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Lack of modification of 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline (MeIQx) rat hepatocarcinogenesis by caffeine, a CYP1A2 inducer, points to complex counteracting influences

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Abstract

2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), one of the most abundant carcinogenic heterocyclic amines in cooked foods, is speculated to be a human liver carcinogen. To test the hypothesis that it is metabolically activated by CYP1A2, we here investigated the effects of caffeine as a CYP1A2 inducer on MeIQx induced rat hepatocarcinogenesis in a medium-term liver bioassay system. Unexpectedly, no modifying effects of caffeine on MeIQx-induced hepatocarcinogenesis were evident, although up-regulation of CYP1A2 and NAT2 were detected. Therefore, mRNAs extracted from GST-P positive foci and the surrounding liver tissue in each group were analyzed to explore mechanisms in detail. The results suggest that suppression of syndecan-2 (Sdc2) and induction of cell cycle arrest through a p21-dependent pathway might have counter-acted any promotion effects of up-regulation of CYP1A2.

Keywords: MeIQx; Caffeine; CYP1A2; NAT2; Syndecan-2; p21; CDK4

1. Introduction

Many epidemiological studies have demonstrated associations between high red meat intake and development of various cancers [1–5] and it is well established that high-temperature cooking procedures such as frying or barbequing of meat and other proteinaceous foods can cause the formation of a group of mutagenic and carcinogenic compounds

Abbreviations MeIQx, 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline; GST-P, glutathione-*S* transferase placental form; HCA, heterocyclic amine; CYP, cytochrome P450; Sdc2, syndecan-2; NAT, *N*-acetyltransferase.

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called heterocyclic amines (HCAs) [6,7]. Humans are constantly exposed to food-derived HCAs, and there is a concern that these compounds may thus be dietary carcinogens [8,9]. Among the HCAs, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) is one of the most abundant and mutagenic formed in cooked meat [10]. Dietary studies conducted in humans have provided evidence of bioavailability and disposition of MeIQx [11–13]. The carcinogenic risk posed by these probable human carcinogens depends not only on the level of exposure, but is also on the existence of modulators such as other dietary factors that might influence their uptake and biotransformation. MeIQx is thought to be metabolically activated to genotoxic intermediates by CYP1A2-mediated *N*-hydroxylation in the liver followed by *N*-acetyltransferase (NAT)-mediated *O*-esterification [14,15]. The resulted metabolites are highly reactive and can covalently bind to DNA [14,16,17].

Caffeine, a major constituent of coffee and tea, is known to have a broad range of biochemical and physiological activities [18]. Several reports have demonstrated inhibitory effects on carcinogenesis in various organ sites of rats and mice [19–22]. However, caffeine is known to be a CYP1A2 inducer in rats [23], and would therefore be expected to modify MeIQx metabolism and hepatocarcinogenesis. Therefore, we investigated the possible interaction of these two environmental chemicals, MeIQx and caffeine, in terms of the modifying effects on rat hepatocarcinogenesis using a medium-term liver bioassay system with glutathione *S*-transferase placental form (GST-P) positive foci as end-point pre-neoplastic lesions in the rat liver [24,25]. Two-stage initiation–promotion assays were well established, and used for many bioassays and research for investigation of the mechanisms. In this study, we investigated gene expression in the pre-neoplastic foci and the surrounding tissue of rat liver induced by the two stage initiation–promotion assay. The medium-term liver bioassay would be suitable for these situations. In addition, microarray technology to monitor the expression of large numbers of genes simultaneously [26], was applied with the integrated 3D-microarray system developed by Olympus Optical Co., Ltd, featuring hybridization, thermal control image capture and image analysis. Real-time signal detection is possible with this approach, allowing quantitation of signals. Although a similar method for hybridization on a 3D-biochip was reported [27,28], the Olympus 3D-microarray system FD10 is the first available commercially in Japan for high throughput gene expression and/or mutation analysis [29]. Since laser microdissection facilitates accurate sampling of specific types of cell or lesions [30,31], it was employed here with microarrays for analysis of gene expression profiles in immunohistochemically distinct cell populations [32].

2. Materials and methods

2.1. Chemicals

MeIQx was synthesized in the NARD Institute (Osaka, Japan) with a purity of $>99.9\%$. Diethylnitrosamine (DEN) was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan) and caffeine was purchased from Wako Pure Chemical Industries (Osaka, Japan) with purities of >99 and $>98.5\%$, respectively.

2.2. Animals

Sixty male F344 rats at 5 weeks of age were purchased from Charles River Japan, Inc. (Atsugi, Japan) and randomly divided into three groups and housed three per plastic cage with hard wood chips as bedding in an air-conditioned specific pathogen free animal room at 23 ± 2 °C and $55\pm5\%$ humidity with a 12-h light/dark cycle. Food (Oriental MF, Oriental Yeast Co., Tokyo, Japan) and tap water were available ad libitum.

2.3. Experimental procedure

All animals received an i.p. injection of DEN at a dose of 200 mg/kg body weight as an initiation procedure. Starting 2 weeks thereafter, they were administered a powdered basal diet (Oriental Yeast) containing 100 ppm MeIQx in combination with 1000 ppm caffeine in their drinking water for 6 weeks (MeIQx + caffeine group). Control groups of rats were given MeIQx alone (MeIQx group) or caffeine alone (caffeine group). Two percent corn oil was added to all diets. The rats were subjected to