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Insights on the antitumor effects of kahweol on human breast cancer: Decreased survival and increased production of reactive oxygen species and cytotoxicity

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ABSTRACT

The present study aims to identify the modulatory effects of kahweol, an antioxidant diterpene present in coffee beans, on a panel of human tumor cell lines. Kahweol inhibits tumor cell proliferation and clonogenicity and induces apoptosis in several kinds of human tumor cells. In the estrogen receptor-negative MDA-MB231 human breast cancer, the mentioned effects are accompanied by caspases 3/7 and 9 activation and cytochrome c release. On the other hand, kahweol increases the production of reactive oxygen species and their cytotoxicity in human breast cancer cells but not in normal cells. Taken together, our data suggest that kahweol is an antitumor compound with inhibitory effects on tumor cell growth and survival, especially against MDA-MB231 breast cancer cells.

1. Introduction

After water, coffee is – along with tea – the most commonly consumed drink in the world. Coffee is made from ground, roasted coffee beans (the dried seeds of coffee plant – *Coffea* – berries). Epidemiological studies associate the consumption of unfiltered coffee with a low incidence of colon and liver cancer [1,2]. A recent meta-analysis of cohort studies reinforces the suggestion that coffee consumption may reduce total cancer incidence [3]. Furthermore, a big prospective analysis concluded that there is a strong inverse association between coffee consumption and risk of lethal prostate cancer [4]. In a recent report, it has been shown that a high daily intake of coffee is associated with a statistically significant decrease in estrogen receptor-negative breast cancer among postmenopausal women [5]. Furthermore, its preventive effects against oxidative stress and DNA damage are well described [6]. Due to its high rate of consumption, coffee is a major source of some antioxidants in the Western diet. Coffee beans contain more than a thousand of compounds [7]. Kahweol (Fig. 1A) is an antioxidant diterpene of coffee beans that is more abundant in unfiltered coffee beverages, such as Turkish and Scandinavian coffee [8], with approximate kahweol contents of 5.4 and 7.2 mg per cup, respectively [9].

In many cases, the biological effects of kahweol have been studied in combination with cafestol, a closely related compound (in fact, kahweol is 1,2-didehydrocafestol). Assays performed with either pure cafestol or a cafestol–kahweol mixture indicated that kahweol contributes specially to increase serum concentration of alanine aminotransferase and has little additional effects on serum concentration of cholesterol, but reduces that of γ-glutamyltransferase [10,11]. In contrast, subsequent studies found out that kahweol really reduces γ-glutamyltransferase but concluded that it is unlikely that kahweol is the component of coffee oil that is responsible for the other liver enzyme level increases [12]. Evidence has accumulated showing anti-oxidant, anti-inflammatory, anti-tumoral and chemoprotective effects of kahweol. As an anti-oxidant, kahweol has been shown to protect against hydrogen peroxide induced oxidative stress and DNA damage, probably via scavenging reactive oxygen species (ROS) [6] and to induce heme oxygenase-1 to control intracellular ROS levels [13]. The anti-inflammatory action of kahweol includes its ability to suppress macrophage cyclooxygenase-2 and inducible nitric oxide synthase expression [14,15] and its modulatory action on NF-kappaB pathway [16]. Our group has shown that kahweol behaves as an anti-angiogenic compound and targets several key inflammatory

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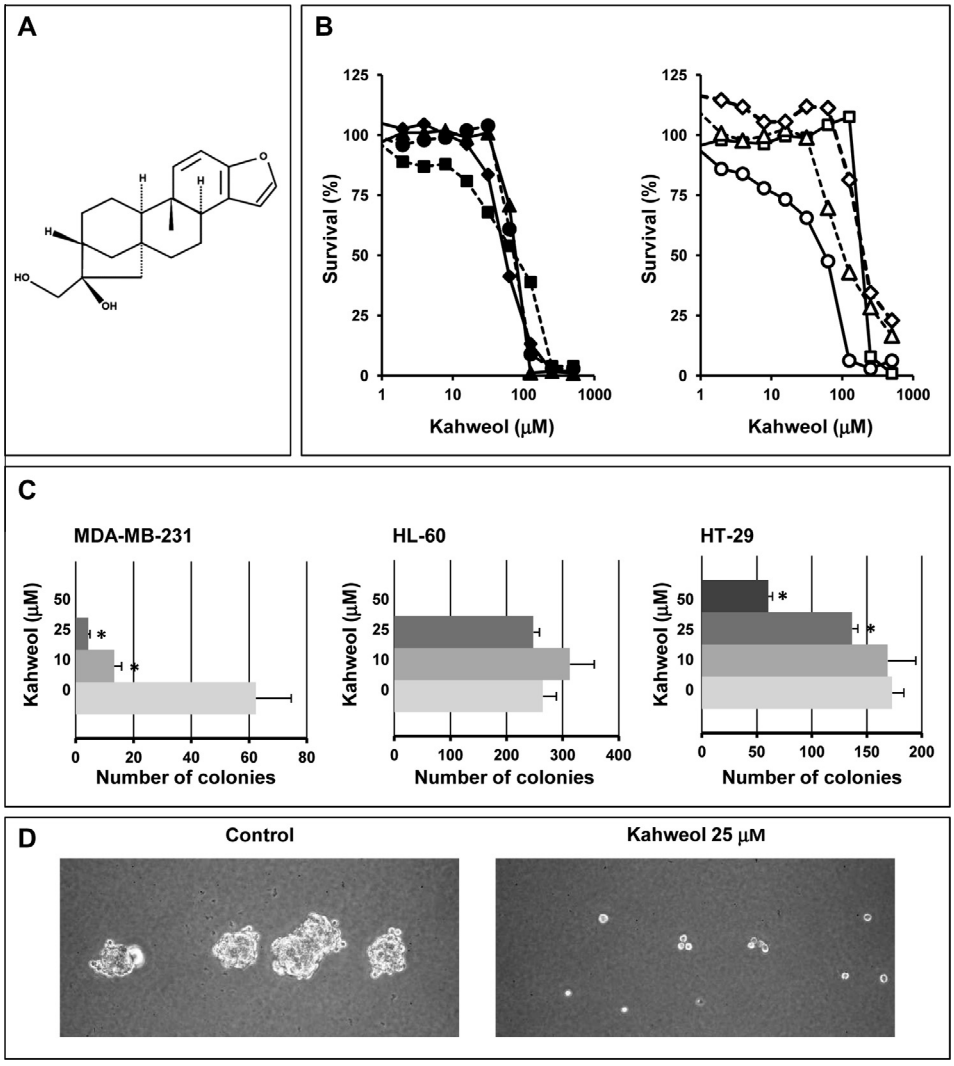

Fig. 1. (A) Chemical structure of kahweol. (B) Kahweol inhibits proliferation of cell lines. Survival curves of tumor cell lines treated with kahweol. Left: HL-60 leukemia (circles), HepG2 hepatoma (squares), HT-29 colon adenocarcinoma (rhombuses), and HT-1080 fibrosarcoma cells (triangles); right: estrogen receptor-negative MDA-MB-231 (circles), estrogen receptor-positive ZR-75-1 (squares) and MCF-7 (triangles) breast carcinoma cells, and human gingival fibroblasts (rhombuses). Concentrations are represented in logarithmic scale. Depicted data are means of values of three independent experiments, each one with quadruplicate samples. (C) Kahweol inhibits the clonogenicity of tumor cells. Histograms show the number of colonies after treatment of human MDA-MB231, HL-60 and HT-29 tumor cells with different concentrations of kahweol given as means ± S.D. of three different experiments. *Statistically significant ($p < 0.01$) as compared to control values, according to a two-tailed Student's *t*-test. (D) The most potent inhibitory effect is observed on MDA-MB-231 cells. The image shows untreated (control) and 25 μM kahweol-treated MDA-MB-231 cell colonies after two weeks of incubation. The image was taken using an inverted microscope under phase contrast at 100× magnification.

biomolecules [17]. Anti-tumor, pro-apoptotic effects of kahweol have been reported for several kinds of cancer cells. Kahweol-induced apoptosis is mediated by STAT3 phosphorylation inhibition in human lung adenocarcinoma A549 cells [18], down-regulation of AKT phosphorylation in human leukemia U937 cells [19], and modulation of the protein expression levels of SP1 regulatory genes in human malignant pleural mesothelioma [20]. Finally, kahweol exhibits Nrf2-mediated chemoprotective effects [21] inducing glutathione S-transferase, γ-glutamylcysteine synthetase, glucoronosyl transferase and O6-methylguanine-DNA methyltransferase [22–24].

Up to date, there is no report comparing kahweol effects on an array of different tumor cells. To contribute to fill this gap is a major aim of the present work. Our results show that, indeed, kahweol is able to inhibit the growth of several human cancer cell types, decreasing their proliferative rates and clonogenicity and inducing their apoptosis. Our data also indicate that the anti-tumoral effect is particularly relevant for the estrogen receptor-negative MDA-MB231 cell line, inducing an activation of the intrinsic pathway of apoptosis. On the other hand, we show that kahweol increases the production of reactive oxygen species and their cytotoxicity in human breast cancer cells but not in normal cells.

2. Materials and methods

2.1. Cell cultures and treatments

Transformed human HL-60 leukemia, HT-29 colon adenocarcinoma, HT-1080 fibrosarcoma, HepG2 hepatoma, and estrogen-receptor positive ZR75-1 and MCF-7 and estrogen receptor-negative MDA-MB-231 breast carcinoma cells were supplied by ATCC and maintained in culture as described by the provider. Human gingival fibroblast primary cultures were maintained in high glucose DMEM and cells in passage 5 were used for the experiments. All culture media were supplemented with 10% FBS and antibiotics. Kahweol was supplied by Santa Cruz Biotechnology,