Communication

Polyphenols in Alcoholic Beverages Activating Constitutive Androstane Receptor CAR

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The constitutive androstane receptor CAR is a xenosensing nuclear receptor that can be activated by natural polyphenols such as flavonoids and catechins. We examined alcoholic beverage phytochemicals for their ability to activate CAR. HepG2 cells were transfected with CAR expression vector and its reporter gene, and then treated with trans-resveratrol, ellagic acid, β-caryophyllene, myrcene, and xanthohumol. A luciferase assay revealed that ellagic acid and trans-resveratrol activated both human and mouse CAR. Since CAR regulates many genes involved in energy metabolism, the possibility exists that these polyphenols would reduce the risk of certain alcohol-induced metabolic disorders with the help of CAR.

Key words: polyphenol; resveratrol; ellagic acid; constitutive androstane receptor; alcoholic beverage

Alcoholic beverages contain a variety of phytochemicals originating from fruit peels (wine), wooden barrels (whisky), or additives (beer and liqueur). These non-alcoholic compounds are called congeners of alcoholic beverages. Constituents of the congeners are potent antioxidants evoking certain physiological activities that can reduce the risk of metabolic disorders associated with chronic alcohol consumption.¹–³ Particularly, resveratrol is well studied in the context of epigenetic regulation of energy metabolism.⁴,⁵ The constitutive androstane receptor (CAR, NR1I3) belongs to the NR1I subfamily of nuclear receptors with its relative, pregnen X receptor (PXR, NR1I2). While it is known as a drug responsive nuclear receptor that makes possible the detoxification of xenobiotics through regulation of the genes involved in this process,⁶ recent studies have revealed expanded roles in regulating the genes related to energy metabolism, ameliorating fat accumulation and insulin resistance.⁷,⁸ We have screened food-derived flavonoids and related polyphenols for their ability to activate CAR in a cell-based assay system.⁹

Among the 29 polyphenols examined, chrysin (5,7-OH flavone) and galangin (3,5,7-OH flavone) were found to elicit activities as strongly as known artificial CAR activators. This incidence was also confirmed in vivo using CAR KO mice, suggesting the possibility that food-derived polyphenols can regulate CAR, alleviating metabolic syndrome. The objective of the present study was to extend the spectrum of known CAR respondents to ingredients of alcoholic beverages. Identification of the novel CAR activators resveratrol and ellagic acid should shed light on the mechanisms of the beneficial effects of congeners in alcoholic beverages.

The structural formulae of the congeners investigated in this study are depicted in Fig. 1. trans-Resveratrol is one of the stilbenes in the peel of the grape variety used in red wine production. Ellagic acid is generated from hydrolysable tannins in the oak barrel during charring and aging process. Beer contains phenolic compounds derived partly from hops (30%) and partly from barley (70%). β-Caryophyllene, myrcene, and xanthohumol are congeners of hop origin. Pure chemicals, ellagic acid (Fluka Biochemika, Switzerland, 45140), trans-resveratrol (Sigma, USA, R5010-500MG), β-caryophyllene (Wako, Japan, 329-53072), and myrcene (Tokyo Chemical Industry, Japan, M0235) were used, except that xanthohumol was supplied as hop extract containing 85% xanthohumol (Hopsteiner, Germany, Xantho-flav-extract). These congeners were subjected to luciferase assay with known CAR activators, as described below (CITCO and TCPOBOP, Sigma).

To detect CAR activation, we used an HepG2 cell-based assay system with the CAR expression vector and the CYP2B6 PBREM reporter.¹⁰ Because the endogenous activity of human CAR in HepG2 cells is low, the PBREM reporter activity in this system depends basically on the activation of exogenously introduced CAR.¹¹ The cells were transfected with mouse or human CAR expression vector, a reporter plasmid (PBREM-TK-pGL3), and a control reporter plasmid (phRL-TK) prior to 48 h of treatment with 0.1% DMSO

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Abbreviations: CAR, constitutive androstane receptor; PBREM, phenobarbital responsive enhancer module
as vehicle or with the various test compounds in the medium at the indicated concentrations. The cell lysates were assayed for luciferase activity. Among the compounds described above, 20 μM ellagic acid and 5 μM trans-resveratrol exhibited significant increases in reporter activity over basal activity (Fig. 2A and B). In the case of human CAR, the values were 1.63-fold for 5 μM galangin (1.90-fold) observed in a previous study.9) Similarly, mouse CAR exhibited activation by 20 μM ellagic acid (1.47-fold) and by 5 μM trans-resveratrol (1.69-fold), approximating the extent due to 5 μM galangin (1.74-fold). When the cells were treated with various doses of these polyphenols, various response curves were obtained (Fig. 3A and B). In the case of both human and mouse CAR, trans-resveratrol caused a maximum response at 5 μM, with decreased responses at 10 μM and 20 μM. No change in cell shape or no decrease in control reporter activity was observed at these concentrations. This is in contrast with the results obtained for chrysin, which showed both of these phenomena.9) As for ellagic acid, both human and mouse CAR exhibited significant responses only at 20 μM, indicating that its agonistic activity is less than that of trans-resveratrol.

The quantities of these polyphenols in alcoholic drinks varies depending on the materials used;1,2,12 they range from 5 to 50 mg per L, whisky or brandy in the case of ellagic acid, and from 1 to 15 mg per L of red wine in the case of resveratrol. There are issues concerning the bioavailability of these polyphenols, since they undergo glyco- or sulfo-conjugation, which decreases their accessibility to inner-cellular components.13,14) Previous studies using human volunteers have indicated that a dose of 40 mg ellagic acid results in the occurrence of 200 μg/L (0.6 μM) of pure ellagic acid in the plasma,15) while it was 25 μg versus 7 μg/L (0.03 μM) in the case of resveratrol.16) Others have reported relatively low penetration ratios.17–19) These concentrations are 200 to 300-fold smaller than the effective concentrations found in our assay. Whether these polyphenols can activate CAR in vivo, especially in the liver where they are metabolized concomitantly with ethanol, is an issue for the future.

The ameliorating effects of ellagic acid and resveratrol on alcohol-induced metabolic disorders have been investigated basically as to their anti-oxidative activity and their effects on specific proteins. Several enzymatic systems for alcohol metabolism have been implicated as sources of reactive oxygen species (ROS).20) The radical-scavenging activities of ellagic acid and resveratrol can antagonize the adverse effects of these ROS.21) Moreover, resveratrol has been reported to activate key
in this process should be performed. We are now investigating as to whether CAR plays some role in regulating fat metabolism such as NAD-dependent protein deacetylase (sirtuins) and adenosine monophosphate activated kinase (AMPK). Because ethanol consumption causes reductions in their activities, which in turn increases adipogenesis and fat infiltration into the liver, the direct or indirect agonistic effects of resveratrol on sirtuins and AMPK might explain its ameliorating effect on alcohol-induced fatty liver. It has also been found that this regulatory network involves peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), which can also interact with CAR. Investigation as to whether CAR plays some role in this process should be performed. We are now conducting a experiment in which wild-type mice are given alcohol with and without these polyphenols to examine their alleviative effects on alcoholic fatty liver. A similar experiment using CAR knockout mice should uncover the contribution of CAR to the beneficial effects of alcoholic drink congeners.

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