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Research paper

Protective effects of kaempferol against reactive oxygen species-induced hemolysis and its antiproliferative activity on human cancer cells

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ABSTRACT

The protective effects of kaempferol against reactive oxygen species (ROS)-induced hemolysis and its antiproliferative activity on human cancer cells were evaluated in this study. Kaempferol exhibited strong cellular antioxidant ability (CAA) with a CAA value of 59.80 ± 0.379 μM of quercetin (QE)/100 μM (EC_{50} = 7.74 ± 0.049 μM). Pretreatment with kaempferol significantly attenuated the ROS-induced hemolysis of human erythrocyte (87.4% hemolysis suppressed at 100 μg/mL) and reduced the accumulation of toxic lipid peroxidation product malondialdehyde (MDA). The anti-hemolytic activity of kaempferol was mainly through scavenging excessive ROS and preserving the intrinsic antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; and glutathione peroxidase, GPx) activities in normal levels. Additionally, kaempferol showed significant antiproliferative activity on a panel of human cancer cell lines including human breast carcinoma (MCF-7) cells, human stomach carcinoma (SGC-7901) cells, human cervical carcinoma (Hela) cells and human lung carcinoma (A549) cells. Kaemperol induced apoptosis of MCF-7 cells accompanied with nuclear condensation and mitochondria dysfunction.

1. Introduction

Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one, Fig. 1A, a natural flavonol, is widely distributed in broccoli, apples, strawberries, and beans [1,2]. It is an active constituent of functional food materials with various pharmacological functions. Previous studies have demonstrated that kaempferol can substantially hinder several inflammation development [2,3]. It could not only block the expression of the interleukin-1 beta (IL-1β) and tumor necrosis factor (TNF) in J744.2 macrophages [4–7], but also interfere with the transference of nuclear factor-κB (NF-κB) into the nucleus, thereby hindering the synthesis of inflammatory proteins [8–10]. Furthermore, kaempferol was reported to exert excellent antidiabetic activity by acting as a partial competitor of peroxisome proliferator-agonist receptor γ (PPARγ) [11]. It could also inhibit the expression of the receptor for advanced glycation end products (RAGEs) and reduce the risk of systemic diabetic complications [12,13]. Additionally, various studies indicated that kaempferol exhibited a broad spectrum of beneficial bioactivities, including cardioprotective, antidiabetic, antiestrogenic, anxiolytic, analgesic, antiallergic and osteoporotic activities [3,14–16].

Reactive oxygen species (ROS) are reactive metabolites of normal cells including highly reactive hydroxyl radical (•OH), superoxide anion radical (O_2^{-}•), singlet oxygen (O_2), and hydrogen peroxide (H_2O_2). ROS-mediated oxidative stress plays pivotal role in the development of many diseases, such as allergy, cardiovascular disease, infectious diseases, neurodegenerative diseases, and ophthalmologic problems [17–21]. Due to the great endangerment of ROS, the antioxidant ability of bioactive compound has become a research focus in the biomedical field. Evidences indicated that the administration of kaempferol remarkably promoted the cell viability against oxidative stress. Kaempferol could enhance the antioxidant ability of normal cells through the regulation of heme oxygenase (HO)-1 expression and mitogen-activated protein kinase (MAPK) pathways [22]. The HO-1 is a ubiquitous redox-sensitive inducible enzyme and the augment of HO-1 expression directly

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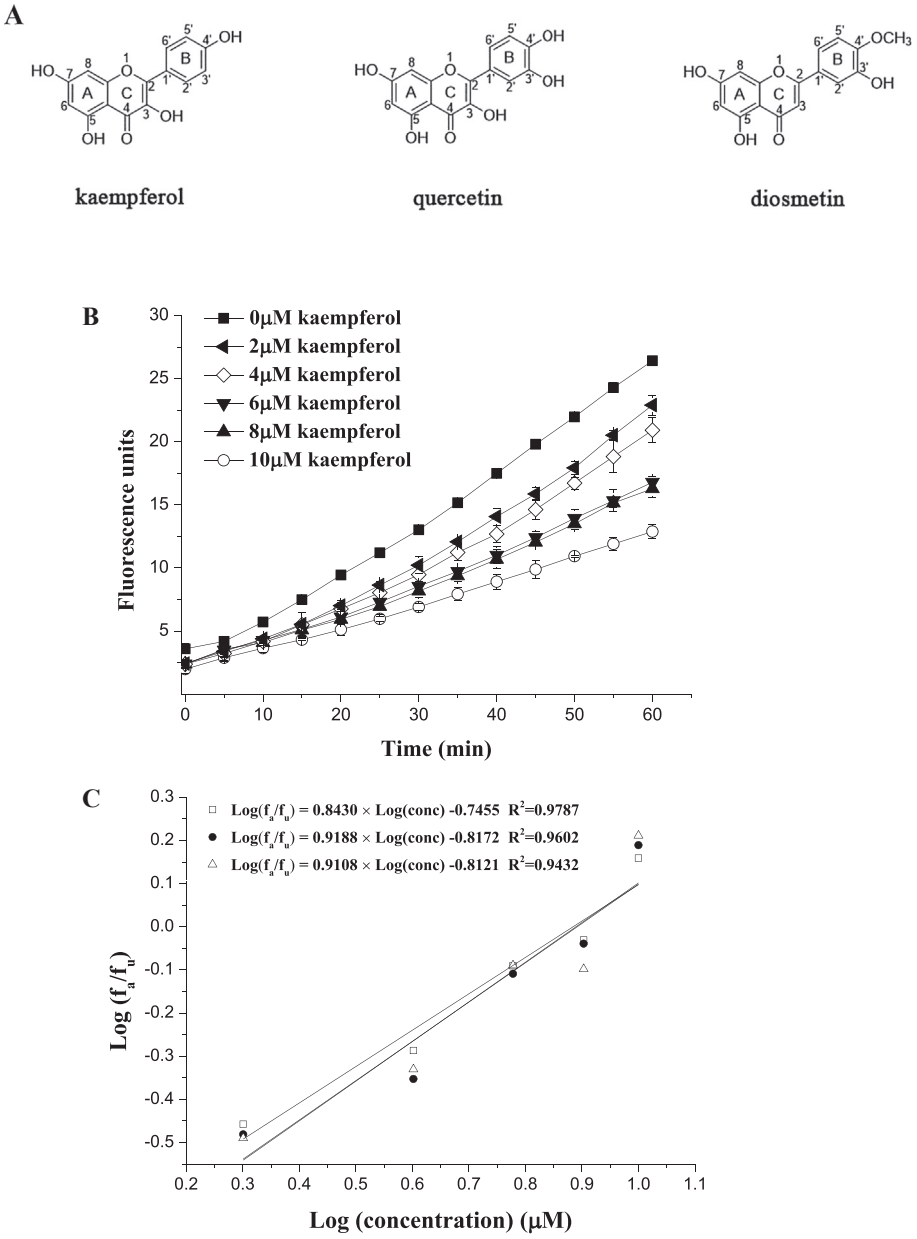

Fig. 1. (A) Chemical structures of quercetin, kaempferol and diosmetin. (B) Time dependence of the DCF fluorescence in the HepG2 cells treated with kaempferol (2–10 μM); (C) the median effect plot for the cellular antioxidant activity of kaempferol, where fa stands for the affected fraction (CAA unit) and fu stands for the unaffected fraction (100 − CAA) by the treatment. The median effect plot is the regression line of log (f_a/f_u) and log (concentration).

increases cell resistance to oxidative injury [23–26]. Although partial molecular mechanism has been elucidated, a majority of intracellular antioxidant detoxifying mechanism of kaempferol has not been investigated. To date, extensive studies about the antioxidant activity of kaempferol based on chemical protocols, such as oxygen radical absorbance capacity (ORAC) assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging method, have been reported [27]. However, these chemical methods do not implicate physiological factors such as pH, temperature, the bioavailability, and metabolism of the antioxidants [28,29]. Therefore, cell-based evaluations of kaempferol under various physiological conditions, are urgently needed.

In the present study, the intracellular antioxidant ability of kaempferol was evaluated using cellular antioxidant activity (CAA) assay and anti-oxidative hemolysis assay. Since the intracellular oxidative stress was initiated by the tumorigenesis [30,31], the antiproliferative effect of kaempferol on four types of human cancer cell lines and its possible underlying mechanisms were uncovered.

2. Results and discussion

2.1. Intracellular antioxidant detoxifying activity of kaempferol

The intracellular antioxidant detoxifying activity of kaempferol was first investigated using the CAA assay, as described in previous literature with some modifications [28]. In this assay, the DCFH-DA (2′,7′-dichlorodihydrofluororescein diacetate) was taken up by HepG2 cells *via* passive diffusion and decomposed by cellular esterases into DCFH (2′,7′-diclorodihydrofluorescin). DCFH was then