Rutin Inhibits Human Leukemia Tumor Growth in a Murine Xenograft Model *In Vivo*

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ABSTRACT: Numerous studies have shown that rutin has anticancer effects. We have previously reported that rutin induced cell cycle arrest and apoptosis in murine leukemia WEHI-3 cells *in vitro* and *in vivo*. However, there are no data showing that rutin inhibits human leukemia HL-60 cells *in vivo* in a murine xenograft animal model. Human leukemia HL-60 cells were implanted into mice and treated with vehicle (1% DMSO), rutin (120 mg/kg of body weight) or vinblastine (120 μg/kg of body weight). Compounds and agents were injected once every four days intraperitoneally (*i.p.*) for 36 days. Treatment with 120 mg/kg of rutin or with 120 μg/kg of vinblastine resulted in a reduction of tumor weight and volume when compared

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with the control groups. Tumor size in xenograft mice treated with 120 mg/kg of rutin was significantly smaller than that in the untreated-control group. These novel findings indicate that rutin inhibits tumor growth in a xenograft animal model. Rutin may be useful in treating leukemia but certainly much more research is needed. Environ Toxicol 00: 000–000, 2011.
Keywords: rutin; human leukemia HL-60 cells; xenograft transplantation; *in vivo*

INTRODUCTION

Leukemia is a major cause of death worldwide. In the United States, about 3.7 per 100,000 people die/year of leukemia (Jensen et al., 2004). It is estimated that about four persons per 100,000 people die from leukemia in Taiwan according to reports from the Department of Health, Executive Yuan, R.O.C. (TAIWAN) (http://www.doh.gov.tw/EN2006/index_EN.aspx). Numerous treatments have been used in leukemia patients but the cure rate remains unsatisfactory.

It is well established that increased consumption of plant-based diets can reduce the risk of cancer (Mahmoud et al., 2000; Mutoh et al., 2000; Wenzel et al., 2000; Hsu et al., 2010) but the exact bioactive agents have not been well-established. For example, in colon cancer clinical studies of chemoprevention have been reported using naturally occurring dietary substances (Kelloff et al., 2000). Herbal based dietary supplements contain many phytochemicals such as flavonoids which may contribute to cancer suppression Rutin, a member of the flavonoid family, possesses anti-inflammatory, antiallergenic, antiviral, and anticarcinogenic properties and it is also a free radical scavenger (La Casa et al., 2000; Kamalakkannan and Stanely Mainzen Prince, 2006). Rutin was found to have chemopreventative activity in several animal models including azoxymethane-induced colon tumorigenesis in mice and rats (Deschner et al., 1993; Matsukawa et al., 1997; Tanaka et al., 1999), dimethylbenz(a)anthracene (DMBA) and *N*-nitrosomethylurea-treated mammary glands of rats and DMBA-treated skin cancer (Verma et al., 1988). There is no available information on effects of rutin on human leukemia cells *in vivo*. Therefore, in the present study, we investigated the effects of rutin on human leukemia HL-60 cells in xenografts of mice *in vivo*.

MATERIALS AND METHODS

Chemicals

Rutin, vinblastine, dimethyl sulfoxide (DMSO), trypan blue and Triton X-100 were obtained from Sigma Chemical Corp. (St. Louis, MO). Rutin and vinblastine were dissolved in 1% DMSO with phosphate buffered saline (PBS).

Cell Culture

The human promyelocytic leukemia cell line (HL-60) was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were plated in 75 cm^2 tissues culture plates in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 Units/mL penicillin and 100 μg/mL streptomycin (Invitrogen/Gibco BRL, Grand Island, NY) and cells were maintained at 37°C in a humidified 5% CO_2 and 95% air (Lin et al., 2007; Lin et al., 2009).

In Vivo Tumor Xenograft Model

Eighteen six-week-old female BALB/$c^{nu/nu}$ mice were obtained from the Laboratory Animal Center of the National Applied Research Laboratories (Taipei, Taiwan). The experimental design of the different treatment groups is shown in Fig. 1. Flanks of mice were subcutaneously (*s.c.*) implanted with HL-60 cells (1×10^7/100 μL culture medium) for a 12-day incubation for solid tumor growth. Animals bearing tumors were randomly assigned to treatment groups (6 mice per group) and treatment initiated when tumors reached volumes of about 200 mm^3 at which time mice were injected intraperitoneally (*i.p.*) once every four days with 30 μL of 1% DMSO control vehicle, rutin (120 mg/kg) and vinblastine (120 μg/kg). All animal studies were conducted according to institutional guidelines approved by the Animal Care and Use Committee of China Medical University (Taichung, Taiwan) (Kuo et al., 2006; Yang et al., 2008; Ho et al., 2009; Ji et al., 2009; Su et al., 2010)

Mice exhibiting tumors were monitored, counted, and the tumor sizes were measured initially after 12 days, with the final measurement taken five weeks after tumor cell inoculation. After xenograft tumor transplantation, tumor size was individually measured once per four days using calipers and tumor volume was estimated according to the following formula: tumor volume (mm^3) = $1/2 \times L \times W^2$ (L, length; W, width). Body weight was measured at various time points. At the end of the experiment, animals from each group were sacrificed. Tumors were removed, weighed, and tumor volumes were calculated as given above (Ho et al., 2009; Ji et al., 2009).

Statistical Analysis

Data are presented as mean ± SD and compared by using the Student's t test, and p values less than or equal to 0.05 were considered significant (***$P < 0.001$).

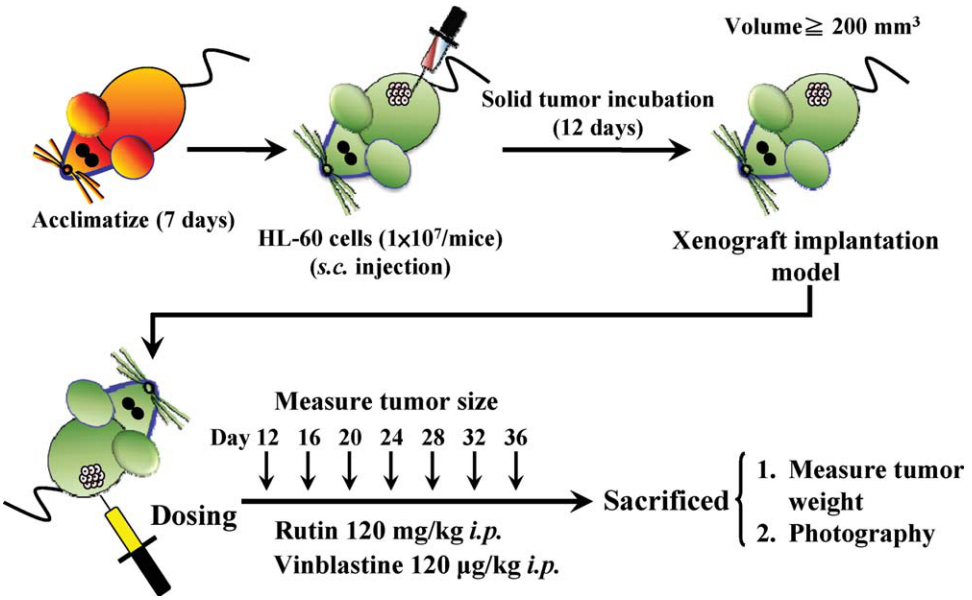

Fig. 1. Experimental design of rutin-suppressed HL-60 tumor in a xenograft animal model. The animals were *s.c.* implanted with HL-60 cells for 12 days until tumor volume reached 200 mm^3 and then randomly divided into three groups. Group 1 was treated with 1% DMSO *i.p.* only. Group 2 were treated with 120 mg/kg rutin *i.p.* and group 3 was treated with 120 μg/kg vinblastine *i.p.* for 36 days. During the treatment, each animal was measure tumor size and weight as described in Materials and Methods. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS

Effects of Rutin on Tumor Size and Weight

Female BALB/$c^{nu/nu}$ mice were intraperitoneally injected with HL-60 cells and then each animal was individually treated with 1% DMSO, rutin and vinblastine for different time periods as shown in Figure 1. A representative animal with tumors is shown in Figure 2(A,B). Mean tumor weight and percent inhibition of tumor occurrence are shown in Table I. Rutin and vinblastine significantly decreased tumor weight compared with the control group (Fig. 2 and Table I). Tumor volume also was suppressed as can be seen in Figure 3. Rutin decreased tumor weight by 62.99% of control (Table I). Comparisons of tumors volumes between the control and rutin or vinblastine treatment groups showed that 120 mg/kg rutin clearly reduced tumor volume and weight when compared with control mice (Table I and Fig. 3). Overall, the tumor mass and tumor growth in the xenograft mice was reduced in the rutin group compared to control mice. In Table I, vinblastine (120 μg/kg) also significantly induced tumor inhibition by ~50%.

DISCUSSION

It is well-known that many compounds from natural plants have chemopreventative and chemotherapeutic efficacy in human cancers (Surh, 2003; Eggler et al., 2008; Pan and Ho, 2008). The discovery of phytomedicinal plants as well as elucidations of their underlying mechanism in anticancer activity is important. Rutin, one of the major representatives of flavonoids, is present in many natural plants (Gong et al., 2010), and it has been shown to cause cell cycle arrest and induce apoptosis in many types of human cancer cell lines (Pu et al., 2004; Kamalakkannan and Stanely Mainzen Prince, 2006; Koda et al., 2008; La Casa et al., 2000). Gong et al. found that rutin protected human vein endothelium cells (HUVEC) against H_2O_2-induced apoptotic cell death (Gong et al., 2010). It that study, the protective effects of rutin was attributed to regulation of glutathione reactive oxygen species (ROS). In another study, it was reported that rutin may reduce the risk of atherosclerosis by inhibiting of low-density lipoproteinoxidation (LDL) oxidation (Milde et al., 2007).

Until this study, there had been no reports on rutin acting on human leukemia HL-60 cells in a xenograft mouse model *in vivo*. HL-60 cells have been used for several years as a model cell line in leukemia studies (Krige et al., 2008). We hypothesized that rutin could inhibit human leukemia tumors *in vivo* in xenograft mice. Rutin inhibited tumor growth in HL-60 cell xenograft mice *in vivo*. These findings differ somewhat from an *in vitro* study where a similar concentration of rutin (50 μM) had significant cytotoxic effects (over 50% cell death) on proliferation of mouse leukemia WEHI-3 cell line as well as inhibition of BALB/c mice intraperitoneally injected WEHI-3 cells *in vivo* (Lin et al., 2009). However, tumor volume and weight in xenograft

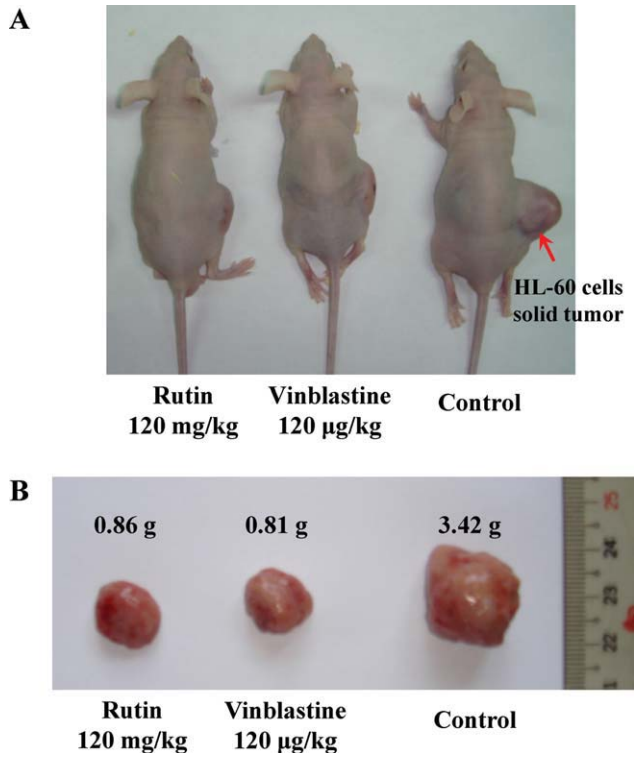

Fig. 2. Representative tumor on the xenograft animal model. Eighteen nude mice were *s.c.* implanted with 1×10^7 HL-60 cells for 12 days and were randomly divided into three groups. Group 1 was treated with 1% DMSO only as a control. Group 2 were treated with 120 mg/kg rutin and group 3 was treated with 120 μg/kg vinblastine once every four days until 36th day after transplantation, and then all animal were sacrificed. A: Representative animal with solid tumor; B: solid tumor and tumor weight. Each animal was measure tumor size and weight during the treatment as described in Materials and Methods. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mice that received 120 mg/kg rutin alone was about 63% less than these of the control group (Fig. 2C). We did not observe toxic effects at the rutin doses administered as evidenced by an absence of changes in body weight (data not shown) or grooming habits.

TABLE I. Effects of inhibition rate (%) of rutin on leukemia solid tumor in the xenograft animal model

Treatment; Dosage	Tumor Weight (g)	Inhibition Rate (%)
Control	2.540 ± 0.695	–
Vinblastine 120 μg/kg	1.284 ± 0.617	49.45
Rutin 120 mg/kg	0.940 ± 0.406***	62.99

Eighteen BALB/c$^{nu/nu}$ mice were subcutaneously transplanted with HL-60 cells (1×10^7 cells/mice) for 12 days and the animals were divided into three groups. At the end of treatment, all mice were sacrificed at 36th day. Each animal was measure tumor weight during the treatment as described in Materials and Methods. Representative tumor weight and inhibition rate (%) were shown in Table I and results expressed was mean ± S.D. and were compared as analyzed by Student's *t*-test. ***$p < 0.001$.The tumors were weighed and observed in groups of control, vinblastine and rutin, respectively.

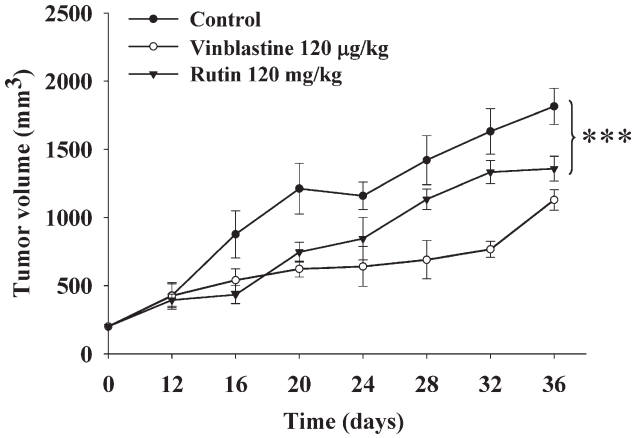

Fig. 3. Inhibitory effect of rutin on an *in vivo* xenograft model. Efficacy of rutin and vinblastine on the growth of HL-60 human leukemia tumor xenografts in BALB/c$^{nu/nu}$ mice. Treatment with theses agents reduced the tumors. Tumor growth is presented as the mean tumor volume (mm^3) ± S.D. Tumor volume was determined by caliper measurements and was calculated as the product of 1/2 × length × width^2. Data presented was mean ± S.D. at 12th–36th day post-tumor implantation, the tumor volumes observed in 1% DMSO as a control group, vinblastine and rutin groups were compared as analyzed by Student's *t* test (***$P < 0.001$) as described in Materials and Methods.

Tumor volume was less in the rutin treated mice compared with controls. Furthermore, the results showed that tumors that received rutin treatment grew slowly, suggesting that complete regression of HL-60 cells xenografts was not achieved using a single treatment. Therefore, this study provided the first *in vivo* evidence for the efficacy of the flavonoid, rutin, on human leukemia HL-60 cells in xenograft mice. Vinblastine treatment also reduced both tumor weight and volume at a concentration of 1000 times lower than rutin. Multiple rutin treatment may be necessary to achieve complete tumor regression. In conclusion, rutin administered once *i.p.* per 4 days at 120 mg/kg was effective in reducing the growth of human leukemia HL-60 tumors in a xenograft mouse model. These findings are the first to study examine effects of rutin as a leukemia preventive agent using a leukemia murine xenograft model.

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