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Rutin Exerts Antitumor Effects on Nude Mice Bearing SW480 Tumor

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Background and Aims. We previously showed that extracts from *Phoradendron serotinum* and *Croton lechleri* exerted *in vitro* cytotoxic and *in vivo* antitumor effects and that their main component was rutin (RTN; 3-rhamnosyl-glucosylquercetin). However, it is unknown whether RTN exerts *in vivo* antitumoral effects on human colon cancer cells. The aim of this work was to evaluate the antitumor effects of RTN on a murine model.

Methods. Cytotoxic effects of RTN on human cancer and non-tumorigenic cell lines were evaluated using the MTT assay. Different doses of RTN were injected intraperitoneally daily into nu/nu mice bearing tumors of SW480 colon cancer cells during 32 days. The growth and weight of tumors were measured. Serum levels of VEGF, survival time, increase in life span and toxicological effects on body weight and organ weight were also analyzed.

Results. RTN showed the highest cytotoxic effects against SW480 cells (IC_{50} = 125 μM) as compared to the other cancer cells lines and decreased, in a dose dependent manner, the tumor volume and weight of mice bearing SW480 tumor. RTN 20 mg/kg, the highest dose tested, lacked toxic effects on body weight and relative organ weight in mice, increased mean survival time by 50 days, and decreased by 55% the VEGF serum levels compared to untreated mice.

Conclusions. RTN exerts *in vitro* cytotoxic effects on SW480 cells, induces *in vivo* antitumor effects, lacks toxic effects on mice bearing SW480 tumor and exerts antiangiogenic properties. © 2013 IMSS. Published by Elsevier Inc.

Key Words: Rutin, Cytotoxic, Antitumor, Angiogenesis, Colon cancer, VEGF.

Introduction

Colon cancer is the third most common cancer worldwide with an incidence of 1.23 million cases and 608,000 deaths in 2008 (1). Family history of colorectal cancer, polyps, inflammatory bowel disease, physical inactivity, obesity, high intake of red meat, smoking, alcohol intake, as well as low fruit and vegetable consumption are involved in the process and development of colon cancer (2). Up to 80% of all colorectal cancer cases and deaths are attributable to diet (3). Therefore, many cases of colorectal cancer and related deaths may be preventable by dietary modifications.

Flavonoids are the most widely distributed group of polyphenols, a class of phytochemicals characterized by their phenolic ring structures, with >5,000 unique compounds found in various fruits, vegetables, grains, nuts, teas and wines (4). The estimated dietary intake of all flavonoids in humans is ~1 g per day (5). A large cohort study of 9,959 men and women in Finland from 1967–1991 found an inverse association between total flavonoid intake and cancer incidence (6). Among these compounds, rutin (RTN; 3-rhamnosyl-glucosylquercetin) (Figure 1) is found in many fruits and vegetables. Previously it was shown that RTN exerted *in vitro* toxic effects on cancer cell lines (7,8) including human colon cancer cells (9). Moreover, it was

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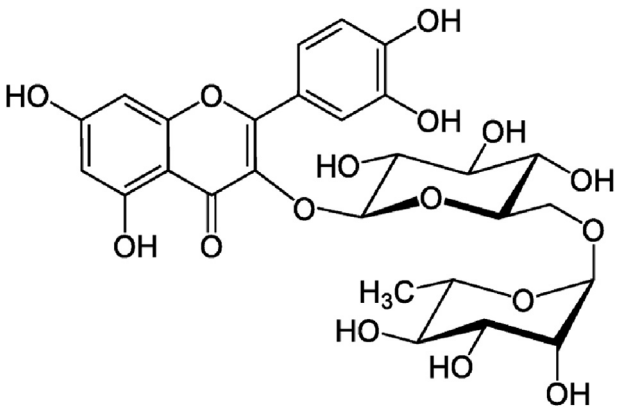

Figure 1. Chemical structure of the flavonoid rutin (RTN).

shown that RTN exerts cytotoxic effects on SW480 cells through mitochondrial pathway caspase-dependent (10).

Several plant extracts such as *Nelumbo nucifera* Gaertn (Nymphaeaceae), *Croton lechleri* Mull. Arg. (Euphorbiaceae) and *Phoradendron serotinum* (Raf.) M. C. Johnst. (Viscaceae), showed *in vivo* antitumor effects, and rutin was one of its major components (11−13). Thus, it is probable that the antitumor effects of these plant extracts could be due to the action of RTN. Furthermore, RTN has shown antitumor effects in some *in vivo* models such as NK/Ly ascites and B16F10 cells (7,8,14). RTN has also shown chemopreventive activity in murine models (15,16). However, the antitumor effects of RTN on human colon cancer cells remain to be studied. This study shows, for the first time, that RTN induces high cytotoxic effects *in vitro* against SW480 cells as well as *in vivo* antitumor and antiangiogenic activities.

Materials and Methods

Materials

DMEM, RPMI and fetal bovine serum (FBS) were purchased from GIBCO BRL (Grand Island, NY), whereas cisplatin (CDDP) was from Accord Farma (Mexico City). Rutin (RTN), obtained from Sigma Chemical Co. (St. Louis, MO), had a 98% purity according to the manufacturer. Sevoflurane was obtained from Abbott Laboratories (Chicago, IL).

Cell Lines and Culture Conditions

Human tumor-derived cell lines from cervical (HeLa), colorectal (SW-480), breast (MDA-MB-231) and liver (HepG2) carcinomas and leukemia (K562) as well as non-tumorigenic human immortalized keratinocytes (HaCaT) cell line were maintained in DMEM supplemented with 7% FBS and antibiotics (100 U/mL penicillin and 100 pg/mL streptomycin). Ovarian (SKOV-3) and prostate (DU-145) carcinoma cell lines were maintained in RPMI medium supplemented with 7% FBS and antibiotics. All human cell lines were obtained from ATCC. All cell cultures were grown at 37°C and 5% CO_2.

Animals

Six-week-old nu/nu mice (weighing 18−23 g) from the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán were used. The experiments were performed following NIH Guidelines for Treatment and Care for Laboratory Animals and the Mexican Official Norm for Animal Care and Handing (NOM-062-ZOO-1999). All procedures carried out in this study were approved by the Research Ethics Committee from the Instituto Nacional de Cancerología (Distrito Federal, México). Mice with free access to food and water were housed in cages with filtered air in a climate and light controlled room with a 12 h light/dark cycle.

In Vitro *Assay*

Cell viability assay. Cells were seeded in 96-well microplates at a density of 5000 cells/well. After 24 h, cells were incubated with concentrations of RTN or CDDP between 0.1 and 300 μM. DMSO at a final concentration of 0.01% was used as vehicle control. After 48 h of treatment, MTT assay was performed and relative viability was calculated as described previously (12). The concentration leading to 50% inhibition of viability (IC_{50}) was also calculated by regression analysis (percent survival vs. log concentration).

In Vivo *Assay*

Antitumor assay. The nu/nu mice were injected s.c. in their backs with SW480 cells (5×10^6). Eight days after tumor implantation, mice ($n = 8$ for each group) bearing tumor received doses of RTN between 1 and 20 mg/kg or CDDP 1 mg/kg, dissolved in 0.1 mL of 0.9% saline solution each, injected i.p. daily over a period of 32 days. In all *in vivo* experiments the animal control group received 0.1 mL of vehicle solution. Tumors were measured using a Vernier caliper and their size (in mm^3) was calculated as follows (17):

$$\text{Tumor volume} = \frac{\text{length} \times \text{width} \times \text{height}}{2}$$

At the end of the experiments, mice ($n = 5$ for each group) bearing tumor were anesthetized by sevoflurane inhalation and their blood was obtained by cardiac puncture. Serum samples were prepared by centrifuging the whole blood at 2000 × g at 4°C for 10 min. Serum levels of VEGF were measured using a commercially available kit (eBioscience; San Diego, CA) according to the manufacturer's