Alcohol dehydrogenase in human tissues: localisation of transcripts coding for five classes of the enzyme

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Abstract Tissue distribution of the five identified classes of human alcohol dehydrogenase was studied by assessment of mRNA levels in 23 adult and four fetal tissues. Alcohol dehydrogenase of class I was found in most tissues, brain and placenta excluded, but expression levels among tissues differed widely. The distribution pattern of class III transcripts was consistent with those of housekeeping enzymes while, in contrast, class IV transcripts were found only in stomach. Transcripts of multiple length were detected for most classes and were due to different gene products arising through the use of different poly-A signals or transcription from different gene loci. Both class II and class V showed a pattern of liver-enriched expression. However, low mRNA levels were detected also in stomach, pancreas and small intestine for class II, and in fetal kidney and small intestine for class V. Significantly higher levels of class V transcripts were present in fetal liver when compared with levels in adult liver, which suggests that human class V is a predominantly fetal alcohol dehydrogenase.

Key words: Human alcohol dehydrogenase; Human; Fetal tissue

1. Introduction

Mammalian alcohol dehydrogenases (ADH, EC 1.1.1.1) comprise a family of enzymes divided into six classes according to function and structure [1]. Today, five classes of human ADH have been identified and an additional class has been defined in rodents [2,3]. Enzymes of ADH classes are capable of ethanol metabolism but display broad substrate specificities, and have been suggested to play a role in cellular detoxification mechanisms [4,5]. Class I, the classical ADHs, are abundant in liver and are the major enzymes in the first step of ethanol metabolism. This class is subdivided into isozymes consisting of three different subunits. The α-subunit is expressed during early fetal development while the expression of the β-subunit appears by midgestation, and the γ-subunit is postnatally expressed [6]. The γ-subunit is the type of human class I ADH that most closely relates to other non-primate mammalian class I ADHs.

Class II ADH activity has been found mainly in liver and the human enzyme shows a high K_m value for ethanol [7]. Class III ADH, identical to glutathione-dependent formaldehyde dehydrogenase, is a poor ethanol dehydrogenase but has been proposed to play a significant role in cytoprotection by metabolism of formaldehyde [8]. Class IV is extra-hepatically expressed with high ethanol metabolising activity in stomach

mucosa [9,10] and so far class V has only been isolated at the DNA level and characterised as a recombinantly expressed enzyme [11,12].

Several studies have reported on the tissue occurrence of ADHs in human tissues [6,10,13–19]. These studies have been carried out at both the transcript and protein levels but have the disadvantage that they, in a single experiment, delineate the distribution of one or a few classes in a restricted number of tissues. Most of the distribution data is available on ADH enzymes of classes I, III and IV, although a total of five different ADH classes are recognised at present. To now further extend the knowledge on the relative tissue occurrence of all human ADHs, we have examined mRNA levels for ADH of classes I–V in an extensive number of adult and fetal tissues, all potential targets of direct or indirect toxic effects of ethanol.

2. Materials and methods

2.1. Cloning of cDNAs encoding ADH of classes IV and V

The class IV ADH cDNA was PCR amplified from human stomach cDNA (Quick-clone, Clontech) using the primers 5′-TTTCA-TATGGGCACTGCTGGAAAAGTTA-3′ and 5′-TTTGGATCCT-CAAAACGTCAGGACCGTTC-3′, designed according to the published gene sequence [20]. Amplified cDNA was cloned into the pCRII vector (Invitrogen) using the TA cloning method [21].

An internal 500-bp fragment of the cDNA coding for human class V ADH [11] was PCR amplified from a human liver cDNA library (Clontech). A 5′ primer, 5′-CCTGAATTCTGAGGGCAATTTT-GT-3′, and a 3′ primer, 5′-GCGAATTCCAATGGCCTCAAAGCA-GAA-3′, were used. Both primers contained *Eco*RI sites for cloning into pEMBL9 vectors. DNA sequence analysis was performed on both PCR products to confirm the authenticity of the cDNAs.

2.2. Northern-blot analysis

Northern-blot filters, containing approximately 2 μg poly-A-enriched RNA from each tissue, were purchased and used according to the recommendations by the manufacturer (Clontech). Class I mRNA was probed with a 400-bp *Bst*EII cDNA fragment from a class I γ cDNA clone [22], and class II with a 440-bp *Taq*I fragment from a class II cDNA clone [23]. A 390-bp *Eco*RI fragment [24] and a 400-bp *Eco*RI fragment ([11], cf. above) served as probes for class III and class V, respectively. Utilising the PCR technique, a 689-bp class IV probe was amplified from the isolated class IV cDNA, using the primers 5′-CAGAAGCAACCCTTCTCCATTG-3′ and 5′-GGTA-GAGTCCTTGGGACTGATAC-3′. In addition, all filters were probed with a 2-kb fragment of human β-actin cDNA. All probes were labelled with [α-^{32}P]dCTP to a specific activity of 5×10^8 cpm/μg (*redi*prime DNA labelling system; Amersham). Hybridisations were performed at 42°C overnight in 50% formamide, 5×SSPE, 10×Denhardt's solution, 2% SDS and 1 mg/ml salmon sperm DNA. Filters were washed with high stringency (0.1×SSC, 0.1% SDS, at 42°C) before exposure to Kodak X-OMAT films for 12–118 h using intensifying screens. The cDNA probes were removed from the filters by washing in 0.1×SSC, 0.5% SDS at 95°C for 10 min before rehybridisation for a new ADH class. To ensure removal of probes the filters were subjected to autoradiography for 46 h between the hybridisations.

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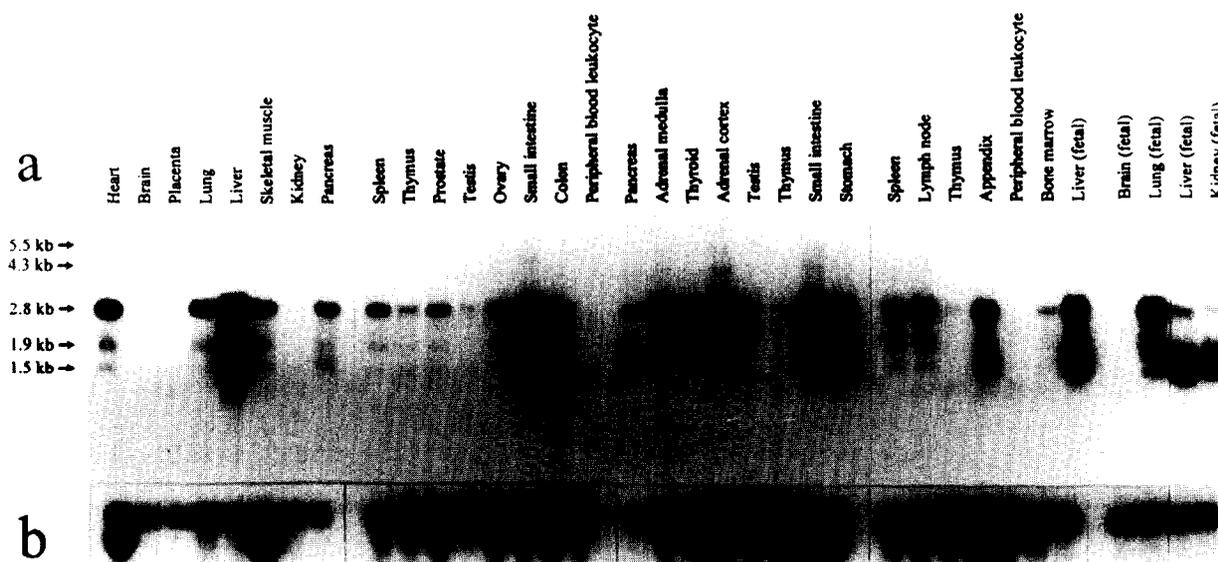
Fig. 1. Northern-blot analysis of class I ADH in human tissues. A: Filters were hybridised with a 400-bp cDNA fragment coding for the γ-subunit of class I. X-ray films were exposed for 44 h. B: Filters were hybridised with cDNA coding for β-actin and X-ray films were exposed for 12 h.

3. Results and discussion

Tissue distribution of the five known classes of human alcohol dehydrogenase was assessed by hybridising filters containing mRNA from 23 adult and four fetal tissues with cDNA probes specific for ADH transcripts of classes I–V (Figs. 1–5). As a control, filters were also probed with a cDNA coding for constitutivly expressed β-actin (Fig. 1b).

Class I transcripts showed a wide distribution, although expression levels varied considerably among different tissues (Fig. 1a). Adult liver showed the highest levels of class I ADH mRNA, but large amounts were also found in stomach, small intestine, colon, adrenal cortex, heart, as well as fetal lung, liver and kidney. In total, transcripts of five sizes were found. Three of these were represented by strong autoradiography signals at 1.5, 1.9 and 2.8 kb, while signals corresponding to 4.3 and 5.5 kb were faint. The probe used for class I ADH, cross-hybridised with transcripts originating from all three class I loci (ADH1-3), which give rise to the α-, β- and γ-subunits [25]. The different sizes of class I transcripts can be explained by transcription from the three different loci and the use of alternative poly-A signals [14,15,26]. A previous study on class I isozyme expression in liver has shown that only the ADH2 locus, coding for the β-subunit, gives rise to transcripts of about 2.8 kb [14]. This implies that the ββ-isozyme is the major class I isozyme in heart, skeletal muscle, pancreas, spleen, prostate, ovary, adrenal medulla, adrenal cortex, thyroid, small intestine, lymph node and lung (Fig. 1a), because the 2.8 kb transcripts predominate in these tissues. The lung has been proposed to be a site for significant extra-hepatic ethanol metabolism, and it has been shown that the ββ-isozyme is the only abundant ADH form in this tissue at all stages of development [6,27]. In the testis, only very faint class I signals were detected. This is in accordance with previous results showing that testicular homogenates have only class III ADH activity [16].

The mRNA coding for the fetally expressed α-subunit corresponds to the 1.5 kb transcript, but message of this size is also shared by mRNA coding for the γ-subunit. The apparent differences in tissue specific expression from the three ADH1-3
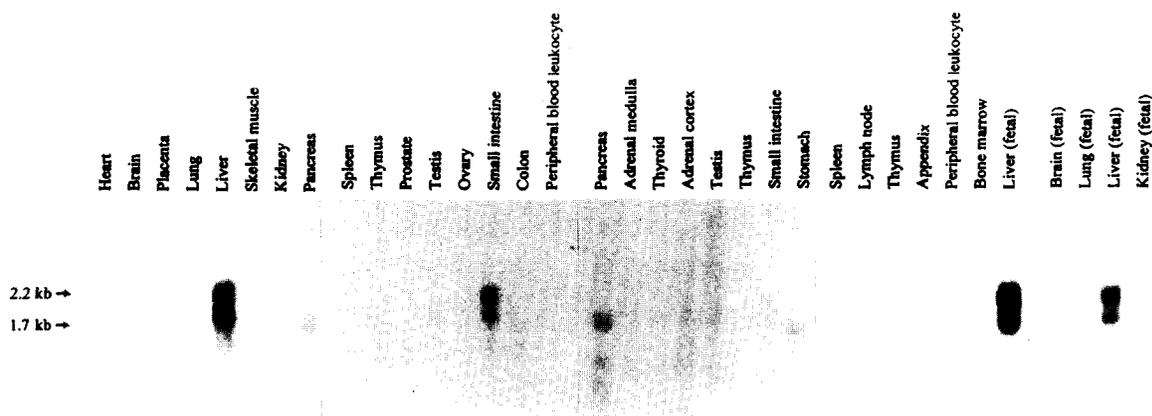
Fig. 2. Northern-blot analysis of class II ADH. Filters were hybridised with a 440-bp cDNA fragment coding for human class II ADH. X-ray films were exposed for 94 h.
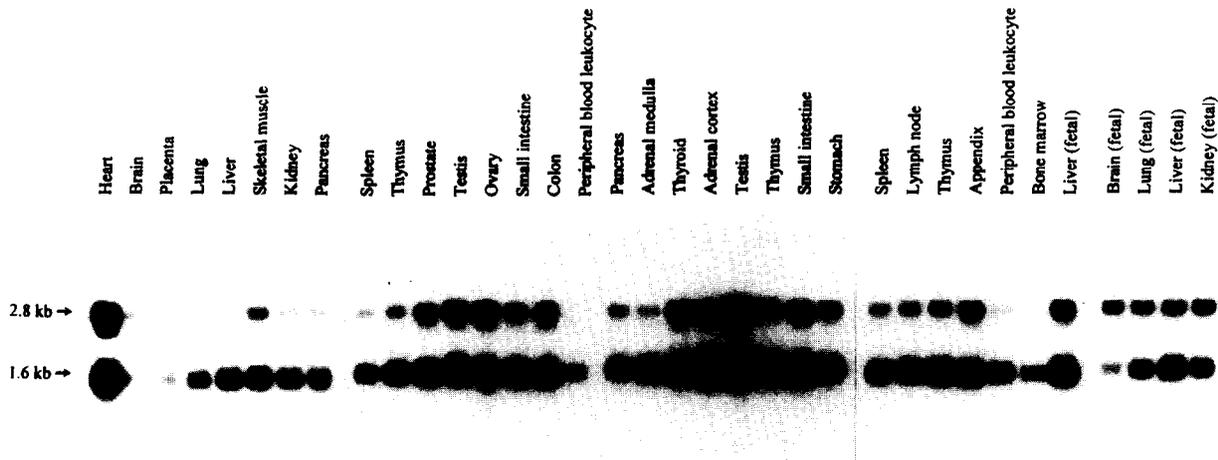
Fig. 3. Northern-blot analysis of class III ADH. Filters were hybridised with a 390-bp cDNA fragment coding for human class III ADH. X-ray films were exposed for 46 h.

loci may be explained by small differences in their proximal promoters, which affect binding affinities for transcription factors [28]. Class I transcripts were detected in fetal kidney, but not in in adult kidney. The lack of class I ADH mRNA in adult kidney is in agreement with a recent study [19], but stands in contrast to both an earlier study which showed the presence of class I ADH ββ-isozyme in human kidney [6] and to a report on tissue-specific gene expression [28].

ADH catalysis of ethanol yields the toxic product acetaldehyde which is further metabolised mainly by aldehyde dehydrogenase (ALDH). It is of interest to notice that the cytosolic aldehyde dehydrogenase, ALDH1, is expressed in similar tissues as class I ADH, while transcripts of the mitochondrial form, ALDH2, are present in every investigated tissue with the exception of placenta [29]. In addition, as found with class I ADH, high levels of ALDH2 expression were found only in the liver.

Control hybridisations with a β-actin probe confirmed that fairly equal amounts of mRNA were present on the filters (Fig. 1b). Pancreas, spleen, thymus, testis, small intestine, peripheral blood leukocyte and fetal liver were represented in duplicates on the filters and shared virtually identical hybridisation patterns when probed for expression of both β-actin and the different ADHs. Class I transcripts, however, differed between the two samples of fetal liver. This was most likely due to the different origin of these two mRNA samples. One

of the fetal liver blots (left fetal liver in Fig. 1a) represents a mRNA pool from 22–26-week fetuses, while the other blot (right fetal liver in Fig. 1a) represents mRNA from 18–24-week fetal livers. It is known that transcripts coding for the β-subunit appear by midgestation, and therefore the appearance of the 2.8 kb mRNA in the blot to the left can be explained.

As opposed to class I, mRNA of class II ADH was expressed mainly in liver, although faint signals could be seen for small intestine, pancreas and stomach (Fig. 2). Our present finding of transcripts in the human small intestine could agree with a previous investigation of class II in rat tissues which showed high levels of mRNA expression also in duodenum [30]. The class II enzyme can catalyse oxido-reduction of a broad range of alcohols and the corresponding aldehydes [7,31]. In addition, both quinone reduction and aldehyde dismutation activities have been observed for this ADH enzyme [32,33]. Because the liver is the major site for turnover of xenobiotic substrates, the limited tissue expression together with the broad substrate specificity imply a possible role for class II ADH in the metabolism of various exogenous compounds.

Class III showed quite a different distribution pattern than the other classes, with transcripts found in all analysed tissues. Inter-tissue variation was very low with the exception of brain and placenta, where levels were significantly lower than aver-
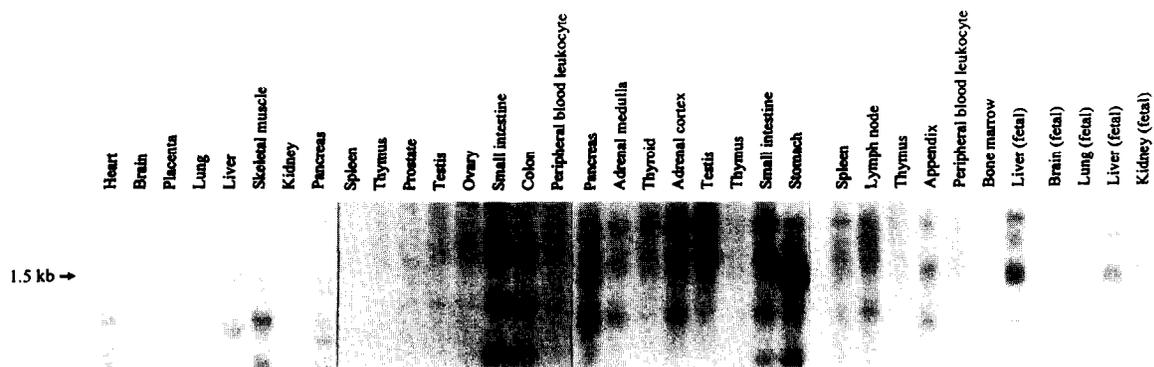
Fig. 4. Northern-blot analysis of class IV ADH. Filters were hybridised with a 689-bp PCR amplified cDNA fragment coding for human class IV ADH. X-ray films were exposed for 74 h.

Heart Brain Placenta Lung Liver Skeletal muscle Kidney Pancreas | Spleen Thymus Prostate Testis Ovary Small intestine Colon Peripheral blood leukocyte | Pancreas Adrenal medulla Thyroid Adrenal cortex Testis Thymus Small intestine Stomach | Spleen Lymph node Thymus Appendix Peripheral blood leukocyte Bone marrow | Liver (fetal) Brain (fetal) Lung (fetal) Liver (fetal) Kidney (fetal)

3.3 kb →

1.6 kb →

Fig. 5. Northern-blot analysis of class V ADH. Filters were hybridised with a 400-bp cDNA fragment coding for human class V ADH. X-ray films were exposed for 118 h.

age (Fig. 3). Like for ADHs of class II and class V, transcripts of two different sizes were found. The ratio between the two transcripts differs widely among tissues. In brain, transcripts of 2.8 kb, but not 1.6 kb, were found while the opposite was found for placenta. These differences in mRNA size are most likely due to the use of different poly-A signals [24], although the functional significance of this length polymorphism is not known. The 5' region of the *ADH5* gene, coding for class III ADH, shows typical characteristics of a constitutively expressed gene with many CpG doublets and lack of both TATA and CAAT boxes [34]. The housekeeping character of class III ADH is confirmed by its ubiquitous expression and is in accordance with its suggested role in formaldehyde scavenging [8,34,35].

In human tissue, class IV ADH activity has been detected mainly in stomach mucosa [9,10], but also in esophagus [17] gingiva and tongue [36]. Similarly, the rat class IV enzyme, denoted ADH-1, has been found in many organs including skin, eyes, ear mucose, nasal mucose, trachea, lungs, buccal mucose, tonge, esophagus, rectum, penis and vagina [37]. These results, like ours of class IV ADH (Fig. 4), suggest a mainly endo/epithelial localisation and are in agreement with earlier transcript distribution results [19]. However, a high background was obtained for the class IV Northern-blot analysis (Fig. 4), probably due to decrease in quality of the filters used after several rehybridisations. The expression pattern of class IV ADH has further been correlated to retinoic acid localisation in mouse [38] and the class IV enzyme may play an integral part of the metabolism of retinol [39].

The class V *ADH* gene has been cloned [11], but the function and substrate specificities of the corresponding enzyme are unknown. Low levels of class V transcript were detected in small intestine and fetal kidney. Significantly higher levels of mRNA, however, were detected in fetal liver as compared to adult liver (Fig. 5). This pattern of expression resembles that of the class I αα-isozyme [6] which strongly suggests that class V ADH is a fetal liver enzyme.

Taken together, the separate transcripts investigated for the human ADH classes display discrete patterns of tissue distribution and indicate that ADH activity is present in all human tissues. Most of the ADH classes were expressed at high levels in the liver, but whether these liver transcripts can be regarded

as representative of amounts of the corresponding enzyme activity remains to be revealed. Clearly, however, liver tissue accounts for the vast majority of ethanol metabolism in the human body, and it is the single tissue in which morphological changes occur early upon alcohol consumption. Although ADH and ALDH activities may be of importance in the concept of ethanol-induced hepatotoxicity, the setup of different classes of ADH with characteristic expression patterns argues in favour of a functional divergence and suggests additional specific functions for all ADH classes other than the oxidation of ingested alcohol.

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