Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Toxicology in Vitro 21 (2007) 685–690
Toxicology in Vitro
www.elsevier.com/locate/toxinvit

Brief communication

# Induction of cytochrome P450 1a1 by the food flavoring agent, maltol

Anwar Anwar-Mohamed, Ayman O.S. El-Kadi *

*Faculty of Pharmacy and Pharmaceutical Sciences, 3126 Dentistry/Pharmacy Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2N8*

Received 23 November 2006; accepted 5 January 2007
Available online 15 January 2007

**Abstract**

Maltol is used extensively as a flavor-enhancing agent, food preservative, antioxidant, and also in cosmetic and pharmaceutical formulations. However, a number of studies have shown that maltol may induce carcinogenicity and toxicity but the mechanisms involved remain unknown. Therefore, we examined the ability of maltol to induce the cytochrome P450 1a1 (Cyp1a1), an enzyme known to play an important role in the chemical activation of xenobiotics to carcinogenic derivatives. Our results showed that treatment of Hepa 1c1c7 cells with maltol significantly induced Cyp1a1 at mRNA, protein, and activity levels in a concentration-dependent manner. The RNA synthesis inhibitor, actinomycin D, completely blocked the Cyp1a1 mRNA induction by maltol, indicating a requirement of *de novo* RNA synthesis through transcriptional activation. In addition, maltol induced aryl hydrocarbon receptor (AhR)-dependent luciferase reporter gene expression in stably transfected H1L1.1c2 cells, suggesting an AhR-dependent mechanism. This is the first demonstration that the food flavoring agent, maltol, can directly induce *Cyp1a1* gene expression in an AhR-dependent manner and represents a novel mechanism by which maltol promotes carcinogenicity and toxicity.
© 2007 Elsevier Ltd. All rights reserved.

*Keywords:* Aryl hydrocarbon receptor; Cytochrome P450 1a1; Maltol; Carcinogenesis

## 1. Introduction

The aryl hydrocarbon receptor (AhR) belongs to the helix-loop-helix protein family, it is found inactive in the cytoplasm attached to a complex of chaperone heat shock proteins 90 (HSP90), hepatitis B virus X-associated protein (XAP2), and p23 (Hankinson, 1995; Dogra et al., 1998; Mimura and Fujii-Kuriyama, 2003). Activation of the AhR upon binding with its ligand, causes translocation of the activated complex to the nucleus. In the nucleus, HSP90 dissociate from the activated AhR which subsequently heterodimerizes with a nuclear transcription factor protein, the aryl hydrocarbon receptor nuclear translocator (ARNT) (Whitelaw et al., 1994). The AhR/ARNT complex then binds to a specific DNA recognition sequence, GCGTG, within the xenobiotic responsive element (XRE), located in the promoter region of a number of receptor regulated genes, including the *CYP1A1* (Denison et al., 1989; Nebert et al., 2004). Among the AhR-regulated genes, *CYP1A1* is the most capable in producing polar, toxic, or even carcinogenic metabolites from various AhR ligands including aromatic and halogenated hydrocarbons (Schrenk, 1998). These metabolites have been shown to be involved in the mediation of a broad range of distinct toxic responses such as immune suppression, endocrine disruption, birth defects, and carcinogenesis (Poland and Knutson, 1982).

Maltol (2-methyl-3-hydroxy-1,4-pyrone) is a naturally occurring substance that is widely used as a flavouring agent. It is formed through thermal degradation of starch or sucrose pyrolysis, and is found in coffee, soybeans, baked cereals, and browned food (Bjeldanes and Chew, 1979). The usual amounts of maltol added to beverages, baked food, ice creams, and candy range from 80 to 110 mg/l (Bjeldanes and Chew, 1979). *In vivo* toxicity studies on male and female rats showed that treatment with maltol in the dose of 1000 mg/kg/day for 9 weeks causes significant weight loss, kidney lesions, and increases the incidence of albuminuria and mortality (Gralla et al., 1969). Similarly, treatment of dogs with maltol 500 mg/kg/day for 21–41 days caused

* Corresponding author. Tel.: +1 780 492 3071; fax: +1 780 492 1217.
*E-mail address:* aelkadi@pharmacy.ualberta.ca (A.O.S. El-Kadi).

0887-2333/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tiv.2007.01.003

significant increase in mortality with severe weight loss, increase in blood urea nitrogen, hepatorenal damage, and mid-zonal hepatic necrosis (Gralla et al., 1969). *In vitro* studies showed that maltol enhanced aluminium toxicity, and by itself is neurotoxic at micromolar concentrations (150 μM) to human and murine neuroblastoma cell lines, and primary murine fetal hippocampal neurons in a dose- and cell-type dependent manner (Hironishi et al., 1996; Johnson et al., 2005). In addition, maltol has been shown to cause mutagenesis in Salmonella typhimurium strains TA-98 and TA-100 using Ames plate assay system, but the mechanism remains unknown (Bjeldanes and Chew, 1979; Shibamoto et al., 1981).

The aim of this study was to investigate the mechanisms by which maltol induces toxicity or carcinogenicity. We therefore examined the effect of maltol on Cyp1a1 mRNA, protein, and enzyme activity in murine hepatoma Hepa 1c1c7 cells. Additionally the involvement of the AhR-dependent signaling pathway was also investigated by using luciferase reporter gene assay, using H1L1.1c2 cells. Here, we provide the first direct evidence for an AhR-dependent induction of *Cyp1a1* gene expression and enzyme activity by maltol.

## 2. Materials and methods

### 2.1. Cell culture and chemicals

Murine hepatoma Hepa 1c1c7 cells (generously provided by Dr. Oliver Hankinson, University of California, Los Angeles, CA), and recombinant mouse hepatoma H1L1.1c2 cells (generously provided by Dr. Michael S. Denison, University of California, Davis, CA), were maintained in standard DMEM supplemented with 10% fetal bovine serum (Sigma–Aldrich Chemical Co., St. Louis, MO). The cells were grown in 75 $cm^2$ tissue culture flasks at 37 °C under a 5% $CO_2$ humidified environment as described previously (Korashy and El-Kadi, 2005). 7-Ethoxyresorufin (7-ethoxy-3H-phenoxazin-3-one, CAS Number: 5725-91-7, purity >99.9%), maltol (2-methyl-3-hydroxy-1,4-pyrone, CAS Number: 118-71-8, purity >99.9%), protease inhibitor cocktail, and anti-goat IgG peroxidase secondary antibody were purchased from the Sigma–Aldrich Chemical Co. (St. Louis, MO). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was purchased from Cambridge Isotope Laboratories (Woburn, MA). CYP1A1 goat polyclonal primary antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Actinomycin D was purchased from Calbiochem (San Diego, CA). Luciferase assay reagents were obtained from Promega Co. (Madison, WI). All other chemicals were purchased from Fisher Scientific Co. (Toronto, Canada).

### 2.2. Effect of maltol on cell viability

The effect of maltol on cell viability was determined using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay as described previously (Gharavi and El-Kadi, 2005).

### 2.3. RNA extraction and Northern blot analysis

After incubation with the test compounds for the indicated time periods, total RNA was isolated from the cells using TRIzol reagent, according to the manufacturer's instructions (Invitrogen Co., CA). Northern blot analysis was performed as described previously (Elbekai et al., 2004).

### 2.4. Protein extraction and Western blot analysis

Twenty-four hours after incubation with the test compound, cells were collected in lysis buffer and the total cellular proteins were obtained by incubating the cell lysates on ice for one hour, with intermittent vortex mixing every 10 min, followed by centrifugation at 12,000*g* for 10 min at 4 °C. Western blot analysis was performed using a previously described method (Gharavi and El-Kadi, 2005).

### 2.5. Determination of Cyp1a1 enzymatic activity

Cyp1a1-dependent 7-ethoxyresorufin *O*-deethylase (EROD) activity was performed on intact, living cells using 7-ethoxyresorufin as a substrate, as previously described (Elbekai et al., 2004; Korashy and El-Kadi, 2004). Enzymatic activity was normalized for cellular protein content, which was determined using a modified fluorescent assay (Lorenzen and Kennedy, 1993).

### 2.6. Measurement of luciferase activity

Recombinant mouse hepatoma H1L1.1c2 cells, stably transfected with integrated XRE-driven luciferase reporter gene plasmid, were grown for 48 h onto 6-well cell culture plates in DMEM culture media. Cells were then washed twice with PBS and incubated for 3 h with, 1 nM TCDD, or with 1 mM of maltol. Luciferase assay was performed according to manufacture's instructions (Promega) and as described previously (Jeuken et al., 2003). Briefly, after incubation with test compounds, cells were washed with PBS and a 200 μl of 1× lysis buffer was added into each well with continuous shaking for at least 20 min, then the content of each well was collected separately in 1.5 ml microcentrifuge tubes, followed by sudden freezing under liquid nitrogen and thawing of the tubes to ensure complete cell lysis. The tubes were then centrifuged to precipitate cellular waste, 100 μl cell lysate was then incubated with 100 μl of stabilized luciferase reagent and luciferase activity was quantified using TD-20/20 luminometer (Turner BioSystems, Sunnyvale CA).

### 2.7. Statistical analysis

The comparative analysis of the results from various experimental groups with their corresponding controls was performed using SigmaStat for Windows (Systat Software, Inc, CA). A one-way analysis of variance (ANOVA) followed by Student–Newman–Keul's test was carried out to assess

Download English Version:

https://daneshyari.com/en/article/2603309

Download Persian Version:

https://daneshyari.com/article/2603309

Daneshyari.com