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Protection against aflatoxin-B_1-induced liver mutagenesis by *Scutellaria baicalensis*

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Abstract

We have measured the inhibition of the mutagenicity of the mycotoxin aflatoxin-B_1 in the liver of the rat by plant material of *Scutellaria baicalensis*, or Huang-qin. The addition of one percent dried Huang-qin to the feed of the animals reduced the mutant frequency of a subsequent administration of aflatoxin-B1 by approximately 60 and 77%, respectively, for two different batches of the plant material. The addition of Huang-qin also increased the expression of the gene for glutathione *S*-transferase A5 subunit by 2.5–3.0-fold, and decreased expression of P450 cytochrome 3A2 by 1.8–2.0-fold. The greater increase of the expression of the GST gene may result in the protection shown by Huang-qin. The sensitivity of the hepatic mitochondria to swelling, a measure of the mitochondrial permeability transition, is increased significantly in animals that are on a diet containing Huang-qin. This may lead to increased sensitivity to apoptosis on treatment with toxic compounds. The two batches of Huang-qin material show differences in both chemical composition and preventive potential. This study demonstrates how a combination of generating and analysis of plant varieties together with a mammalian assay for efficacy may improve the search for better plant-based prevention of cancer initiation.

Keywords: Mutagenicity; Plants; *Scutellaria*; Chemoprevention; Transgenic; *lacI*

1. Introduction

More than 25% of the pharmaceuticals commonly available by prescription are derived from leafy plants while numerous other whole plant preparations are used for the treatment of human diseases throughout the developed and developing world [1]. However, herbal preparations are mixtures of various plants, and contain hundreds of potentially active ingredients [2]. This may present a problem as most ingredients are not well characterized and some are potentially toxic. This is exemplified by several instances of reported organ failures

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and even fatal toxicity [3,4], as well as many instances of other adverse effects. An additional concern is the possibility of interactions between herbal components and pharmaceutical drugs [5]. Nevertheless, the beneficial potential of many herbs is undeniable and characterization of their beneficial as well as toxic properties is urgent and important.

The Chinese herb *Scutellaria baicalensis* Georgi (Huang-qin) is a popular and widely used herb in traditional Chinese medicine. It has anti-inflammatory and anti-pyretic effects, and is used in mixtures for skin disorders, jaundice, hepatitis, and inflammatory diseases [6,7]. In addition, some secondary metabolites of *S. baicalensis*, including baicalin and wogonin have been shown to have apoptotic and anti-tumor properties [8]. Extracts from the roots (Radix) of *S. baicalensis* have protective effects against the genotoxicity of various chemicals in bacterial assays, including *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and aflatoxin-B_1 [9]. Flavonoids from *S. baicalensis* were recently shown to inhibit proliferation of various human hepatoma cell lines [7].

Plants have the potential to provide us with many beneficial compounds. In the current study the potential antimutagenic effects of *S. baicalensis* were assessed in an animal model system. We provided rats with feed containing 1% of one of two mixtures of ground up *S. baicalensis*, with different compositions of relevant secondary metabolites. Subsequently the animals were exposed to aflatoxin-B_1 by oral gavage. The induction of mutation was assessed in the liver, the primary target organ for aflatoxin mutagenicity and carcinogenicity, by using a rat model with the retrievable bacterial *lacI* gene that acts as a target gene for mutation. In addition to mutation, we measured the effects of *S. baicalensis* on the expression of genes involved in aflatoxin metabolism, and the effects on the sensitivity of hepatic mitochondria to the permeability transition.

2. Materials and methods

2.1. Chemicals and plants

Several optimized germplasm lines of Huang-qin (*Scutellaria baicalensis*; Richter's Herbs, Goodwood, Ont., Canada) were previously developed and the contents of baicalin, baicalein, wogonin, and melatonin determined (Murch et al., in press, in Plant Science). Huang-qin lines were obtained from clonally propagated de novo shoots regenerated from wild-harvested seedlings [10,11]. Mixtures of roots and shoots of several lines were combined to obtain enough material for the experiment. Plant tissue was frozen in liquid N_2, freeze dried to complete dryness for 24 h (Labconco, Caltec Scientific Ltd., Toronto, Ont., Canada) and ground in a coffee mill (Braun) to a fine powder. The average dry weight of the plantlets was 11.6%.

The contents of relevant chemicals in the material of two mixtures used in this study are mentioned in the Section 3. Aflatoxin-B_1 was obtained from Sigma (St. Louis, MO, USA). All reagents used during packaging and plating of phage were supplied by Stratagene (La Jolla, CA, USA).

2.2. Animal treatment

F344 *lacI* transgenic BigBlue® transgenic rats were obtained from Stratagene (La Jolla, CA) and bred to produce sufficient numbers of female animals for this study. The rats were housed individually and fed AIN-93G diet throughout the study. Seven- to ten-week-old female rats were randomly assigned into several groups (control, aflatoxin, aflatoxin-Huang qin A, and aflatoxin-Huang qin B). Aflatoxin was administered at 0.5 mg/kg body weight (suspended in corn oil) by oral gavage. Control animals received corn oil only. The body weight of the animals was measured at the time of aflatoxin administration. Dried and powdered Huang-qin material was provided in the feed as 10 gm/kg feed (powdered feed with plant material, water added and dried into cookies). Preventive treatments started 3 weeks prior to gavage with aflatoxin and lasted until sacrifice, 2 weeks after the aflatoxin treatment. Food and drinking water were supplied ad libitum.

2.3. Mutant recovery

All rats were killed by CO_2 asphyxiation and cervical dislocation 2 weeks after the aflatoxin B_1 injection. The liver was removed, flash frozen in liquid nitrogen and stored at $-80\,^{\circ}C$. Genomic DNA was isolated using a dialysis method [12] and packaged into bacteriophage particles with Transpack™ packaging extract (Stratagene) according to the manufacturer's instructions [12,13]. Phage particles were screened for