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Short Communication

Hydroxylation of delta-9-tetrahydrocannabinol by human peripheral blood monocytes in tissue culture

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Introduction

Decreased lymphocyte function in marijuana smokers and abnormalities of lymphocyte function observed following *in vitro* administration of cannabinoids suggest the immunosuppression of immune cells when exposed to constituents of *Cannabis sativa* [1–3]. Treatment of macrophages and monocytes with delta-9-tetrahydrocannabiniol (d9THC) led to decreased phagocytosis, abnormalities in cellular ultrastructure, and an increase in the oxidation of arachidonic acid to hydroxy-fatty acids and leukotrienes [4].

The authors are interested in the effects of cannabinoids on macrophage functions, since the macrophage plays a central rôle in the modulation of the immunological system. While monocytes and macrophages secrete a wide variety of regulatory substances such as peptides, eicosanoids, immune RNA, and enzymes, this important cell can also modify exogenous substances such as tumour antigens and present the products to lymphocytes [5–7]. The monocyte/macrophage is an avid user of molecular oxygen and is capable of producing reactive oxygen species such as superoxide anion, hydroxyl free radical and singlet oxygen, and can produce hydroperoxides of fatty acids and cholesterol [8, 9]. The authors have sought to identify oxidized metabolites of human monocytes to d9THC *in vitro*.

Material and Methods

Human peripheral blood moncytes were prepared from buffy coats by Ficoll-hypaque density centrifugation followed by 2 h of adherence to plastic Petri dishes. Non-adherent cells were washed, and serum-free media containing 2 $\mu g\ ml^{-1}$ of d9THC with 0.5 $\mu g\ ml^{-1}$ of ether delipidated bovine serum albumin (BSA) were aded. BSA can trap oxidized lipids and prevent their interaction and possible incorporation into cells. Following 48 h of incubation, both cells and supernatants were extracted with ethyl acetate–ethyl ether–hexane (10:10:10, v/v/v) three times. The extract was concentrated under nitrogen and then applied to silica gel G plates for thin-layer chromatography. Zones corresponding to cannabinoid standards were scraped and extracted from the silica with ethyl ether–hexane (10:20, v/v), and the solvent evaporated with nitrogen. The samples were dissolved in 100 μl of hexane and injected into a Finnigan MAT combined gas chromatograph–mass spectrometer. Metabolites were separated with a 15 m SE-30 capillary column. The gas chromatographic conditions were as follows: column

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temperature 200 to 250°C at 4°C min^{-1}; Carrier gas, N_2 at 18 ml min^{-1}; injector temperature, 300°C. The mass spectrometer settings were as follows: 70 eV; current, 0.5 μamp; interface, 200°C; 40 scans min^{-1}. Fragmentation patterns were compared with patterns obtained with known standards and with structures available in the literature and the NIH library.

Results and Discussion

The majority of metabolites of d9THC in humans involve the addition of hydroxyl groups to the major ring or side-chain, oxidation of the allylic carbon at C-7, and oxidation of the side-chain to the carboxylate form [10]. The authors have found similar oxygenation by human monocytes treated with d9THC in tissue culture. Figure 1 indicates the structure of d9THC. Figure 2 shows cannabinol, a common metabolite that has been found in a variety of other mammalian systems [11, 12]. Figure 3 shows the tentative structure of a compound, mol. wt 312, that may be an intermediate in the formation of cannabinol. d9THC is converted with dehydrogenation and double bond formation to cannabinol. This unusual intermediate has not been described in other systems [13]. Figure 4 reveals the structure of a dihydroxylated cannabinol. The authors have tentatively identified it as an oxidized metabolite of cannabinol. In Fig. 5 the conversion of a mono-hydroxylated form of the intermediate to cannabinol was observed. Analysis of metabolites of d9THC revealed a predominance of hydroxylated by-products.

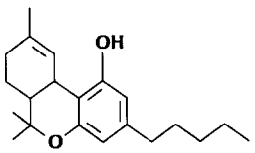

Figure 1
Structure of delta-9-tetrahydrocannabinol.

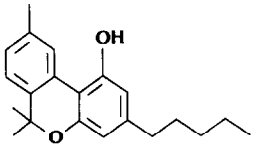

Figure 2
Structure of cannabinol.

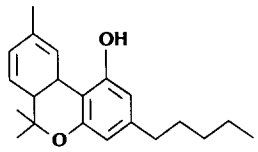

Figure 3
Proposed structure of new intermediate.

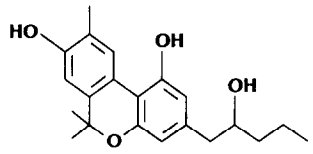

Figure 4
Dihydroxyl derivative of cannabinol.

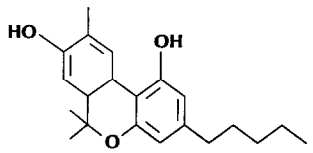

Figure 5
Mono-hydroxylated product of new intermediate.

When macrophages are stimulated, oxygen is converted to active species such as hydroxyl radical, superoxide anion, and singlet oxygen. It is possible that these reactive intermediates serve to interact with membranes, since unsaturated lipids often serve as substrates for these reactive molecules. It is not known whether the cannabinoid metabolites are converted in a similar way, or if they are metabolized by other systems such as the P-450 microsomal oxidase system, or other non-haem oxygenases. The significance of these products may be related to their ability to act as immuno-regulatory substances. Oxidized fatty acids, and hydroxy-derivatives of cholesterol can act as potent immunodulators [14]. Further investigations on the immunoregulatory potential of d9THC and its oxygenated metabolites are continuing in the authors' laboratory.

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