved at −80°C until used.

Materials [α-^{32}P]dCTP and an oligolabeling kit were obtained from Amersham Corp. (Buckinghamshire). NADPH was from Boehringer Mannheim (Mannheim). All other reagents were of the highest analytical grade available.

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[5] Abbreviations used: LEC, Long-Evans with a cinnamon-like color; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; SSC, standard saline-citrate; LEA, Long-Evans with an agouti color.

SDS-PAGE and immunoblotting of the protein Various tissue samples and hepatoma tissues were homogenized in 4 volumes of PBS with a Polytron homogenizer. After centrifugation at 105,000*g* for 60 min, the supernatant fraction was used for further analyses. Protein concentrations were determined by the Bradford method.[17] Proteins were fractionated by 10% SDS-PAGE according to Laemmli[18] and then transferred onto nitrocellulose membranes using a Sartoblot IIS semi-dry electroblotter (Sartorius, Goettingen, Germany). The blots were blocked in 4% bovine serum albumin for 30 min, then incubated for 2 h at room temperature with a 1:25 dilution of anti-rat lens aldose reductase serum[5] or anti-rat liver aldehyde reductase serum.[19] The nitrocellulose membranes were washed three times for 10 min each, then the blots were incubated with 1:1000 diluted, affinity-purified, peroxidase-conjugated goat anti-rabbit IgG (Dako, Glostrup, Denmark). The immunoblots were again washed three times in PBS and developed in PBS using 4-chloro-1-naphthol as a substrate.

RNA preparation and Northern blotting The cDNA fragments encoding rat aldose reductase described previously[5] were used as probes. Total RNA was prepared from rat liver preserved at −80°C according to Chomczynski and Sacchi,[20] and quantitated by measuring the absorbance at 260 nm. Thirty μg of total RNA was heat-denatured at 65°C for 15 min in the presence of 50% formamide and the running gel buffer (40 m*M* morpholinopropanesulfonic acid (Mops), 10 m*M* sodium acetate, and 1 m*M* EDTA, pH 7.0), and then electrophoresed on 1% agarose gel containing 2.2 *M* formaldehyde. The size-fractionated RNAs were transferred onto Zeta-Probe membranes (Bio-Rad, Richmond, CA) for 20 to 40 h by capillary action, and the blotted RNAs were immobilized on the membranes by incubation for 1 h at 80°C. After hybridization with a ^{32}P-labeled aldose reductase cDNA probe at 42°C in the presence of 50% formamide, the membranes were washed twice at 55°C with 2× SSC and 0.1% SDS for 80 min. The Kodak XAR films were exposed for 1–3 days with an intensifying screen at −80°C. The blots were stripped and reprobed with a rat aldehyde reductase cDNA[19] using the hybridization and washing conditions described above.

RESULTS

Tissue distribution of aldose reductase and aldehyde reductase in LEC and LEA rats Because LEC rats develop hepatitis followed by spontaneous hepatocellular carcinoma,[13] livers from LEC rats were examined at various developmental stages after birth. LEA rats, which are a sibling line of LEC rats, but do not develop hepatitis or hepatocellular carcinoma, were used as controls in some experiments. Immunoblot analysis was carried out for some tissues from LEC rats and LEA rats using anti-rat lens aldose reductase serum[5] and anti-rat liver aldehyde reductase serum.[19] In LEA rats at 47 weeks of age, aldose reductase was detected in all tissues except for liver (Fig. 1), as reported in other strains.[5,21] The liver of LEC rats at 40 weeks of age, however, expressed a significant amount of aldose reductase. There was essentially no difference observed in aldehyde reductase expression between LEC and LEA rats.

Age-related changes of aldose reductase gene expression in LEC rat liver We then examined expression levels of aldose reductase and aldehyde reductase in the livers of LEC and LEA rats at different ages (Fig. 2). While the level of aldehyde reductase was similar in LEC and LEA rat livers, prominent differences were observed in aldose reductase. In LEC rat liver, a marked increase in aldose reductase was found at 23 weeks and 40 weeks, while it was undetectable in LEA rat liver. Thus, the expres-

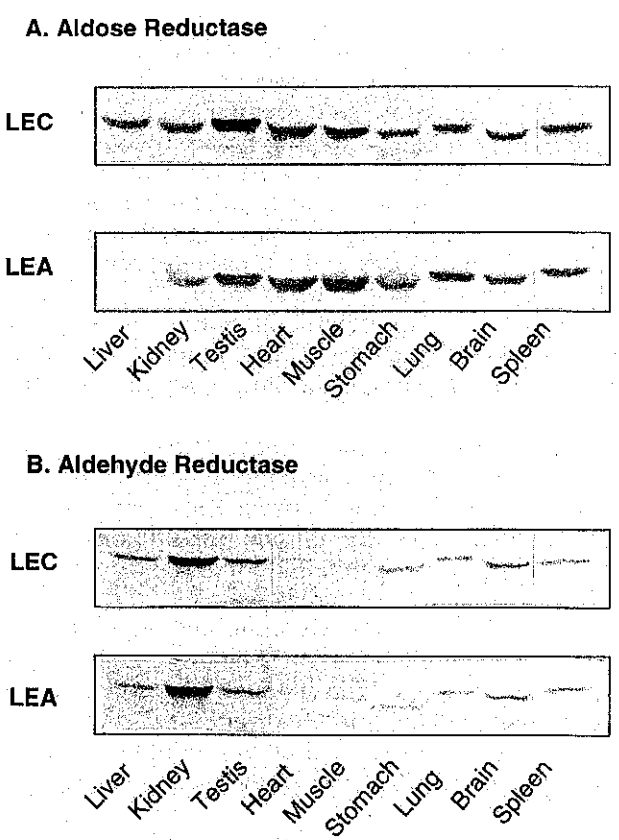

Fig. 1. Immunoblot analysis of proteins from various tissues of LEC and LEA rats. Protein (40 μg) from tissues of LEC at 40 weeks and LEA at 47 weeks of age was subjected to immunoblot analysis. Antisera against rat aldose reductase (A) or rat aldehyde reductase (B) were used as the primary antibodies.

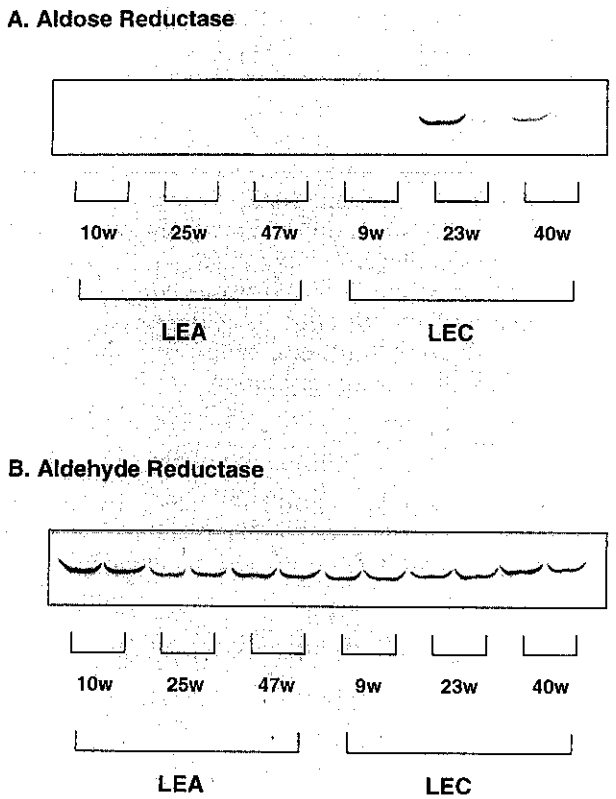

Fig. 2. Immunoblot analysis of proteins from livers of LEC and LEA rats at different stages. Immunoblotting was carried out in duplicate for each age of rats using antisera against rat aldose reductase (A) or rat aldehyde reductase (B) as described in the legend to Fig. 1.

sion of aldose reductase occurred at the stage of acute hepatitis and was maintained until the stage of chronic hepatitis.

Detection of aldose reductase gene expression by Northern blot analysis To examine aldose reductase gene expression at the transcriptional level, we examined the mRNA by Northern analysis of total RNAs isolated from livers of LEC and LEA rats, using an aldose reductase cDNA as a probe. Fig. 3 shows that the aldose reductase mRNA was induced in LEC rat liver at 16 weeks of age, with high expression in cancerous lesions and lower expression in non-cancerous lesions. No changes, however, were observed in LEA rat liver up to 47 weeks of age.

DISCUSSION

Because LEC rats spontaneously develop hepatitis followed by hepatocellular carcinoma,[13] they provide a unique system with which to investigate the development of the diseases and accompanying changes in gene expression. In this study, we found that in LEC rats the aldose reductase gene was highly expressed during the development of the hereditary hepatitis and hepatoma in comparison with LEA rat controls. A dramatic increase in gene expression was observed at 16 weeks of age, which corresponds to the stage of development of acute hepatitis. A large population of affected hepatocytes dies at this stage. This is followed by regeneration of the liver. Kupffer cells are scavenger cells in the liver, and proliferate in LEC rats at the stage of acute hepatitis.[22] Since var-

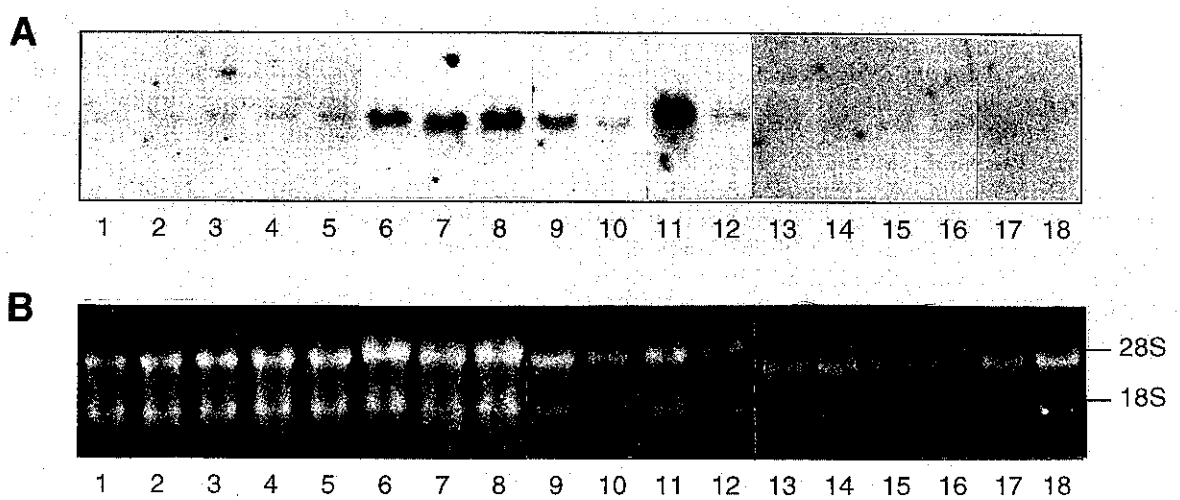

Fig. 3. Northern blot analysis of RNA from LEC and LEA rat livers. LEC rat livers at 8 (lanes 1–3), 9 (lanes 4 and 5), and 16 weeks of age (lanes 6–8), cancerous lesion (lane 9) and non-cancerous lesion (lane 10) at 56 weeks of age, cancerous lesion (lane 11) and non-cancerous lesion (lane 12) at 60 weeks of age, and LEA rat livers at 13 (lanes 13 and 14), 25 (lanes 15 and 16), and 47 (lanes 17 and 18) weeks of age. Total RNA (30 μg) was separated on 1% agarose gel followed by staining with ethidium bromide (B). Northern blotting was carried out using rat aldose reductase cDNA (A).

ious enzymes belonging to the aldo-keto reductase gene superfamily are known to be induced by, and to catalyze reduction of, cytotoxic aldehydes,[8-10] the enhanced expression of aldose reductase should protect cells from such aldehyde compounds. A high level of this enzyme was maintained up to the stage of cancer development.

It is well known that aldose reductase gene expression is regulated by hyperosmotic pressure,[23] and enhanced by hyperglycemia occurring during diabetes mellitus.[24] Although the promoter regions of rat and human aldose reductase genes have been cloned and partially characterized, the underlying mechanism of gene regulation is not yet clear. In cancerous lesions, angiogenesis is up-regulated to supply oxygen and energy sources. Because the metabolism in cancer cells is augmented, resulting in more glucose and oxygen consumption, there is an enhanced production of aldehyde compounds. This may explain the enhanced aldose reductase expression in such cells, for the detoxification of cytotoxic compounds, as we previously hypothesized.[5]

In addition to changes in the aldose reductase gene, LEC rats display various abnormalities such as age-related changes in glycosyltransferase,[25] in the expression of drug-metabolizing enzymes,[14-16] and in DNA methyltransferase.[26] Abnormal maturation of T cells[27] and selective suppression of IgG2a subclass in LEC rats are also observed.[28] The simplest explanation for both these observations is that a common mechanism participates in the gene regulation. Some of these alterations in LEC rats might result from copper accumulation in the liver, as a consequence of a defect in the copper-transporting ATPase gene Atp7b.[12] However, they might only be secondary effects of other gene products, such as cytokines and growth factors, which are induced by heavy metal ions or redox regulation.

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