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Alcohol and cancer: the role of cytochrome P-4502E1

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ABSTRACT

Chronic alcohol consumption is a risk factor for tumours of the oral cavity, pharynx, larynx, oesophagus, liver, colorectum and the female breast. Various mechanisms contribute to alcohol-mediated carcinogenesis, including the action of cytochrome P-4502E1 (CYP2E1). CYP2E1 is one of 57 cytochrome P450 enzymes that are responsible for over 90% of the redox reactions of chemicals, including drugs, vitamins, steroids, chemical carcinogens, and industrial compounds. CYP2E1 is an important constituent of microsomal ethanol oxidation and is significantly induced by chronic ethanol consumption, which results in an increase of ethanol oxidation. CYP2E1 is present in almost all tissues, but predominantly in the liver. The increase of CYP2E1 following chronic ethanol consumption results in an enhanced activation of a variety of environmental procarcinogens present in food and especially tobacco smoke. CYP2E1 is also responsible for the degradation of retinol and retinoic acid (RA), which is associated with changes in cell differentiation, cell proliferation and apoptosis, leading to a precancerous cell cycle behaviour. Subsequently, CYP2E1 generates reactive oxygen species (ROS), which either bind directly to DNA or which result in lipid peroxidation (LPO) with LPO products such as 4-hydroxy-nonenal and malondialdehyde. These compounds can bind to DNA and may form highly carcinogenic etheno DNA-adducts. Thus, the induction of CYP2E1 by chronic alcohol ingestion is an important factor to trigger ethanol-mediated carcinogenesis.

KEYWORDS

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CANCER
CYTOCHROME P4502E1
PROCARCINOGENS
RETINOIC ACID
REACTIVE OXYGEN SPECIES
ETHENO DNA-ADDUCTS

INTRODUCTION

Chronic alcohol is a risk factor for the development of various cancers, including cancer of the mouth, pharynx, larynx, oesophagus, colo-rectum, liver, and female breast. Although some progress has been made in the last years to identify mechanisms of alcohol-mediated carcinogenesis, many questions still remain unsolved. The International Agency for Research on Cancer (IARC) in Lyon, France, has classified alcohol-containing beverages as carcinogenic and has identified acetaldehyde (AA), the first metabolite of ethanol oxidation as a carcinogen[1]. In vitro and in vivo animal studies, as well as studies in humans, have clearly shown that AA is mutagenic and carcinogenic and that AA plays an important role in upper gastrointestinal carcinogenesis[2,3]. In addition to the action of AA, other

mechanisms are also important. This is especially true for the liver. These mechanisms include oxidative stress, epigenetic modifications caused by a disturbed methyl transfer, a loss of retinol and retinoic acid, and ethanol-induced changes of intracellular signal pathways to name only a few[4-7].

Our laboratory has focused, for almost 40 years, on the role of cytochrome P4502E1 (CYP2E1) in alcohol-mediated carcinogenesis. This review discusses multiple mechanisms by which alcohol-induced CYP2E1 stimulates hepatic and extrahepatic cancer initiation and promotion.

CYP2E1 AND ETHANOL OXIDATION: PHYSIOLOGIC PROPERTIES, INDUCTION, AND INHIBITION

Cytochrome P450 enzymes are responsible for over 90% of the redox reactions of chemicals, including drugs, vitamins, and chemical carcinogens[8-10]. Fifty-seven P450 genes are present in humans[10]. One of these cytochromes P450s is cytochrome P4502E1 (CYP2E1), which among others, is involved in ethanol oxidation. However, the major pathway for ethanol oxidation involves alcohol dehydrogenase (ADH). Seven isoenzymes of ADH exist; some of them are polymorphic, and they are located in the cytoplasm of the cell[11]. In addition, ethanol is also metabolized by microsomes. Orme-Johnson and Ziegler[12] demonstrated an NADPH-dependent ethanol oxidation by microsomes from rats and rabbits. Lieber and DeCarli were the first who not only isolated this enzyme system, but also noted an increase in the activity of hepatic microsomal ethanol oxidation induced by chronic ethanol consumption, which was found to be inhibited by carbon monoxide[13,14]. This system was named microsomal ethanol oxidizing system (MEOS), and its dependency on CYP2E1 was clearly demonstrated[13,14]. Mezey et al[15] and Miwa et al[16] further confirmed that this ethanol oxidation needs CYP2E1. Finally, CYP2E1 was isolated, purified, and characterized from rabbits[17], rat, and human livers[18], and the c-DNAs for CYP2E1 were cloned[19,20]. A detailed description of the exact chemical reaction involving iron and various redox steps is described elsewhere[21].

Concerning ethanol as a substrate, it is of considerable importance that chronic ethanol consumption results in an increased metabolism of ethanol to acetaldehyde due to an increased activity of the MEOS with CYP2E1 as an important constituent[13,14]. This increase in hepatic CYP2E1 is significant[22] and explains some of the negative consequences with this oxidative pathway. Actually, ethanol does not induce CYP2E1. The increase of CYP2E1 following chronic ethanol consumption is due to a stabilization of the enzyme and not to an increased synthesis[23]. CYP2E1 can be detected immunologically in human biopsies from various tissues, but activities cannot be measured due to the small amounts of tissue available. Although most of CYP2E1 is located in the liver, CYP2E1 is also present in extrahepatic tissues such as the oesophagus[24], the small intestine[25,26], the colon[27,28], the pancreas[29], the broncho-pulmonaryepithelium[30,31], and the brain[32]. In rodents, we have found a two- to three-fold increase of CYP2E1 following alcohol consumption in the mucosa of the small-[25,26] and large intestine[27], as well as in the lungs[30,31].

Total body CYP2E1 activity can be determined by using the chlorzoxazone (CZ) test[33,34]. CZ, a muscle relaxant, is administered, and after a certain period of time, CZ and its metabolite, 6-OH-CZ, is measured in the blood. Since CYP2E1 is responsible for this hydroxylation, the ratio of CZ/6-OH CZ represents the relative activity of CYP2E1. Using this test, it could be shown, in volunteers, that even a dose of 40 g of ethanol daily over 4 weeks leads to a significant induction of CYP2E1[35]. These experiments resulted in important conclusions: 1) at 40 g of ethanol daily after one week, there is a significant induction of CYP2E1; 2) this induction increases further over the following weeks; 3) the intensity of this induction varies inter-individually. Although most of the volunteers revealed an induction, some did not. The reason for this different effect of ethanol on CYP2E1 is still unclear. 4) The CYP2E1 induction decreases fast following abstinence within days. Again, it was noted that the time until normalization of CYP2E1 following alcohol withdrawal was individually different[35].

Various factors modify the induction of CYP2E1 by ethanol. CYP2E1 of hepatic microsomes reveals a significant gender effect. Hepatic microsomes of female rats have an increase of benzo(a)pyrene (BP) hydroxylase activity by 42% as compared to male rats. The activity of BP hydroxylase is determined by CYP2E1. In the Ames test, these microsomes activate BP to a mutagen much stronger as compared to microsomes from male rats[36]. In addition, age modifies CYP2E1[37]. A loss of microsomal and mitochondrial function occurs with age. Therefore, it is not surprising that the induction of CYP2E1 by alcohol is significantly reduced in 36-month-old Fischer rats as compared to 12-month-old Fischer rats or to very young animals[37]. Animal experiments have also shown that the administration of a fat-enriched diet results in the induction of CYP2E1, which can be inhibited by tomato extract[38].

As already highlighted, chronic alcohol consumption also results in a significant induction of CYP2E in the

oesophageal mucosa of humans, which correlates with the amount of alcohol consumed[24]. Furthermore, an induction of CYP2E1 was also noted in the mucosa of the colon in patients with alcohol-related liver disease (ALD)[39,40]. It was concluded that oxidative stress due to CYP2E1 induction results in mucosal damage with an increased penetration of endotoxins from the gut to the liver, contributing to ALD[40].

Clomethiazole (CMZ) is the only effective CYP2E1 inhibitor[41]. CMZ is a central nervous acting agent, which is used in some countries for alcohol detoxification therapy. CMZ is a non-competitive CYP2E1 inhibitor with a Ki of 12 µM[42]. In vivo and in vitro studies have investigated the effect of CMZ on CYP2E1 in humans[42-44]. The activity of CYP2E1 was determined by using the CZ test. The effect of CMZ was investigated in 10 individuals with alcohol abstinence and 24 patients with alcohol use disorder (AUD) in a clinical setting. Patients with AUD received either CMZ (1.3-2.3 g/day) or clorazepate (100-300 mg/day) to prevent alcohol withdrawal syndrome. In all patients with AUD, CYP2E1 was found to be elevated as compared to non-drinkers. Already one day following the administration of a single dose of CMZ, all patients revealed an almost complete inhibition of CZ hydroxylation, and CYP2E1 was almost undetectable[42]. Pharmacokinetic studies demonstrated that CMZ-mediated CYP2E1 inhibition could be detected at very low CMZ blood concentrations. In addition, the effect of CMZ on CYP2E1 was also investigated in human liver microsomes. A Dixon Plot Analysis found a non-competitive inhibition with a Ki of 12 µM[42]. Finally, a prospective, controlled clinical trial demonstrated that the inhibition of CYP2E1 by CMZ resulted in an improvement of serum transaminase levels in patients with ALD[45].

ACTIVATION OF PROCARCINOGENS BY CYP2E1

CYP2E1 catalyses not only ethanol oxidation via MEOS, but also the metabolism of various drugs and xenobiotics, including procarcinogens. Pilot studies from the laboratory of Charles Lieber reported an enhanced activation of various procarcinogens to their final carcinogenic metabolites by microsomes from rats after chronic ethanol ingestion. Table 1 summarizes various procarcinogens that are known to be activated to their final carcinogens in various tissues by CYP2E1[46-50]. Their activation is significantly enhanced following chronic ethanol consumption. The Ames test was applied to investigate whether this increased activation of procarcinogens resulted in an increased mutagenicity of the compounds. Using this test, microsomes from chronic ethanol-fed animals and their pair-fed controls have been incubated with a procarcinogen and a special strain of Salmonella typhimurium (TA 1530). This Salmonella strain is unable to grow on a histidine-deficient medium. However, when the strain receives a mutation caused by the activated procarcinogen, the strain begins to grow on a histidine-deficient medium. The number of colonies reflects the intensity of procarcinogen activation by the microsomes.

Our predominant interest was focused on nitrosamines since it has been shown that, for example, dimethylnitrosamine (DMN) is activated to its final carcinogen by various isoenzymes, all of them need CYP2E1[10]. The involvement of CYP2E1 in the metabolic activation of DMN has been clearly shown by Wrighton et al[51] and by Yang et al[52]. The capability of ethanol to activate DMN demethylase is of special interest. This activation is observed through a wide range of DMN concentrations from 0.3 to 100 mM[36], while other microsomal DMN enzyme inductors, such as phenobarbital, 3-methylcholanthrene, and polychlorinated biphenyls, increase the activity of DMN demethylase isoenzymes with high DMN concentrations (> 40 mM) and decrease the activity of low Km-DMN-demethylase[53,54]. This ethanol effect is explained through the induction of CYP2E1, and a selective affinity for DMN could be explained for ethanol-induced CYP2E1[36]. These DMN concentrations are so low that it is presumed that ethanol ingestion may also influence the in vivo metabolic activation of pathophysiologic DMN concentrations. The enhanced activity of hepatic DMN-demethylase was associated with an increased capacity of liver microsomes from ethanol-fed rats to activate DMN to a mutagen in the Ames test[36]. Dietary ethanol also enhanced the concentrations of O6-methyldesoxyguanosine adducts in DMN-treated rats[55]. Most importantly, microsomes from patients with AUD possesses also an increased capacity to activate DMN[56].

In addition to DMN, an increased activation of various different procarcinogens after chronic ethanol consumption has also been observed (Table 1)[57]. The induction of CYP2E1 by ethanol is tissue-, substrate-, gender-, and species-dependent. For example, in the small intestine, alcohol stimulates the microsomal activation of BP and tryptophane pyrolisate, but not of tobacco pyroliysate, while pulmonary microsomes of ethanol-fed rats have an increased capacity to activate promutagens in tobacco pyrolysate, but an effect towards BP or tryptophane pyrolysate was not observed[58]. Although the mutagens activated in tobacco pyrolysate are not exactly known, it is of interest that pulmonary microsomes of chronically ethanol-fed rats

show an increased activation of the tobacco mutagen N-nitrosopyrolidine[58]. On the other hand, intestinal microsomes of ethanol-fed rats were found to be more potent to activate 2-aminofluoren as compared to control microsomes[26], while hepatic microsomes did not[59].
The enhanced intestinal activation of procarcinogens following chronic ethanol ingestion may increase the bioavailability of these compounds and may lead to an increased concentration of these carcinogens in the portal vein and finally in the systemic circulation. The most important of tobacco pyrolysate is nitrosopyrolidine, which is significantly activated by oesophageal microsomes following alcohol ingestion[60]. These results are of considerable relevance since patients with AUD also smoke heavily. Both factors act synergistically on carcinogenesis of the upper alimentary tract.
Knockout mouse models demonstrated further the significance of CYP2E1 in carcinogen metabolism. CYP2E1 knockout mice did not show any benzene-associated toxicity or genotoxicity, as compared to wild-type animals[61].

Table 1. Procarcinogens activated by CYP2E1.

2-acetylaminofluorene
2-aminofluorene
4-aminobiphenyl
aflatoxins
pyrolysates of amino acids
tobacco pyrolisate
benzo(a)pyrene
dimethylhydrazine
nitrosamines (dimethlynitrosamine, diethylnitrosamine)
nitrosopyrrolidine
vinylchlorid
benzene
bromobenzene
tetrahydrochloride
cyclophosphamide
isoniazid
methylazoxymethanol

THE ROLE OF CYP2E1 ON THE METABOLISM OF RETINOIDS AND ITS RELEVANCE IN CARCINOGENESIS

Retinoic acid (RA) is an important factor to regulate cell growth, apoptosis, and cell differentiation. Reduction of RA leads to uncontrolled cell proliferation, loss of cell differentiation, and dysregulated apoptosis that affects tumour promotion. More details are reported elsewhere[6,62].

It has been shown that chronic alcohol consumption results in a significant decrease of retinol in the liver of patients with ALD[63]. This is clinically associated with night blindness and sexual dysfunction. To study the mechanisms by which alcohol results in a loss of retinol and RA hepatic microsomes from rats fed alcohol chronically and controls were incubated with RA. Microsomes from ethanol-fed rats had an increased capacity to break down RA with the generation of polar RA metabolites such as 18-hydroxy-RA and 4-oxo-RA, which were not seen when microsomes from control rats were incubated[64].
Since the in vitro metabolism of RA could be inhibited by CMZ and CYP2E1 antibodies, it was concluded that CYP2E1 is responsible for RA degradation. Chronic ethanol feeding resulted in CYP2E1 induction and low hepatic RA levels, which were normalized by the administration of CMZ. CMZ-mediated improvement of hepatic retinol and RA65 was also associated with the normalization of cell proliferation and cell cycle behaviour[66].
It is possible that the CYP2E1 induction contributes to the loss of RA and retinol following chronic ethanol consumption, even when alcohol is absent from the system. This may explain why chronic and excessive alcohol ingestion is a risk factor not only for the liver, but also for extrahepatic cell proliferation and carcinogenesis, since CYP2E1 is also inducible in the oesophagus, the stomach and the colon (see above). The change in retinoid homeostasis results in a change in the retinoid-receptor signal through an up-regulation of the c-jun-N-terminal kinase (JNK) signal pathway[67]. Both reduced RA concentrations and oxidative stress due to CYP2E1 induction activate the JNK pathway. This pathway leads to an induction of the transcription factor-activator protein 1 (AP-1) and an increase in c-fos and c-jun, strong stimulators of carcinogenesis. Chronic alcohol feeding increases these proteins by a factor of 14[68].
In summary, chronic alcohol administration leads to apoptosis, cellular proliferation, immune-function changes, and inflammation due to a loss of RA and oxidative stress. Retinoid signalling and oxidative stress normalize when CYP2E1 is inhibited. This is associated with a reduced cancer risk. The additional administration of ß-carotene, retinol or RA, in a setting with highly induced CYP2E1, may generate toxic and/or apoptotic metabolites due to an enhanced degradation of RA[69].
Indeed, in rats, administration of ß-carotene or vitamin A together with alcohol results in an increase of apoptosis and hepatic cellular damage[55]. In animal experiments, the administration of high doses of lycopens leads to an increase in ethanol-induced CYP2E1 with elevated hepatic TNFα-mRNA concentrations and in-

flammation[70]. These data show an interaction between alcohol consumption and dietary supplementation of retinoids, between CYP2E1 and TNF-α, especially in animals that received alcohol and high doses of lycopens[71].
In a cancer prevention multi-centre study, it was shown that individuals who smoke and ingest more than 11 g of alcohol per day have an increased lung cancer risk when they take 30 mg of ß-carotene per day. This study was initiated because it was detected, in animal experiments, that ß-carotene reduces the risk of lung cancer[72,73]. Due to the unexpected results, the multi-centre study had to be stopped before time[72].

GENERATION OF REACTIVE OXYGEN SPECIES AND ETHENO DNA ADDUCTS THROUGH ETHANOL METABOLISM VIA CYP2E1

One pathogenic feature of chronic alcohol consumption is the generation of oxidative stress. Ethanol metabolism via CYP2E1 results in the generation of acetaldehyde, and of reactive oxygen species (ROS) such as H_2O_2, OH-, and carbohydrate-centred OH-[2,74-76]. Although it has been questioned whether CYP2E1 could produce large scale levels of ROS[10], many in vitro and in vivo experiments underline the important role of CYP2E1 in ROS production and especially of localized ROS generation in the liver, e.g., mitochondria[2,74-76]. Under normal conditions, the transcription factor Nrf2 regulates a potent anti-oxidative defence system (AODS), which eliminates ROS[77]. However, this AODS is damaged by ethanol (for more details see[2]).
ROS generated by CYP2E1 not only activates the JNK pathway as discussed above, it also leads to lipid-peroxidation (LPO) with the LPO products, malondialdehyde and 4-hydroxynonenal (4-HNE). 4-HNE can bind to adenosine or cytosine, forming highly carcinogenic excyclic etheno DNA-adducts[78-81]. In cell experiments with CYP2E1 overexpressed HepG2 cells, it could be clearly shown that the adenosine derivate 1, N^6-etheno-2'deoxyadenosine (edA) correlated significantly with the levels of CYP2Σ and 4-HNE. This adduct formation was inhibited by the CYP2E1 inhibitor CMZ[81].
Exocyclic etheno DNA adducts have also been found in the liver of patients with ALD. These adducts also correlated significantly with 4-HNE and CYP2E1[81].
To evaluate the role of CYP2E1 in ALD and alcohol-mediated cancer, it is important to modify CYP2E1 activities and to study alcohol effects in the presence and absence of CYP2E1. One approach is the use of CYP2E1 knockout mice and/or CYP2E1 transgenic mice. When alcohol was given to CYP2E1 knockout mice, their markers of oxidative stress were found to be reduced, and liver histology was improved as compared to wild-type mice[82-84]. A similar improvement in hepatic histology was found when CMZ was administered to block CYP2E1[85]. On the other hand, animals that overexpress CYP2E1 show an increased severity of liver disease after alcohol treatment[86,87].
To study the effect of CYP2E1 on hepatocarcinogenesis, diethylnitrosamine (DEN) was given for tumour induction. Following initiation of hepatocarcinogenesis with 20 mg DMN per kg body weight, the animals received an alcohol and a control diet for 6 to 10 months with and without CMZ as a CYP2E1 inhibitor. Chronic alcohol consumption over 10 months resulted in the development of hepatic adenomas in almost all animals with DMN, while none of the rats receiving ethanol, DMN and CMZ developed hepatic tumours[88]. In this animal model, chronic alcohol feeding increases the expression of TNFα and NF_kB, while the concomitant application of CMZ normalizes hepatic proliferation and reduces the number of preneoplastic foci and adenomas[89].
The importance of CYP2E1 in the generation of the etheno DNA-adducts and as a trigger for fibrosis was extended by studying these parameters in biopsies from 97 patients with ALD. A highly significant correlation between CYP2E1, etheno adducts, and the degree of fibrosis was found[90]. This observation confirms earlier studies on CYP2E1 overexpressed HepG2 cells in which alcohol incubation resulted in an enhanced activation of hepatic stellate cells and in an enhanced fibrogenesis, most likely due to ROS and oxidative stress[91,92].
Since hepatic fibrosis is closely linked to the development of HCC, the contribution of CYP2E1 to stimulate hepatic fibrosis adds another CYP2E1-driven mechanism in the pathogenesis of HCC.
It is noteworthy that the induction of CYP2E1 already at the stage of fatty liver not only enhances oxidative stress, but also increases the sensitivity of hepatocytes towards lipopolysaccharides[93] and TNFα[94]. This CYP2E1 enhanced, LPS- and TNFα-mediated liver damage is JNK- and p38MAPK dependent[95].
In addition to the liver, oesophageal biopsies were taken adjacent to cancer lesions from patients with alcohol-mediated oesophageal cancer. In the healthy mucosa adjacent to cancer, again etheno DNA-adducts correlated significantly with 4-HNE and CYP2E1[24]. Thus, CYP2E1 seems to be a causal factor in the generation of these highly carcinogenic adducts involved in carcinogenesis. Since most of the oesophageal cancers are due to high alcohol intake, it is not surprising that the amount of alcohol consumed correlated significantly with CYP2E1[24].
Ethanol also results in epigenetic changes. These changes are associated with the activation of the ca-

nonical Wnt pathway stimulating β-catenin-dependent tumor growth and CYP2E1 transcription[96,97].

SUMMARY AND CONCLUSIONS

The role of CYP2E1 in ALD and alcohol-mediated cancer can be summarized as follows (Figure 1):
a) Chronic ethanol consumption increases CYP2E1 in the liver, but also in extrahepatic tissues.
b) CYP2E1 is involved in the activation of a variety of dietary and tobacco-borne procarcinogens.
c) CYP2E1 is involved in the degradation of retinol and retinoid acid, retinoids essential for the balance of cell differentiation, cell regeneration, and apoptosis.
d) CYP2E1 produces oxidative stress by generating ROS, leading to DNA damage and in the liver to fibrosis, a precancerous condition.
e) Alcohol activates the canonical Wnt pathway that may render β-catenin-dependent tumor growth and stimulate CYP2E1 transcription.

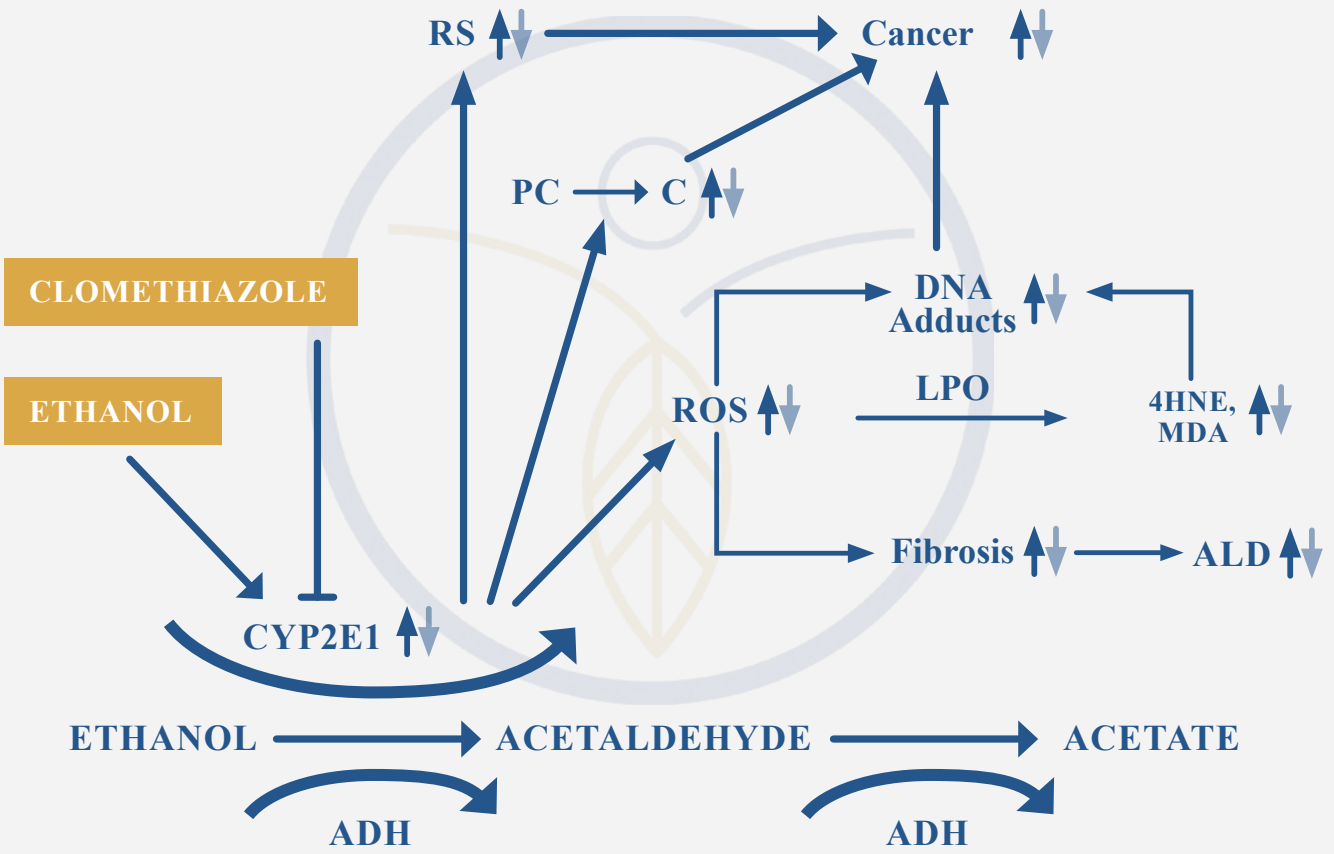

Figure 1. The role of Cytochrome P4502E1 in ethanol-mediated carcinogenesis and its inhibition by Clomethiazole. Ethanol induces CYP2E1 that activates a number of procarcinogens to their final carcinogenic metabolites. CYP2E1 also decreases retinoic acid (RS), resulting in a loss of cell differentiation leading to cellular hyper-proliferation, which favours carcinogenesis. CYP2E1 also generates reactive oxygen species (ROS). ROS stimulates carcinogenesis through 1) direct binding to DNA forming DNA-adducts, 2) lipid-peroxidation (LPO) with LPO-products such as 4-hydrxynonenal (4HNE) or malondialdehyde (MDA) and the generation of etheno-DNA-adducts, and 3) enhanced fibrogenesis leading to alcoholic liver disease and hepatocellular cancer (HCC). Clomethiazole inhibits CYP2E1 and thus blocks all CYP2E1 mediated reactions and pathways, and thus inhibits carcinogenesis. Induction of CYP2E1 by ethanol and all consequences are marked by blue arrows. Clomethiazole and its inhibition of CYP2E1 are shown by grey arrows. ADH= alcohol dehydrogenase; ALDH= acetaldehyde dehydrogenase; RS= retinoic acid; ROS= reactive oxygen species; LPO= lipidperoxidation; 4HNE= 4-hydroxynonenal; MDA= malondialdehyde; ALD= alcoholic liver disease; PC= procarcinogen; C= carcinogen

Conflict of Interest

The authors declare that they have no conflict of interest.

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