

Boron Trifluoride Etherate on Silica-A Modified Lewis Acid Reagent (VII). Antitumor Activity of Cannabigerol Against Human Oral Epitheloid Carcinoma Cells

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(Received November 24, 1997)

Geraniol (1), olivetol (2), cannabinoids (3 and 4) and 5-fluorouracil (5) were tested for their growth inhibitory effects against human oral epitheloid carcinoma cell lines (KB) and NIH 3T3 fibroblasts using two different 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and sulforhodamine B protein (SRB) assay. Cannabigerol (3) exhibited the highest growth-inhibitory activity against the cancer cell lines.

Key words : Cannabinoid, 5-Fluorouracil, Cannabigerol, Antitumor activity, Human oral epitheloid carcinoma cell, MTT assay, SRB assay

INTRODUCTION

The cannabinoids, the active constituents of the herbaceous plant *Cannabis sativa* L. (marihuana, hashish, bhang), have been known to affect many biological systems (Martin, 1986). Delta-9-tetrahydrocannabinol (delta-9-THC) belongs to a class of compounds known as cannabinoids which are responsible for the psychoactive properties of marijuana. The cannabinoids produce in man and animals a complex pattern of pharmacological effects some of which are unique to this class of compounds (Mechoulam, 1986; Dewey, 1986). Dronabinol (Marinol, Roxane Laboratories, Columbus, OH) is delta-9-tetrahydrocannabinol formulated in sesame oil. It was approved in the U.S. in 1986 for treatment of cancer chemotherapy-induced nausea and vomiting refractory to other agents (Plasse *et al.*, 1991). Watson and his colleagues (1983) reported that cannabigerol (3) and cannabinol methyl ether were not sensitizers. Most of the cannabinoids were found to be allergenically cross-reactive. In addition, it was shown that the presence of a free 1'-hydroxyl group was required for sensitization, but not to elicit a response in sensitive animals. Eisohly *et al.* (1982) have reported that cannabigerol type compounds having a methyl side chain in most cases exhibit an increased

antifungal and antibacterial activities.

In a recent paper (Baek *et al.*, 1996), Cannabigerol (3) was evaluated for the antitumor efficacy against mouse skin melanoma cells and showed a significant activity with IC₅₀ value of 31.30 μ M *in vitro* MTT assay. The aim of this present study was to determine whether cannabinoids (3 and 4) and anticancer agents possess the growth-inhibitory activity against human oral epitheloid carcinoma cells. The effects of 5-fluorouracil (5) were also examined for comparison.

MATERIALS AND METHODS

IR spectra were recorded on a Perkin-Elmer 457 grating infrared spectrophotometer. ¹H-NMR spectra were obtained on a Bruker WH-200 and WH-300 pulsed FT spectrometers. Chemical shifts are given in parts per million downfield from Me₄Si internal standard. Mass spectra were recorded on a Varian Mat CH-5 mass spectrometer. Analytical TLC was performed by using commercially available silica plates (polygram sil N-HR/UV₂₅₄), and the plates were visualized with fast blue phenol reagent. Medium pressure liquid chromatography was performed on an ALTEX glass column, 1 meter long, diameter 9 mm internal using an FMI pump and silica gel 60 (230~400 mesh) purchased from Merck. Fractions were collected with LKB 2070 or LKB 7000 fraction collectors at a rate of 2~10 ml/min. Tumor cells for the experiments were

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obtained from Korean Cell Line Bank in the Seoul National University. *In vitro* anticancer-drug RPMI-1640 medium supplemented with 10% FBS, streptomycin 0.1 mg/ml and penicillin 100 units/ml at 37°C in 5% carbon dioxide. Cells were dissociated with 0.25% trypsin just before transferring for experiment and were counted by Hemcytometer.

Preparation of cannabigerol (3)

Cannabigerol (3) thus obtained was identified by comparison of its spectral data (TLC, MS, NMR and IR) with those published or by direct comparison with an authentic sample (Baek *et al.*, 1995).

Preparation of cannabidiol (4)

Cannabidiol (4) was kindly provided by Professor R. Mechoulam, in the Department of Natural Products, at School of Pharmacy, Hebrew University, Israel.

Evaluation of antitumor activity

The antitumor activity of geraniol (1), olivetol (2), cannabinoids (3 and 4) and 5-fluorouracil (5) was determined by the modification of the literature methods (Mosmann, 1983; Carmichael *et al.*, 1987; Keeper *et al.*, 1991).

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide(MTT)-microculture assay: The assay is dependent on the cellular reduction of water-soluble MTT (Sigma Chemical Co. St. Louis, M.O.) by mitochondrial dehydrogenase of vial cells to a blue water-insoluble formazan crystal product which can be measured spectrophotometrically (Mosmann, 1983; Carmichael *et al.*, 1987). Human oral epitheloid cancer cell lines were cultured in RPMI-1640 medium (Gubco La-

boratories) containing 10% fetal bovine serum. Exponentially growing tumor cells (5×10^5) were cultured for 48 hrs at 37°C in a humidified 5% CO₂ incubator in the presence or absence of cannabinoids (3 and 4) and 5-fluorouracil (5).

Sulforhodamine B protein (SRB) assay

The SRB assay was performed essentially according to the method of Skehan *et al.* (1991). The methods of plating and incubation of cells were identical to those cells of the MTT assay.

Evaluation of toxicity: Cytotoxicity assay: In order to determine the cytotoxicity mediated by compounds (1, 2, 3, 4 and 5) MTT and SRB assays were used (Carmichael *et al.*, 1987; Skehan *et al.*, 1991).

Morphology: Changes in the morphology of KB cells cultured in a medium with compounds (3, 4 and 5) were documented by microphotography (Fig. 1 and 2).

Statistical analysis: All values, expressed as mean \pm S.D., were statistically analyzed through analysis of Student's t-test. The P value less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

The present study shows the *in vitro* growth inhibitory activities of geraniol (1), olivetol (2), cannabinoids (3 and 4) and 5-fluorouracil (5) as a reference compound against KB cell lines (Fig. 1 and 2). 5-Fluorouracil (5-FU) is commonly used as a therapeutic agent to treat cancers of the large bowel. However, therapy with 5-fluorouracil (5) as a single agent has only limited success. Additive agents or modulators for the 5-fluorouracil (5) effect are needed for

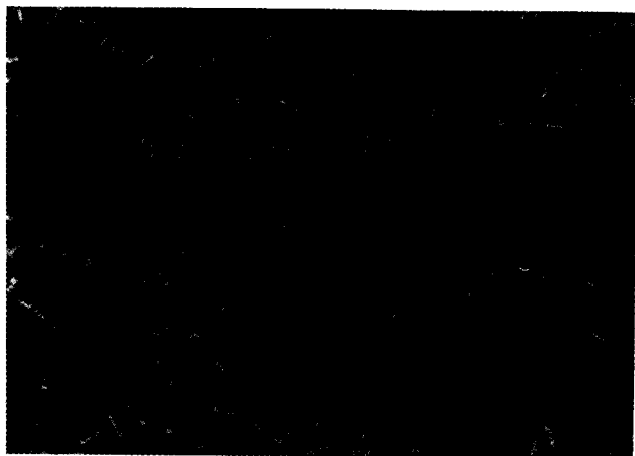


Fig. 1. Inverted photomicrograph of KB cells treated with MTT for an additional 3 hrs after incubation in unmodified medium (control) for 2 days $\times 400$. Most cells had abundant cytoplasm and cytoplasmic process.

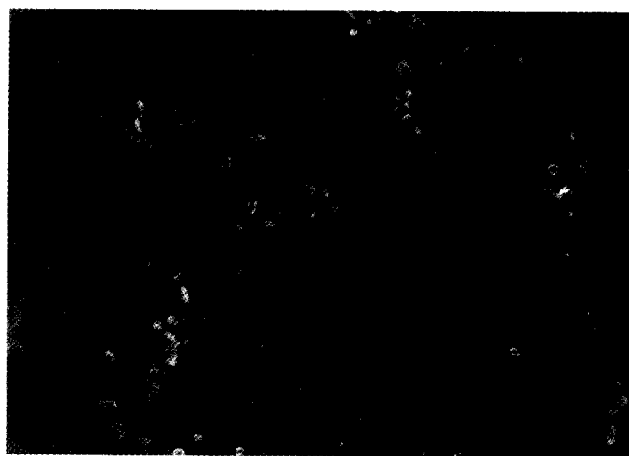


Fig. 2. Inverted photomicrograph of KB cells treated with MTT for an additional 3 hrs after incubation in 100 μ M cannabigerol containing medium (control) for 2 days $\times 400$. Most cells were formed cell cluster and number of cells were decreased.

Table I. The antitumor activities of geraniol (1), olivetol (2), cannabinoids (3 and 4) and 5-fluorouracil (5) on KB cell lines. Comparison of CD_{50} for cannabinoids (3 and 4) and 5-fluorouracil (5)-SRB assay, MTT assay

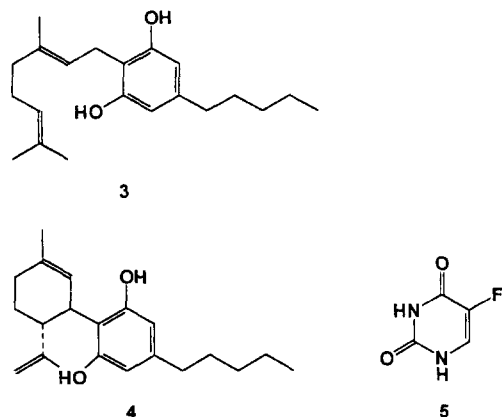
Compounds ^a	IC_{50} (μM) ^b	
	MTT assay	SRB assay
CBG	31.30	76.54
CBD	273.41	523.81
5-FU	145.91	326.19
Olivetol	105.10	116.69
Geraniol	482.74	931.47

^aEach compound was examined in four concentrations in triplicate experiments.

^b IC_{50} represents the concentration of a compound required for 50% inhibition of cell growth.

more effective treatment of this highly resistant malignancy (Boersma, *et al.*, 1993).

Table I shows the potent anti-tumor activities of geraniol (1), olivetol (2), cannabinoids (3 and 4) and 5-fluorouracil (5) against KB cells. In general, the antitumor activities of these compounds (1, 2, 3, 4 and 5) were dose-dependent over the micromolar concentration range 1 to 100 μM , and the susceptibility of KB cells to these compounds was quite different (Table I). The comparison of IC_{50} values of these compounds in tumor cell lines shows that their susceptibility to these compounds decreases in the following order: CBG>OLVTL>5-FU>CBD>GRNL in MTT assay, CBG>OLVTL>GRNL>CBD>5-FU in SRB assay (Table I). Cannabigerol (3) (Formukong, *et al.*, 1989) was the most effective growth inhibitor of KB cell lines, producing an IC_{50} of about 31 μM in MTT assay and 77 μM in SRB assay. The sulforhodamine B protein stain assay was compared with the tetrazolium (MTT) colorimetric assay for *in vitro* chemosensitivity testing of KB cells. The SRB assay appeared to be more sensitive than the MTT assay, with a

**Fig. 3.** The structures of cannabigerol (3), cannabidiol (4), and 5-fluorouracil (5).**Table II.** The cytotoxicities of geraniol (1), olivetol (2), cannabinoids (3 and 4) and 5-fluorouracil (5) on NIH 3T3 fibroblast cell lines. Comparison of CD_{50} for cannabinoids (3 and 4) and 5-fluorouracil (5)-SRB assay, MTT assay

Compounds ^a	CD_{50} (μM) ^b	
	MTT assay	SRB assay
CBG	60.46	82.98
CBD	36.27	60.25
5-FU	41.27	75.90
Olivetol	105.10	116.69
Geraniol	482.74	931.47

^aEach compound was examined in four concentrations in triplicate experiments.

^b CD_{50} represents the concentration of a compound required for 50% cytotoxic dose of cell growth.

better linearity with cell number and higher reproducibility. Olivetol (OLVTL, 2), the simplest compound of this series of phenolics, was more potent than 5-fluorouracil (5) as a reference compound. This compound is structurally related to cannabinoids (3 and 4). However, the antitumor activity of cannabidiol (4) exhibit less activity than olivetol on KB cells (Fig. 3).

Table II shows the cytotoxic activities of geraniol (1), olivetol (2), cannabinoids (3 and 4) and 5-fluorouracil (5) against NIH 3T3 fibroblasts. In general, the cytotoxic activities of these compounds (1, 2, 3, 4 and 5) were dose-dependent manner over the concentration range 1 to 100 μM , and the susceptibility of NIH 3T3 fibroblasts to these compounds was quite different (Table II). The comparison of CD_{50} values of these compounds in NIH 3T3 fibroblasts shows that their susceptibility to these compounds decrease in the following order; CBD>5-FU>CBG>OLVTL>GRNL in MTT assay and SRB assay (Table II). However, cannabigerol (3) was the least cytotoxic effect of NIH 3T3 fibroblast, producing a CD_{50} of about 60 μM in MTT assay and 83 μM in SRB assay. Cannabigerol (3) was more potent than 5-fluorouracil (5) as a reference compound. The activity of cannabidiol (4) exhibits more active than that of 5-fluorouracil (5) on NIH 3T3 fibroblast (Table II). Cannabigerol (3) is structurally related to geraniol, a known inhibitory effect (Baik *et al.*, 1988), inhibited the least effective growth-inhibitory activity against the tested cancer cell lines. The compounds used are known to inhibit the activity of several enzymes of the arachidonate cascade including cyclo-oxygenase and lipoxygenase (Evans *et al.*, 1987) and to both stimulate and inhibit phospholipase A_2 activity (Evans *et al.*, 1987). The actions of the cannabinoids on membrane associated enzymes are complex and dose-dependent. Although these actions are possibly associated with the ability of the cannabinoids to act as anticancer agents, (Plasse *et al.*, 1991), specific structural alterations may be critical in det-

erminating enzyme targets. Cannabigerol (**3**) has been selected as lead compounds for further examinations (Fig. 3).

ACKNOWLEDGEMENT

This work was supported by the Wonkwang University. We thank Prof. R. Mechoulam, in the Department of Natural Products, at School of Pharmacy, Hebrew University for kindly providing us with sample of authentic cannabidiol.

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