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Acetaldehyde burst protection of ADH1B*2 against alcoholism: An additional hormesis protection against esophageal cancers following alcohol consumption?

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Abstract

This account of recent work presented at the 4th International Symposium on Alcohol Pancreatitis and Cirrhosis reports animal studies aimed at determining the role of the "acetaldehyde burst", generated shortly upon ethanol intake, as the mechanism of protection against alcoholism conferred by the ADH1B*2 polymorphism. Literature studies discussed suggest an additional role of the acetaldehyde burst on the paradoxical (hormesis) protection of the ADH1B*2 polymorphism against esophageal cancers in alcoholics.

A recent review of the alcohol and alcoholism field (Crabbe et al., 2006) addressed the relevance of 93 putative protective genetic polymorphisms against alcoholism. With such wealth of information the reviewers indicate: "*despite the importance of inherited contributions, we know for certain that only two genes affect the alcoholism risk*". These are the genes involved in the metabolism of alcohol and acetaldehyde.

Protection against alcoholism by alcohol dehydrogenase and aldehyde dehydrogenase polymorphisms

For historical reasons which may help understanding the direction that research in this field has taken, we start with the metabolism of acetaldehyde.

Early studies showed that a dominant point mutation in ALDH2 reduces by 85% the activity of the tetrameric enzyme ALDH2*2/ALDH2*1 (Crabb et al., 1989; Yoshida et al., 1984). Individuals who carry the *ALDH2*2* polymorphism are markedly protected (66 to 99 %) against alcohol abuse and alcoholism (Chen et al., 1999; Harada et al., 1982; Higuchi, 1994; Thomasson et al., 1991; Tu and Israel, 1995). It has been well established that the active enzyme aldehyde dehydrogenase ALDH2*1 (or its polymorphic inactive ALDH2*2) are present in the mitochondria of all tissues (Goedde et al., 1980; Yoshida, 1984). When

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alcohol is consumed by homozygous or heterozygous subjects for *ALDH2*2*, marked elevations in venous blood acetaldehyde are observed (Mizoi et al., 1983; Peng et al., 2007) where elevated acetaldehyde likely leads to dysphoric reactions and to a heightened perception of intoxication (Mizoi, 1983) which reduce subsequent alcohol intake. Such elevations in venous acetaldehyde in individuals who carry the *ALDH2*2* allele stem not from one, but from two factors that are often not considered: (i) the spillout of acetaldehyde from the liver into the general circulation and (ii) a reduced metabolism of acetaldehyde in peripheral tissues. Thus, in individuals carrying the ALDH2*2 polymorphism *all tissues* are *constantly* exposed high levels of acetaldehyde. One of the tissues exposed to high levels of acetaldehyde is the esophagus; in addition the esophagus has high levels of alcohol dehydrogenase-4 (see Vaglenova et al., 2003) which, although with a high K_m for ethanol, is active at high concentrations of alcohol present in the esophagus.

It has been well documented that alcoholics who continue to drink despite carrying the *ALDH2*2/ALDH2*1* genotype are 10-times more prone to develop esophageal cancers than alcoholics who are *ALDH2*1/ALDH2*1* homozygous (Chen et al., 2006; Yokoyama et al., 2001; Yokoyama and Omori, 2003). The mechanism that has been proposed for this effect is the binding of acetaldehyde to DNA (Matter et al., 2007; Murakami et al., 2009; Nagayoshi et al., 2009), which upon constant high exposure to acetaldehyde might induce mutations in procarcinogenic or antiapoptotic genes. Conversely, individuals who carry the fast ADH1B*2 are markedly (and paradoxically) *protected* against esophageal and upper respiratory cancers, both in Asian and in European populations (Chen et al., 2006; Ding et al., 2009; Hashibe et al., 2006, Yokoyama et al., 2001). The obvious question is: what is the mechanism of such protection? Do these individual display elevated acetaldehyde levels upon ethanol consumption?

Some alcohol dehydrogenase (ADH1) polymorphisms, including the fast *ADH1B*2*, have been shown to protect against alcoholism (see meta-analysis by Zintzarras et al., 2006), although the mechanism of this effect has not been elucidated. Protection that can reach levels as high as those afforded by the *ALDH2*2* polymorphism have been documented recently for the *ADH1B*2* (47His) which codes an enzyme that is one to two orders of magnitude more active than the typical ADH1B*1 (47Arg). These studies show protections up to 80% against alcoholism in *ADH1B*2* homozygous individuals (Chen et al., 2009; Kim et al., 2008). Very puzzling to investigators have been the reports that blood acetaldehyde is not elevated following alcohol ingestion even in *ADH1B*2* homozygous individuals, while in the same studies, with the same methodologies, it was clearly elevated in heterozygous *ALDH2*2/ALDH2*1* subjects who ingest alcohol (Mizoi et al., 1994; Peng et al., 2007). We suggest that this is likely due to two factors: (i) Venous (mainly antecubital) rather than arterial blood is normally sampled. As indicated above, peripheral tissues in individuals carrying the *ALDH2*2* mutation are not able to remove acetaldehyde from the circulation, while *ALDH2*1* homozygous are able to do so, such that in *ADH1B*2* individuals who are also *ALDH2*1* homozygous, acetaldehyde disappears from the venous blood; (ii) a second factor unraveled in studies in the animals presented below is that arterial blood must be sampled at very short times after ethanol is consumed or administered.

Studies by Rivera-Meza et al. (2010) addressed, in an animal model, the mechanism of *ADH1B*2* protection against alcoholism. Wistar-derived UChB rats selectively bred for 70-80 generations (Quintanilla et al., 2006) for their high ethanol consumption were administered a cDNA mutated by recombinant DNA techniques to encode a rat analog of the human *ADH1B*2* (rat ADH-47His). This cDNA was incorporated into an adenoviral vector (size about 70 nM), which is able pass through the large pores of liver capillaries (fenestra of 100-500 nM), showing marked hepatic tropism when administered systemically, while is not being able to pass through the pores of capillaries in other tissues, being below

15 nM. A preferential delivery of adenoviral vectors into hepatocytes is also aided by the high perfusion of the liver (some 25% of heart output). When the adenoviral vector was administered intravenously, only the liver expressed the *ADH* cDNA, as shown by increases in liver ADH activity, but not that in other tissues (Figure 1).

Administration of ethanol to rats transduced with the rat *ADH1B*2* analogue cDNA (rADH-47His) led to a 90% increase in hepatic ADH activity. Also aiming at increasing the normal liver ADH activity, the administration of the normal rat ADH (rADH-47Arg) coding cDNA resulted in a 32% increase in hepatic ADH activity. Upon ethanol administration, animals with higher ADH activities showed marked increases surges ("burst") in *arterial* acetaldehyde concentrations which were respectively 5 and 3.5 times higher than those generated by control animals that received an empty adenoviral vector (Fig 2). Noteworthy, the acetaldehyde "burst" lasted 15 minutes (Rivera-Meza et al., 2010). Acetaldehyde surges (bursts) of this type have been shown to depend both on the activity of liver ADH as well as on the *initial* high levels of plasma and liver pyruvate (and likely other NADH reoxidizing substrates), which are reduced as ethanol metabolism proceeds (see Quintanilla et al., 2007). The question then arises as to whether such increases in acetaldehyde would reduce ethanol administration in animals bred for generations as alcohol drinkers. The answer was positive; increases in ADH activity and in arterial acetaldehyde levels upon ethanol metabolism led to marked reductions in ethanol volition (Figure 3), but primarily in animals that received rat analog of human *ADH1B*2* (Rivera-Meza et al., 2010). Furthermore, alcohol intake was inversely related (R^2= 0.99) to the peak acetaldehyde levels achieved (Rivera-Meza, 2009) (Fig 4). These studies in an animal model strongly suggest that a sharp but brief increase in arterial acetaldehyde is likely the responsible for the aversion to alcohol in humans that carry the *ADH1B*2* polymorphism. The question remains as to the 10-fold higher prevalence in esophageal cancer in ALDH2*2/ALDH2*1 alcoholics versus a 50 to 90% protection in ADH1B*1/ADH1B*2 individuals.

Toxin exposure and Hormesis

Of note are the differences in the two mechanisms by which acetaldehyde would reduce alcohol intake in humans; a *brief increase* (limited exposure) in arterial acetaldehyde in *ADH1B*2*/ADH1B*2 individuals versus a *sustained* exposure in systemic and cellular acetaldehyde in *ALDH2*2* subjects. An explanation for the difference in esophageal cancer (protective *versus* inducing) under these two genotypes is offered below.

It is now well established that some toxins or stressors are beneficial at low doses while damaging at higher doses, an effect known as *hormesis* (Calabrese and Baldwin, 2003). While not all mechanisms of hormesis are understood, conditions such as short physiological challenges or low doses of ionizing radiation are known to elicit general protective and reparative mechanisms, including an enhanced DNA repair (see Feinendegen, 2005; Kang et al., 2008; Li et al., 2007; Schwartz et al., 2008). On the basis of the present study, it may be suggested that in *ADH1B*2* drinkers, protection against cancers might result from an activation of repair mechanisms induced by only a limited exposure to acetaldehyde. In *Drosophila*, exposure to low concentrations of acetaldehyde increases the longevity of flies at lethal doses, providing a clear example of hormesis (Parsons, 1989). Also, extended longevity has been reported in mice that consume moderate -but not high- amounts of alcohol throughout their adult life (Schmidt et al., 1987). Most hormesis mechanisms for a variety of toxins display a "J" or "U" types of dose-response curves, as expected from a protection at low toxin exposures but damage at high exposure levels.

It should be noted that in addition to esophagus, other tissues such as mouth, pharynx, stomach and colon also develop cancers in alcoholics (Thygesen et al 2009). Salaspuro

(2009) has proposed that the generation of acetaldehyde from bacterial and yeast flora at different levels of the GI tract, including its transport by saliva into oropharyngeal laryngeal tissues, may have an important impact in the generation of cancers in these tissues. If such a mechanism also contributes to the development of esophageal cancer, is of interest that the ADH1B*2 polymorphism also protects this organ against acetaldehyde generated by microflora, thus increasing the validity of a hormetic effect generated by a short while high exposure to acetaldehyde.

It has not escaped to us that minor alcohol ingestion (e.g., one standard drink per day) has been consistently shown to reduce mortality by a variety of causes, showing typical “J” type curves (Rehm and Bondy, 1998; Sasaki, 2000). It is not unlikely, on the basis of the above findings, that a limited exposure to acetaldehyde regardless of ADH genotype may generate, in all subjects, protective hermetic effects against many conditions that lead to cell dysfunction and death.

Acknowledgments

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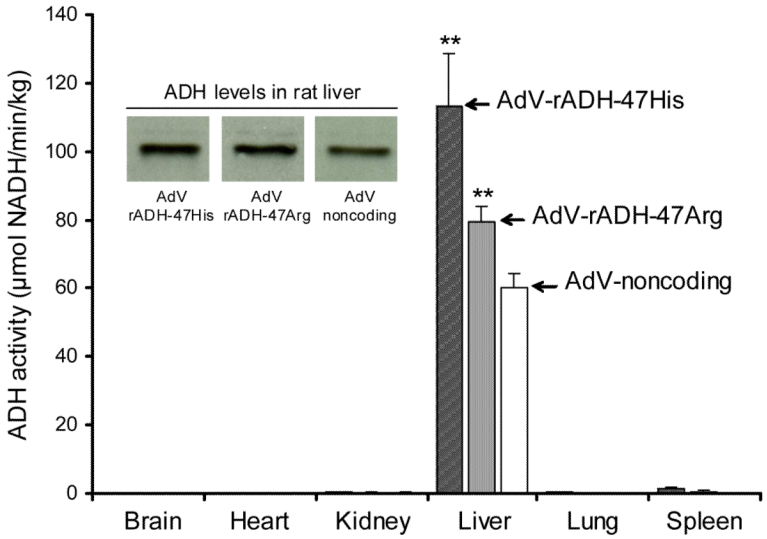

Figure 1. Alcohol dehydrogenase activity in different tissues of rats treated with adenoviral vectors coding for the mutated rADH-47His or the wildtype rADH-47Arg enzymes

Three weeks after administration of the adenoviral vectors, the animals were killed and the ADH activity was determined in brain, heart, kidney, liver, lung and spleen. Bars represent mean ± SE ADH activity in tissues (µmol NADH/min/kg body wt.); 5 animals/group. Rats treated with AdV-rADH-47His showed an 88% increase in liver ADH activity *vs*. control animals; rats treated with AdV-rADH-47Arg showed an increase of 32%. Inset: liver ADH levels of rats treated with AdV-rADH-47His or AdV-rADH-47Arg were similar, and both were higher than levels in rats treated with the AdV-noncoding control vector. ** $P<0.01$ for AdV-rADH-47His *vs*. AdV-noncoding ($t=3.4$, df=8) and AdV-rADH-47Arg *vs*. AdV-noncoding ($t=3.3$, df=8); Student's *t* test. (Rivera Meza et al., 2010)

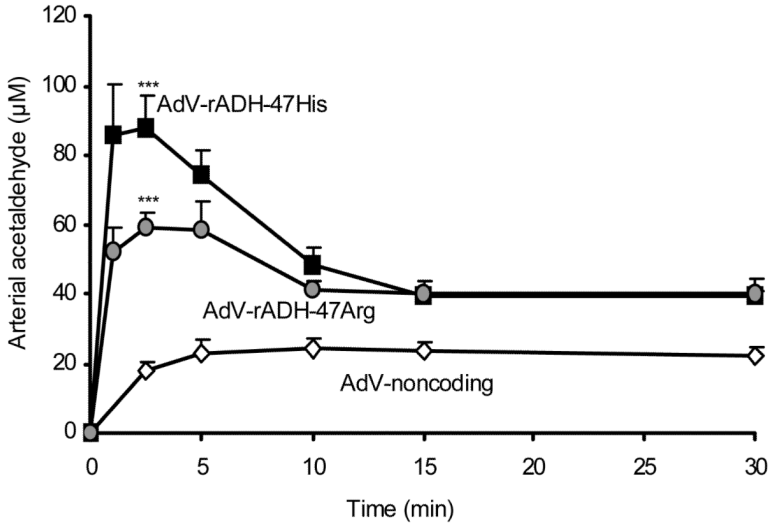

Figure 2. Arterial blood acetaldehyde concentrations after administration of ethanol to UChB rats treated with adenoviral vectors coding for the mutated rADH-47His or the wild-type rADH-47Arg enzymes

Three weeks after administration of the adenoviral vectors, animals were injected with ethanol intraperitoneally at a dose of 1 g/kg, and acetaldehyde levels were measured in arterial blood (carotid artery) by gas chromatography. Points represent mean ± SE blood acetaldehyde concentration; 5 animals/group. At 2.5 minutes after ethanol administration, maximal concentrations of acetaldehyde in animals that received AdV-rADH-47His and AdV-rADH-47Arg were 5-fold (t=7.2, df=8) and 3.5-fold (t=8.2, df=8) higher than those of control animals that received AdV-noncoding, respectively. ***P<0.001. ANOVA for repeated measures revealed significant differences between treatments [F(2,14)=11.6, P<0.01]. *Post hoc* comparisons revealed significant differences (P<0.05) between AdV-rADH-47His and AdV-noncoding and also between AdV-rADH-47Arg and AdV-noncoding. No statistically significant differences were found between AdV-rADH-47His and AdV-rADH-47Arg. (Rivera-Meza et al., 2010).

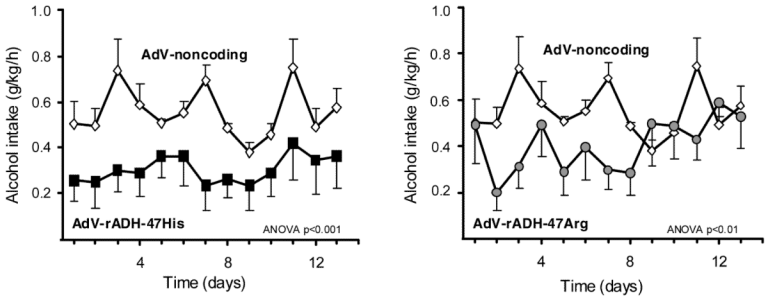

Figure 3. Voluntary ethanol intake of UChB rats treated with adenoviral vectors coding for the mutated rADH-47His or the wild type rADH-47Arg enzymes

Fifteen rats from the high-drinker UChB line were injected with 5×10^{12} vp/kg of one of the adenoviral vectors AdV-rADH-47His, AdV-rADH-47Arg, or AdV-noncoding as control. Ninety-six hours after administration of the adenoviral vector (day 1), the animals were allowed access to a 10 % ethanol solution for only 1 h each day. Water was continuously available. Points represent means ± SE of daily ethanol intake during the 1 h access to ethanol; 5 animals/group. Rats treated with AdV-rADH-47His (left panel) showed a 50% reduction in voluntary alcohol intake *vs*. control animals [ANOVA, $F(1,25)= 71.3$, $P<0.001$], whereas in rats treated with AdV-rADH-47Arg (right panel), the reduction was 30 % [$F(1,25)=8.7$, $P<0.01$]. (Rivera-Meza et al., 2010).

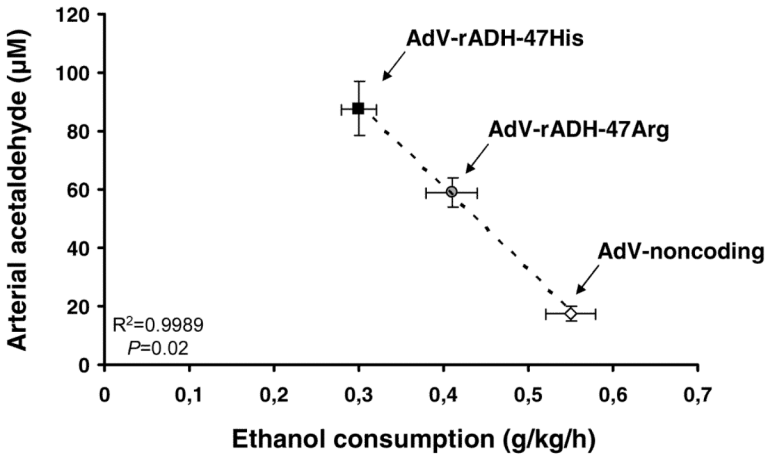

Figure 4. Inverse relationship between voluntary ethanol intake and maximal (peak) acetaldehyde levels attained in Wistar-derived UChB rats treated with adenoviral vectors coding for the mutated rADH-47His or the wild-type rADH-47Arg enzymes

Peak acetaldehyde levels attained after an ethanol dose (1 g/kg i.p) from data shown in Figure 2 *versus* average voluntary alcohol intake are derived from data as shown in Figure 3. Acetaldehyde levels and voluntary alcohol intake variables are inversely related with an R^2=0.99 (*P*=0.02). (Rivera-Meza, 2009).