Available online at www.sciencedirect.com
ScienceDirect
Chemico-Biological Interactions 169 (2007) 80–90
Chemico-Biological Interactions
www.elsevier.com/locate/chembioint

Combined metadoxine and garlic oil treatment efficaciously abrogates alcoholic steatosis and CYP2E1 induction in rat liver with restoration of AMPK activity

Sung Hwan Ki [a], Jae Hoon Choi [a], Choon Won Kim [b], Sang Geon Kim [a,*]

[a] College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, South Korea
[b] Department of Diagnostic Medicine Laboratory, Hanyang University Medical School, Seoul, South Korea

Received 14 March 2007; received in revised form 22 May 2007; accepted 22 May 2007
Available online 2 June 2007

Abstract

Alcoholic steatosis is the earliest and most common response to heavy alcohol intake, and may precede more severe forms of liver injury. Accumulation of fat, largely triglyceride, in hepatocytes results from the inhibition of fatty acid oxidation and excessive oxidative stress involving CYP2E1. This study evaluated the therapeutic effects of metadoxine, garlic oil or their combination on alcoholic steatosis. Feeding rats an alcohol-containing diet for 4 weeks elicited an increase in hepatic triglyceride content and induced CYP2E1. The concurrent administration of metadoxine and garlic oil (MG) to rats during the last week of the diet feeding efficaciously abrogated both fat accumulation and CYP2E1 induction as compared to the individual treatment at higher doses. Histopathology confirmed the ability of MG combination to inhibit lipid accumulation. Blood biochemistry verified improvement of liver function in rats treated with MG. Alcohol administration resulted in a decrease in AMP-activated protein kinase-α (AMPKα) phosphorylation, which was restored by MG treatments. Recovery of AMPK activity by MG was supported by an increase in acetyl-CoA carboxylase phosphorylation. Hepatic fatty acid synthase (FAS) expression was markedly decreased after alcohol consumption, which correlated with a decrease in AMPK activity and a commensurate increase in lipid content. Combined MG treatments caused restoration of the FAS level. These results demonstrate that the combination of MG effectively treats alcoholic steatosis with CYP2E1 inhibition, which may be associated with the recovery of AMPK activity, promising that the combination therapy may constitute an advance in the development of clinical candidates for alcoholic steatosis.

Keywords: Metadoxine; Garlic oil; Alcoholic steatosis; CYP2E1; AMPK

1. Introduction

Chronic alcohol consumption increases hepatic accumulation of fat, largely triglyceride (TG), and lead to hepatic steatosis, which is the earliest and most common response to heavy alcohol intake. The mechanisms by which alcohol ingestion causes fatty liver seem to be complex. Alcohol-induced fat accumulation in hepatocytes may result from increase of TG synthesis, inhibition of fatty acid oxidation and excessive oxidative stress. A decrease in the activity of AMP-activated

Abbreviations: ACC, acetyl-CoA carboxylase; ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; AST, aspartate aminotransferase; CYP2E1, cytochome P450 2E1; FAS, fatty acid synthase; GGT, γ-glutamyl transferase; metadoxine, pyridoxol L-2-pyrrolidone-5-carboxylate; MG, metadoxine and garlic oil; TG, triglyceride

* Corresponding author at: College of Pharmacy, Seoul National University, Sillim-dong, Kwanak-gu, Seoul 151-742, South Korea. Tel.: +82 2 880 7840; fax: +82 2 872 1795.
E-mail address: sgk@snu.ac.kr (S.G. Kim).

0009-2797/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2007.05.008

protein kinase (AMPK), a conserved intracellular energy sensor, may be responsible for the disruption of fatty acid oxidation pathway [1]. Hepatic steatosis may render the liver more susceptible to oxidative damages, in particular to endotoxins [2], which promote the pathogenesis of alcoholic hepatitis or liver fibrosis [3,4]. Therefore, recovery from fatty liver would help decrease susceptibility to liver fibrosis. However, there is as yet no clearly established pharmacologic therapy for reversing fatty liver.

Cytochome P450 (CYP2E1) is a specific alcohol-inducible form of cytochrome P450, and primarily renders liver injuries after alcohol ingestion [5]. CYP2E1 induction is considered to be an early and necessary step in the pathogenesis of alcohol-induced steatosis. It has been shown that CYP2E1 expression levels were increased during the alcohol-dependent metabolic changes or the early liver injuries such as steatosis and steatohepatitis [6]. CYP2E1 not only catalyses alcohol metabolism, but also metabolizes other xenobiotics including acetaminophen and carbon tetrachloride [7,8], resulting in oxidative stress. The oxidants produced by CYP2E1 can promote toxicities such as protein oxidation, damage to the DNA and lipid peroxidation [5]. Increase in oxidative stress by alcohol contributes to fat accumulation in the liver due to redox state changes and production of reactive products such as acetaldehyde.

Both pyrrolidone carboxylate and pyridoxine are the well-known agents that are useful for the treatment of alcoholics and alcoholic liver diseases [9,10]. Metadoxine is pyridoxol L-2-pyrrolidone-5-carboxylate, which is the ion-pair between pyrrolidone carboxylate and pyridoxine. In an animal model, metadoxine treatment increases the clearance of alcohol and acetaldehyde, reduces the damaging effect of free radicals, and thereby restores cellular ATP and GSH levels [11–13]. Nevertheless, the molecular target of metadoxine therapy has not been clearly defined. Although clinical trials partly support the effect of metadoxine for steatosis [12], the therapeutic efficacy needs to be verified by other experimental approaches [14]. Hence, successful treatment of hepatic steatosis or steatohepatitis may require combination of metadoxine with other agents.

Allium species comprises organosulfur compounds. The organosulfur ingredients existing in garlic oil (GO) exert chemoprotective effects against chemical-induced carcinogenesis in rats [15,16]. The chemoprotective effect of GO is explained in part by the induction of phase II detoxifying enzymes [17,18]. Moreover, studies have shown that GO or garlic extracts exert protective effects through the inhibition of CYP2E1 responsible for the production of reactive metabolic intermediates from a wide variety of organic molecules [19]. For example, the induction of CYP2E1 by pyrazine was blocked in animals by concomitant treatment with a relatively high dose of GO (e.g., 500 mg/kg body weight) [20]. Previously, we also showed that feeding mice a diet containing a GO extract inhibited the ability of carbon tetrachloride to increase hepatic TG and cholesterol contents [21]. In spite of the findings that CYP2E1 is involved in the pathogenesis of alcohol-induced liver injury and that chemical inhibition of CYP2E1 is plausible, GO has never been tried to treat alcoholic steatosis. Furthermore, the fact that CYP2E1 inhibition necessitates high doses of GO intake may deter its application for alcoholic liver injuries.

In view of the previous reports showing the differential mechanistic basis of metadoxine and GO for hepatic effects, we attempted to evaluate the potential therapeutic efficacy of their combination on alcoholic steatosis and CYP2E1 induction under the assumption that the two agents might act on sequential steps in the obligate pathologic progress to hepatic steatosis. We found that the combination of metadoxine and GO (MG) efficaciously inhibits alcohol-induced hepatic TG accumulation and improves liver function as compared to individual agent alone. Moreover, the combination of MG recovered the activity of AMPK in alcohol-fed rats, which might account for the pharmacologic efficacy of MG for steatosis.

2. Materials and methods

2.1. Materials

Metadoxine and GO were provided from PharmaKing Pharmaceutical Co. (Seoul, Korea). Lieber-DeCarli liquid diet was purchased from Dyets, Inc. (Bethlehem, PA). Anti-CYP2E1 antibody was supplied form Oxford Biomedical Research (Oxford, MI). Antibodies that specifically recognize phosphorylated AMPK and phosphorylated acetyl-CoA carboxylase (ACC) were obtained from Cell Signaling (Beverely, MA). An antibody directed against fatty acid synthase (FAS) was supplied from BD BioSciences (San Diego, CA). Horseradish peroxidase-conjugated goat anti-rabbit IgG and goat anti-mouse IgG were provided from Zymed Laboratories Inc. (San Francisco, CA).

2.2. Animals and diets

Animal studies were conducted in accordance with the institutional guidelines for care and use of labora-