Available online at www.sciencedirect.com
SCIENCE DIRECT®
Cancer Letters 235 (2006) 69–74
CANCER Letters
www.elsevier.com/locate/canlet

Lack of enhancing effects of sodium nitrite on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary carcinogenesis in female Sprague–Dawley rats

Yasuki Kitamura, Megumi Yamagishi, Kazushi Okazaki, Fumio Furukawa, Takayoshi Imazawa, Akiyoshi Nishikawa, Masao Hirose*

Division of Pathology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

Received 3 February 2005; received in revised form 5 April 2005; accepted 6 April 2005

Abstract

A number of heterocyclic amines (HCAs) have been shown to exert enhanced carcinogenic and mutagenic potential when given simultaneously with sodium nitrite ($NaNO_2$). In the present experiment, effects of combined treatment with $NaNO_2$ and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), one of the most prevalent carcinogenic HCAs in the human environment, were assessed with regard to mammary tumor induction in female Sprague–Dawley (SD) rats. Animals at 6 weeks of age were given intragastric doses of 100 mg/kg body weight of PhIP twice a week for 4 weeks, during which period 0 or 0.2% $NaNO_2$ was administered in the drinking water. Control rats received 0.2% $NaNO_2$ alone for the 4 weeks or non-supplemented water during the entire 48 week experimental period, without carcinogen treatment. The first tumor in the PhIP + $NaNO_2$ group appeared significantly later than with PhIP alone, and during the experimental period, the incidence, multiplicity and volume of mammary tumors in this group tended towards decreased, although values did not significantly differ at the terminal sacrifice. These results indicate that $NaNO_2$ does not enhance PhIP-induced rat mammary carcinogenesis, rather possessing some potential for inhibition.

Keywords: 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP); Heterocyclic amine; Sodium nitrite ($NaNO_2$); Mammary carcinogenesis; Sprague–Dawley (SD) rats

1. Introduction

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), one of the most prevalent mutagenic and carcinogenic heterocyclic amines (HCAs) in cooked

* Corresponding author. Tel.: +81 3 3700 9818; fax: +81 3 3700 1425.
E-mail address: m-hirose@nihs.go.jp (M. Hirose).

0304-3835/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2005.04.004

meat and fish [1–3], exerts carcinogenicity in the small and large intestine, mammary glands, prostate, lymphoma and pancreas of rodents [4–7]. DNA adduct formation by activated metabolites in the target organs is considered a major causal factor for its carcinogenicity in experiment animals, as with other HCAs [1,8,9], and PhIP has been found in urine after intake of cooked food [4], the urine samples having mutagenic activity [10]. In addition, HCA-DNA adducts have been demonstrated in human tissues [11] and some reports documented a positive correlation between the ingestion of cooked meat and risk of breast cancer [12–14]. Based on carcinogenicity data in experimental animals, supporting genotoxicity data, PhIP is anticipated to be a human carcinogen [7].

Sodium nitrite ($NaNO_2$) is used as a color fixative and preservative in meats and fish, and is also contained in vegetables [15]. Regarding interactions between HCAs and $NaNO_2$, non-IQ type HCAs which possess a 2-aminopyridine structure, such as 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2), 2-amino-3-methyl-*9H*-pyrido[2,3-*b*]indole (MeAαC) and 2-amino-5-phenylpyridine (Phe-P-1), were found to be inactivated by nitrite under acidic conditions in the mutagenicity assays in vitro, while IQ-type HCAs which have a 2-aminoimidazole structure, such as 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), appear resistant to such effects [16]. Indeed, Lin et al. demonstrated the mutagenicity of IQ and MeIQ to be strongly enhanced by the addition of nitrite under acidic conditions in both the presence and absence of rat S9 mix in vitro [17]. Recently, we found that the numbers and areas of GST-P positive rat liver foci induced by 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) were strongly increased [18] and the multiplicity of IQ-induced rat colon tumors was clearly elevated by combined treatment with $NaNO_2$ [19]. However, combined effects of PhIP and nitrite have not so far been reported in vivo.

In the present experiment, the effects of $NaNO_2$ on PhIP-induced mammary carcinogenesis when given in combination were therefore examined in female Sprague–Dawley (SD) rats.

2. Materials and methods

2.1. Chemicals

PhIP and $NaNO_2$ were obtained from Toronto Research Chemicals Inc. (Ontario, Canada) and Wako Pure Chemical Industries, Ltd (Osaka, Japan), respectively.

2.2. Animals and diets

Fifty female Crj:CD(SD) SPF rats, at 6 weeks of age, were obtained from Charles River Japan, Inc. (Atsugi, Japan), and assigned to four groups. They were housed in plastic cages (five rats/cage) with soft chips for bedding in a room with a barrier system and maintained under the following conditions: temperature (23±2 °C), relative humidity (60±5%), ventilation frequency (18 times per hour) and a 12-h illumination per day. Normal powder diet (CRF-1, Oriental Yeast Co., Tokyo, Japan) and tap water were available ad libitum. The Animal Care and Utilization Committee for the National Institute of Health Sciences, Japan, approved the protocols for this study.

2.3. Experimental design

The experimental design is shown in Fig. 1. The animals were randomly divided to give equal weight distributions into group 1, consisting of 20 animals,

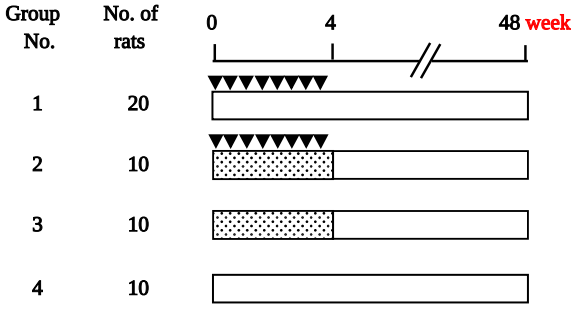

Fig. 1. Experimental design. Female SD rats were given intragastric administrations of 100 mg/kg body weight of PhIP (▼) twice a week for 4 weeks, and received water containing 0 (□) (Group 1) or 0.2% $NaNO_2$ (▩) (Group 2), respectively, for the period of carcinogen exposure. Other groups received 0.2% $NaNO_2$ (▩) alone for 4 weeks (group 3) or water without supplement (□) during the entire experimental period (group 4), without carcinogen exposure. All animals were killed at week 48.