Available online at www.sciencedirect.com
SCIENCE DIRECT
Food and Chemical Toxicology 44 (2006) 42–55
www.elsevier.com/locate/foodchemtox

Genetic toxicity studies with genistein

R. Michael McClain [a,*], Erich Wolz [b], Alberto Davidovich [c], Jochen Bausch [b]

[a] McClain Associates, 10 Powder Horn Terr, Randolph, NJ 07869, USA
[b] DSM Nutritional Products Ltd., Wurmisweg 576, CH-4303 Kaiseraugst, Switzerland
[c] DSM Nutritional Products, 45 Waterview Blvd., Parsippany, NJ 07054, USA

Received 5 March 2005; accepted 8 June 2005

Abstract

Genistein is a phytoestrogen that occurs naturally in the diet especially in soybeans and soy-based foods. Genistein and related phytoestrogens are of interest as chemopreventive agents for a variety of diseases and cancers based on epidemiologic evidence of reduced cancer rates in populations with a high intake of soy. Although soy and its constituents have been consumed at high levels in Asian populations without apparent adverse effects, concern has been raised of potential adverse effects due to estrogenic and other activities of the isoflavones. In these studies, genistein was evaluated for mutagenicity and clastogenicity in vitro in the *S. typhimurium* assay (Ames Test), the mouse lymphoma assay and in vivo in the micronucleus test in mice and rats.

There was no evidence for a mutagenic effect in the in vitro *S. typhimurium* assay with and without metabolic activation (S9). In the in vitro mouse lymphoma assay, genistein increased resistant mutants with and without metabolic activation (S9), which were predominantly small colonies indicating that genistein acts as a clastogen. Three independent in vivo micronucleus tests were performed in Moro mice, RAIf rats and Wistar rats. MORO male and female mice were treated orally for 14 days at doses up to 20 mg/kg/day. RAIf and Wistar male and female rats were treated orally at doses up to 2000 mg/kg without an increase in micronuclei in treated mice or rats.

It is concluded that genistein was not mutagenic in the *S. typhimurium* assay or mutagenic or clastogenic in vivo in the mouse and rat micronucleus test. In the mouse lymphoma assay, genistein induced an increase of predominantly small colonies indicating that genistein acts as a clastogen. This observation is in agreement with published data on the inhibitory action of genistein on topoisomerase II, which is known to lead to chromosomal damage with a threshold dose response.

Keywords: Genistein; Soy; Phytoestrogen; Genotoxicity; Mutagenicity; Clastogenicity; Ames test; Mouse lymphoma test; Micronucleus test

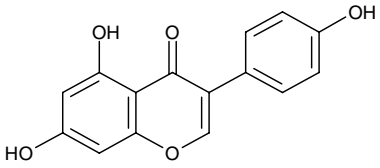
1. Introduction

Genistein is a phytochemical that occurs naturally in the diet and is found in a wide variety of plant-derived foods. The structure of genistein resembles that of endogenous estrogens (Fig. 1) and it has the capability of binding to the estrogen β receptor, but with a much lower affinity than estradiol. Genistein can compete with estradiol for binding to the receptor and once bound can increase the expression of estrogen responsive genes (Miksicek, 1995). Because of its ability to exert estrogenic activity, genistein and related compounds are referred to as phytoestrogens.

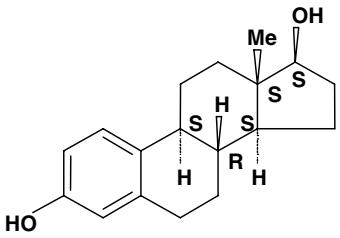
The phytoestrogens from plant sources can be classified as isoflavones, coumestans, and lignans. These substances are found in a wide variety of food derived from plants and the isoflavone group of phytoestrogens is found predominantly in soybeans and foods containing soy products (Knight and Eden, 1994). The major isoflavones are genistein, daidzein and glycitein, which in their natural state exist primarily as glycosides (Sirtori,

* Corresponding author. Tel.: +1 973 895 1363; fax: +1 973 895 1393.
E-mail address: michaelmcclain@msn.com (R.M. McClain).

0278-6915/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2005.06.004



Genistein (Bonestein™) is 4',5,7-Trihydroxyisoflavone ($C_{15}H_{10}O_5$) with a molecular weight of: 270.24. CAS No. [446-72-0]



Estradiol is Estra-1,3,5(10)-triene-3,17-diol (17β) ($C_{18}H_{24}O_2$) with a molecular weight of: 272.38. CAS No. [50-28-2]

Fig. 1. Structure of genistein.

2001). After oral ingestion, the glycosides are hydrolyzed by bacteria in the large intestine and absorbed. The aglycones are then glucuronidated and sulfated in the liver and undergo enterohepatic recirculation and are excreted primarily in the urine (Barnes et al., 1996).

Soy has been a major component of the diet for several Asian populations for centuries, thus soy and its components, such as genistein, have been extensively consumed without any apparent adverse effects. Indeed there has been considerable interest in soy and its components, such as genistein, for potential chemopreventive effects based on several epidemiologic studies suggesting reduced cancer rates associated with the consumption of soy-based foods. Several cancers such as breast, prostate and colon and several diseases including osteoporosis, hypercholesteremia, menopausal symptoms, atherosclerosis and heart disease have been of particular interest (Goldwyn et al., 2000; Suthar et al., 2001a,b).

The issue of genotoxicity of genistein has been raised (Kulling and Metzler, 1997; Kulling et al., 1999). In numerous reports of studies conducted in vitro, genistein has been demonstrated to be clastogenic and also in a few of these studies, mutagenic. The positive findings for genotoxicity have only been observed in in vitro systems and have been generally negative in in vivo systems. The mechanism of genistein genotoxicity is considered to involve the inhibition of topoisomerase II, an enzyme involved in DNA replication. Genistein binds to and stabilizes the topoisomerase–DNA complex inhibiting re-ligation and resulting in DNA strand breaks (Kaufmann, 1998; Markovits et al., 1989).

In the current studies, genistein was evaluated for mutagenic and clastogenic activity in vitro and in vivo including in vitro bacterial reverse mutation assay in *S. typhimurium*, the in vitro mouse lymphoma (ML/TK) assay and three independent in vivo micronucleus assays in mice and two strains of rat. The studies reported in this manuscript confirm the general lack of mutagenicity of genistein in *S. typhimurium* (Ames Test) and the lack of clastogenicity or aneuploidy in 3 separate in vivo micronucleus assays in mice and rats. In vitro, genistein increased predominantly small colony formation in the mouse lymphoma assay indicative of a clastogenic rather than a point mutation inducing effect.

2. Material and methods

2.1. Ames test (methods)

An in vitro evaluation of the mutagenic potential of genistein in bacteria (Ames Test) was performed with and without an exogenous metabolic activating system (S9) (Albertini, 1995). Genistein (batch 15574B-42-2) was a beige powder with a purity of 99.5% and was stored in a refrigerator (below −4 °C); protected from light, under nitrogen. The S9 metabolic fraction was prepared from male albino rats induced by combined IP treatment with phenobarbital and β-naphthoflavone (Ames et al., 1975; Matsushima et al., 1980). The *S. typhimurium* strains TA1535, TA97, TA98, TA100, and TA102 were obtained from B.N. Ames and are described elsewhere (Ames et al., 1975; Levin et al., 1982a,b; Maron and Ames, 1983). The sensitivity of the *S. typhimurium* strains was verified using the