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Immunosuppressive effect of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) through the inhibition of T-lymphocyte proliferation and IL-2 production

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Abstract

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is the predominant heterocyclic amine formed in cooked meat and fish and causes cancers in the colon, the mammary glands, and the lymphoid organs. In the present study, we investigated the immunological impact of PhIP using thymocytes isolated from Balb/c mice and a murine thymocyte-derived cell line, EL4. Treatment of the thymocytes with PhIP moderately inhibited T-cell mitogen-induced cell proliferation and interleukin (IL)-2 secretion. Reverse transcription-polymerase chain reaction (RT-PCR) analysis demonstrated that PhIP attenuated IL-2 mRNA expression in the thymocytes and EL4 cells stimulated with phytohemagglutinin (PHA) plus phorbol 12-myristate 13-acetate (PMA). In vitro transient transfection assay using a reporter gene construct containing IL-2 promoter showed that the decrease in the steady-state IL-2 mRNA level by PhIP is partially due to the attenuation of IL-2 mRNA synthesis at the transcriptional level. Furthermore, an electrophoretic mobility shift assay showed that PhIP inhibited DNA binding activity of nuclear factor for immunoglobulin κ chain in B cells (NF-κB), activator protein-1 (AP-1) and nuclear factor of activated T cells (NF-AT), which are known to be responsible for IL-2 transcriptional activation. Concomitantly, PhIP inhibited the PMA/PHA-induced generation of reactive oxygen species (ROS) involved in activation of the transcription factors. These results suggest that PhIP has potential immunosuppressive effects by inhibiting T-cell proliferation and IL-2 expression through down regulation of ROS generation and thereby inhibiting NF-κB, AP-1 and NF-AT activation.

Keywords: 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; Heterocyclic amines; Interleukin-2; Reactive oxygen species

Abbreviations: AFB1, aflatoxin B1; AP-1, activator protein-1; CAT, chloramphenicol acetyltransferase; CP, cyclophosphamide; cpm, counts per minute; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; EMSA, electrophoretic mobility shift assay; HCAs, heterocyclic amines; IL, interleukin; MFI, mean fluorescence intensity; NF-AT, nuclear factor of activated T cells; NF-κB, nuclear factor for immunoglobulin κ chain in B cells; PHA, phytohemagglutinin; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction

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1. Introduction

Overcooked meats and fish frequently contain substantial amounts of potent mutagens and is recognized as a significant risk factor in certain human cancers (Sugimura, 1997; Knize et al., 2002; Sinha, 2002; Nowell et al., 2004). Previous studies demonstrated that the consumption of overcooked meat and fish has a strong association with human neoplastic diseases (Steffensen et al., 1997), particularly tumors of the colon (Pool-Zobel and Leucht, 1997; Glaab et al., 2000; Moonen et al., 2004) and rectum (Totsuka et al., 1996; Wakabayashi et al., 1997), which led to the discovery of a large family of heterocyclic amines (HCAs). Thus, considerable effort has been devoted to assess the risk of mutagenesis and carcinogenesis in humans exposed to the HCAs. Indeed the importance and relevance of these studies were confirmed by the fact that most HCAs examined in rodent bioassays have been found to induce tumors (Adamson et al., 1996; Christian, 2002).

For most HCAs, the site of neoplastic diseases tends to be the liver although extrahepatic sites also proved to be a target. It is intriguing that the exception to this is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), which appears to induce tumors in lymphoid tissues of mice (Sorensen et al., 1996) as well as colon (Doi et al., 2005; Ikeda et al., 2005), mammary gland (Roberts-Thomson and Snyderwine, 2000; Christian, 2002; Yu et al., 2002; Shan et al., 2004) and prostate (Norrish et al., 1999; Shirai et al., 1999, 2000, 2002) in rats, with the exclusion of the liver. PhIP is the most prevalent among the HCAs recognized as carcinogens in the human diet (Felton et al., 1986).

Although the role of PhIP in certain cancer initiation has been thoroughly studied, much less is known about the effects of PhIP on the immune system. Since PhIP is capable of altering the status of various lymphoid tissues, it is possible that PhIP affects and modulates certain immunological pathway. In this study, we attempted to clarify the mechanism responsible for the modulation of IL-2 mediated T-cell activation and mitogen-induced thymocyte proliferation by PhIP.

2. Materials and methods

2.1. Chemicals and reagents

PhIP was purchased from Wako Chemical Industries Ltd. (Osaka, Japan) and LPS (*Salmonella typhosa*) from Sigma–Aldrich (St. Louis, MO). All reagents for RT-PCR were purchased from Promega (Madison, WI), except for the recombinant *Taq* DNA polymerase (rTaq) and dNTP (a mixture of dGTP, dATP, dTTP, and dCTP), which were from Takara Bio Inc. (Otsu, Shiga, Japan).

2.2. Animals

Specific pathogen-free 4–6 weeks old female Balb/c mice were purchased from Charles River Japan Inc. (Hino Breeding Center, Japan). On arrival, randomized mice were transferred to cages (five mice per cage) containing a sawdust bedding and quarantined for 1 week. The mice were given food (Purina Certified Lab Chow) and water ad libitum, and used for experimentation when their body weight reached 17–20 g. The conditions of the animal care facility were kept at 21–24 °C and 40–60% relative humidity with a 12 h light/dark cycle. All animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

2.3. Preparation of thymocytes

Thymuses were aseptically isolated from the mice and single cell suspension was prepared as previously described (Kaminski et al., 1994). Single cell suspension of thymocytes was washed with serum-free RPMI (GIBCO BRL, Gaithersburg, MD) and then cultured in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-mercaptoethanol, 2 mM L-glutamine and 10% fetal bovine serum (FBS) at 37 °C in a 5% CO_2-humidified incubator.

2.4. Culture of EL4 cell line

A murine thymoma cell line, EL4, was purchased from the American Type Culture Collection (Manassas, VA). The cells were cultured with the RPMI 1640 complete medium as described above.

2.5. Cell proliferation assay

Thymocytes (2×10^5 cells/well) were stimulated with mitogen (5 μg/ml of PHA or 50 nM of PMA plus 5 μg/ml of PHA) in the presence or absence of PhIP (2–20 μg/ml) in a 96-well microtiter plate (Falcon, Beckton Dickinson Labware, Franklin Lakes, NJ) and were incubated at 37 °C in a 5% CO_2-humidified incubator for 72 h. Cells were pulsed with 1 μCi/well of [^{3}H]-thymidine for the last 6 h of culture. Tritium incorporation was measured by scintillation counting. Results were expressed as mean counts per minute (cpm) ± standard deviation (S.D.) of triplicate cultures for each data point.

2.6. Measurement of IL-2 secretion

The thymocytes were cultured in triplicate in 48-well cell culture plates. The cell culture media were collected 24 h post-stimulation and quantified for IL-2 by a conventional sandwich ELISA as previously described (Han et al., 1998).