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Studies on the toxicity of gambogic acid in rats

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ABSTRACT

Aim: To study the chronic toxicity of gambogic acid (GA), the major active ingredient of gamboges, a brownish to orange resin extracted from the *Garcinia hanburyi* (family *Guttiferae*) in Southeast Asia, using Sprague–Dawley rat as an animal model and provide further theoretical support for clinical applications of this promising natural anticancer agent.
Methods: GA was administered orally at dosages of 120, 60 and 30 mg/kg once every other day for a total of 13 weeks. Then we carried out the chronic toxicity studies including general body parameters, hematological, serum biochemistry, histopathological, and viscera examination.
Results: The results from the studies demonstrated that rats treated with high dose (120 mg/kg) of GA for a long time can lead to the damage on the kidney and liver. An innocuous dose was established to be 60 mg/kg after administration to rats for a total of 13 weeks at a frequency of one administration every other day. This dose was approximately 18.0 (body weight) or 9.6 (body surface area) times higher then that of the dose (200 mg/60 kg, every other day) used for human trials.
Conclusions: The studies demonstrated that the toxicity targets in the rats were the kidney and liver. These results provide further theoretical support for clinical applications of this promising natural anticancer agent.

1. Introduction

Gambogic acid (GA, Fig. 1) is the major active ingredient of gamboges (Auterhoff et al., 1962), a brownish to orange resin extracted from the *Garcinia hanburyi* tree (genus *Garcinia* of family *Guttiferae*) in Southeast Asia. Traditionally, GA has been used as a coloring material in folk medicine, due to its unique color and broad spectrum of cytotoxic activities (Asano et al., 1996). Recent studies from several laboratories including ours have demonstrated that this natural product possesses potent anticancer activity both *in vitro* and *in vivo* (Wu et al., 2004; Zhao et al., 2004; Guo et al., 2006). The potent anticancer activity of GA is mainly attributed to its activation of the impaired apoptosis pathways in cancerous cells via down-regulation of telomerase (Guo et al., 2006; Jun et al., 2006; Yu et al., 2006). However, knowledge concerning the potential side effects and toxicity of this promising anti-tumor agent is surprisingly limited. This limitation retards the approval of clinical applications of this natural product in chemotherapy of cancer patients. Here, we studied the chronic toxicity of GA in Sprague–Dawley rats as a model on the basis of the GLP (Good Laboratory Practice) of the People's Republic of China. It is expected that the results presented in this paper will help to establish appropriate dosage, frequency, and treatment duration in clinical applications of this natural product.

2. Materials and methods

2.1. Plant material and preparation of GA

The gamboge resin (lot. 20000526) of *G. hanburyi* was purchased from Jiangsu Provincial Medicinal Materials Company, China. GA was isolated and purified according to the established methods previously reported (Zhao et al., 2004). Preparations with GA content of 95% or higher were used in all experiments as solutions in 0.5% carboxymethyl cellulose Na (CMC-Na). All other reagents were purchased from commercial sources without any further purification or modification unless otherwise indicated.

2.2. Experimental design

Sprague–Dawley rats of both sexes with weights of 110–130 g were obtained from Shanghai Sipper-bk Animal Co., Ltd. (China). The animals without gender mixing were kept in stainless steel cages that hold five animals in each with light–dark cycles, 22 ± 2 °C and 55–65% humidity. The room was regularly disinfected by UV light. All the housing condition and test operation were regulated by the GLPs of the People's Republic of China. The animals were divided

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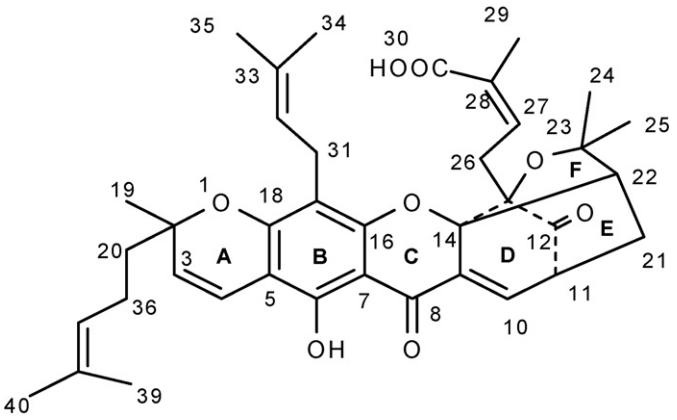

Fig. 1. Molecular structure and atom numbering scheme of GA ($C_{38}H_{44}O_8$, mol. wt.: 628.75).

into four groups of 20 rats each (10 females and 10 males). GA was dissolved in 0.5% CMC-Na and administered by gavage at dosages of 120, 60 and 30 mg/kg (groups I, II and III, respectively) once every other day for a total of 13 weeks. The control group (group IV) was given 0.5% CMC-Na containing no GA. Twenty-four hours after the last administration, 10 rats (5 females and 5 males) from each group were sacrificed by decapitation and various parameters were measured and compared with the values obtained prior to treatment. The 10 remaining animals in each group were carefully observed for an additional 4 weeks with necessary food and water supply. They were then sacrificed and various parameters determined as described above.

2.3. General body parameters

Experimental animals were weighed once every week. Their body temperature was measured (at anus without animals anesthetized) at the points before and after administration and in the recovery period. The objective signs of color pattern, cleanliness, behavior, food intake, urine, manure, psychic states, eye and porous channel secretions were measured and recorded every day.

2.4. Blood analysis

Blood samples (1 ml each animal) were collected by tail bleeding and taken into the tubes containing EDTA (1.5 mg) and heparin (0.125 mg) for hematological and biochemical analyses. The levels of blood glucose (Kouitcheu Mabeku et al., 2007), protein (Lowry et al., 1951), leukocyte, urea nitrogen (Natelson et al., 1951), cholesterol (Parekh et al., 1976), bilirubin, alanine aminotransferase, aspartic acid aminotransferase, alkaline phosphatase (Lott, 1977), creatinine and so on were determined by HITACHI-7020 automatic biochemistry analyzer (HITACHI, Japan).

2.5. Autopsy study

Specimens were collected at two different stages of the experiment with the first and second groups of samples obtained 24 h and 4 weeks, respectively, after the last GA administration. The animals were sacrificed by decapitation and dissection. During the process of dissecting, the parenchymatous organs' color, texture, lump and so on were carefully examined. The color and integrity of the cavities' mucosa were also examined. In the meantime, the weight of the heart, liver, spleen, lung, kidney, adrenal gland, thymus, thyroid gland, brain, uterus, testis, and prostate were measured and recorded. The organ–body index was calculated according to the following formula:

$$\text{Organ–body weight index } (\%) = \frac{\text{Wet organ weight}}{\text{Body weight}} \times 100\%$$

2.6. Histopathological study

Histopathological investigation was done according to methods described in the literature (Vasilescu et al., 1955; Akdogan et al., 2003; Abd-Elhamid, 2004). In addition to the organs mentioned above, the pancreas, chorda spinalis, hypophysis, cranial nerve, absorbent gland, bladder, and bone marrow (chest bone) were also studied. Briefly, small organ pieces (3–5 mm thick) were fixed in 10% formal-saline (0.9% NaCl in 10% formaldehyde) for 24 h and washed in running water for another 24 h. Samples were dehydrated by passing through 50, 70, 90, and 100% alcohol (analytical pure dehydrated alcohol) over a 2-day period, and were then cleaned in benzene to remove alcohol until the tissues became transparent. This was followed by staining with hematoxylin–eosin, and thorough examination using a light microscope.

2.7. Convalescence stage study

Ten animals from each group were further observed for 4 weeks after the last GA administration. They were then sacrificed and various parameters determined as described above.

2.8. Statistical analysis

All results are reported as mean ± S.E.M. Statistical evaluations of the data were initially tested by the homogeneity of variances. Data with homologous variances were further tested by the Student's *t*-test, and *P*-values less than 0.05 were considered significant. Aspin–Welch statistical analysis was employed to evaluate data sets with non-homologous variances.

3. Results

3.1. Effect of chronic oral administration of GA on the general behavior of the rats

The responses, such as slobbering, astasia, and anepithymia, were observed among animals in the high dose group (120 mg/kg). The symptoms described above disappeared in the convalescence stage. Interestingly, GA administration of 60 and 30 mg/kg did not bring any notable changes to the animals except for some slobbering. Animals in the two groups (60 and 30 mg/kg) displayed normal physical behavior compared with those in the control group. All animals were weighed once a week. The weight of animals receiving high dosages of GA decreased dramatically in weeks 1–5 (for female, $P < 0.05$, Fig. 2) and weeks 1–15 (for male, $P < 0.01$, Table 1) after GA injection compared with those in the control group during the corresponding period. This body weight loss did not reverse in the 4-week convalescent period. No other animals had extraordinary changes in weight. Body temperature and the food intake of the animals treated did not seem to change compared with those in the control group (data not shown).

3.2. Effect of chronic oral administration of GA on the hematological and biochemical parameters of rats

The effect of chronic oral administration of GA on the hematological and biochemical parameters is presented in Tables 2 and 3. After the last GA treatment, compared with the controls, the levels of leukocyte of the animals treated with different dosages (120,