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CANNABIDIOL METABOLITES: FORMATION AND BIOLOGICAL ACTIVITY
An overview of the literature

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INTRODUCTION

Among the ~110 cannabinoids isolated from hemp (*Cannabis sativa*), Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have been the most thoroughly studied substances. Synthetic THC has been available for three decades as a medicine, and pharmaceutical grade herbal cannabis as well as formulations of cannabis extracts containing THC and CBD in well-defined ratio have also been registered as medicines in several countries.[1]

Cannabidiol (**Fig. 1**), one of the major ingredients of *C. sativa*, was first isolated from American hemp[2] and Egyptian hashish[3] in 1940, its structure was determined by Mechoulam & Shvo in 1963.[4] Historical, preclinical laboratory and human case reports as well as a plethora of anecdotal accounts combined with the relative safety of CBD prompted the exploration of the therapeutic potential of CBD against a range of diseases.[5–9] The promise of CBD in treating drug-resistant epilepsy in children has recently brought this natural product into the focus of the scientific community, the media as well as politicians and regulatory bodies, in particular in the USA.[10–14]

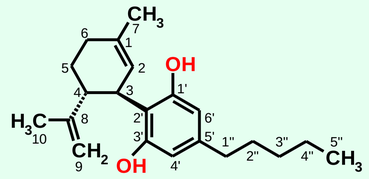


Figure 1. **The chemical structure of CBD**

The chemistry, pharmacology of CBD and the various molecular targets this non-psychotropic cannabinoid interacts with have been reviewed.[15–18] Some information on the pharmacokinetics of CBD in experimental animals and humans is also available.[19–21] However, unlike the parent compound, the biological activity of CBD metabolites has received less attention. A recent review summarized the biological properties of the main THC metabolite.[22] This poster reviews the pharmacokinetics of CBD and the scarce information on the biological properties of its metabolites.

HUMAN PHARMACOKINETICS OF CBD UPON VARIOUS ADMINISTRATION ROUTES

Due to extensive Phase I metabolism, the pharmacokinetics of CBD is complex and the bioavailability of oral CBD is low across species.[19,20] In general, the most abundant metabolites are water-soluble hydroxylated 7-COOH derivatives (***Fig. 2***) that are excreted either intact or as glucuronide conjugates. The route of administration affects the pharmacokinetics of CBD and, in humans, high intra- and inter-subject variability is not unusual. It is of note that a large portion of the administered CBD is excreted intact or as its glucuronide.

In individuals **smoking** of a single cigarette containing ~19 mg CBD, average peak blood plasma level was 110 ng/ml (at 3 min post-dose), the half-life was ~31 h, and the systemic availability was estimated as 31% (range: 10–42 ng/ml; n = 5).[23]

An early study using intravenous **injection** of 20 mg [^{3}H]CBD observed that 33% of the total radioactivity, mostly unchanged CBD accompanied by several oxygenated metabolites (TLC), was excreted in the feces in 72 h.[24] A more detailed investigation found that intravenous injection of 20 mg CBD resulted in peak plasma level of 686 ng/ml (at 3 min post-dose) that rapidly dropped to 48 ng/ml at 1h; the half-life was ~24 h and bioavailability was estimated to be 6%.[23]

Oral administration of a blend of 40 mg CBD + 20 mg THC resulted in low peak plasma levels of ~5 ng/ml for each drug at 1.5–3 h.[25] Similar low peak plasma levels (range: 0.3–2.6 ng/ml) were noted 1 h after oral ingestion of a cannabis extract containing 5.4 mg CBD and 10 mg THC.[26] Chronic administration of large doses of CBD does not result in elevated mean blood concentrations: in a 6-week trial using daily oral doses of 700 mg CBD, plasma levels of the drug remained in a low, narrow range of 6–11 ng/ml throughout the trial and the elimination half life was 2–5 days.[27] A recent study recorded respective mean blood concentrations of 4.7 and 17 ng/ml of CBD after 1 and 2 h oral intake of a single dose of 600 mg CBD.[28] Peak plasma CBD concentrations as high as 221 ng/ml 3 h after concomitant oral administration of 800 mg CBD + 1 mg/kg fentanyl (i.v.) have been reported.[29]

As a part of an extensive series of studies of cannabis based medicine extracts (e.g., Sativex®) the pharmacokinetics of a total dose of 20 mg CBD in **sublingual** drops was studied.[30] The highest plasma concentration was 2 ng/ml at 130 min post-dose. Similar values were obtained for a 1:1 mixture of THC : CBD applied either in sublingual drops or as aerosol; when applied via a nebuliser (10 mg THC + 10 mg CBD), however, the peak plasma level was 9.5 ng/ml at 36 min and the half-life was 66 min.

Upon **oromucosal** application of low (5.4 mg THC + 5.0 mg CBD) and high (16.2 mg THC + 15.0 mg CBD) Sativex® doses, the mean peak plasma levels were 1.6 ng/ml at 3.7 h and 6.7 ng/ml at 4.0 h, respectively.[31,see also Ref. 32]

The human **skin** permeation of CBD solutions was investigated *in vitro*[33] and various CBD-formulations for transdermal and **intranasal** administration have been studied also in rodents.[34,35]

According to an analysis of *in vivo* distribution of cannabinoids in post-mortem cases, high CBD concentrations were found in bile (up to 63 ng/ml) and muscle (up to 32 ng/g) tissues; the relatively high CBD-content of the brain (up to 6.7 ng/g) was unexpected.[36,see also Ref. 37]

HUMAN METABOLISM OF CBD

Since the first identification from rat liver homogenate of 7-OH-CBD and 3”-OH-CBD (for numbering, see ***Fig. 1***) in 1973,[38] metabolism studies in mammals, including humans, using various types of administrations indicated considerably species variability. Being a good substrate of cytochrome P450 (CYP450) mixed function oxidases, CBD undergoes extensive hydroxylation at multiple sites and further oxidations result in complex metabolic profiles; altogether some 100 CBD metabolites have been identified.[19] Compared to THC, the metabolism of CBD is unusually complex. The major metabolites of CBD were derivatives of CBD-7-oic acid hydroxylated at the side chain.

Following initial excretion studies,[24,39] about 40 oxygenated Phase I metabolites / biotransformation products have been characterized typically in human urine (**Fig. 2**).[19,40–42] The structures of main metabolites, identified in the urine of a distonic patient chronically treated with 600 mg daily oral doses of CBD are shown in ***Fig. 2a***.[40,41] Minor metabolites found in the urine or produced by human liver *in vitro* are listed in ***Fig. 2b***. (The concentration of CBD was 12.1% of the total excreted cannabinoids.)

Of the various CYP450 isoforms, CYP3A4 and CYP2C19 appear to be main isoforms responsible for the formation of 6-OH, 7-OH and 4”-OH CBD metabolites, while CYP1A and CYP3A isoforms are involved in the 1”-and 2”-hydroxylations of CBD.[42] Glucuronidation of CBD at the phenolic oxygen at 1’-position is a major Phase II metabolic step in humans.[40,43,44]

Figure 2a. **Chemical structures and abbreviations of major (>1%) human metabolites of CBD**[40,41] (numbering as in *Fig. 1*)

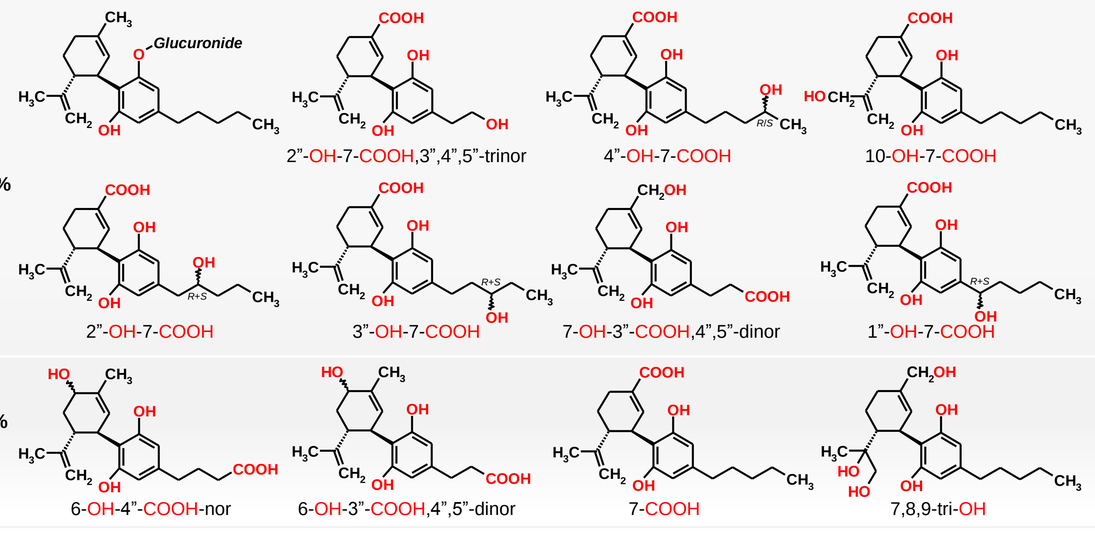
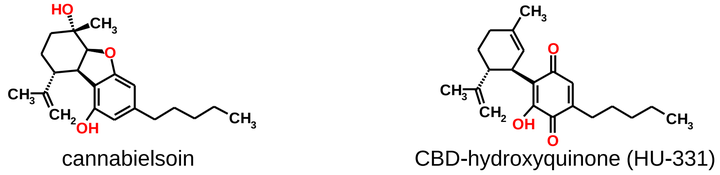


Figure 2b. **Abbreviated names of minor (>1%) or trace human metabolites of CBD** (numbering as in *Fig. 1*)

4”-COOH,5”-nor; 7-OH; 7-OH-4”-COOH,5”-nor; 7-COOH-8,9-dihydro-diOH; 3”-COOH,4”,5”-dinor; 2”-COOH,3”,4”,5”-trinor; 7-OH,1”-COOH,2”,3”,4”,5”-tetranor; 2”,7-diOH,3”,4”,5”-trinor; 6,7-diOH; 2”,6-diOH,3”,4”,5”-trinor; 6-OH; 7-OH,5”-COOH; 1”-COOH,2”,3”,4”,5”-tetranor; 6-OH,1” 2”,3”,4”,5”-tetranor; 1”-COOH,2”,3”,4”,5”-tetranor; 6-OH; 6-OH-5”-COOH; and 6,7-diOH-5”-COOH; 1”-OH; 2”-OH; 3”-OH; 4”-OH; 5”-OH (all detected in human liver microsomal preparation[42])

Additional biotransformation products detected in human urine: Δ^8-THC, Δ^9-THC[93] and cannabinol.

Figure 3. **Chemical structures of potential human metabolites of CBD** (see text for explanation)



BIOLOGICAL ACTIVITY STUDIES

Studies *in vitro*

An early study[45] on the inhibition of the binding of a quaternary amine analogue of Δ^8-THC to rat brain neuronal membranes by a series of cannabinoids showed 10-OH-CBD to be slightly less active than CBD (K_i = 94 and 73 nM, resp.; THC: K_i = 27 nm). Cannabinoid CB1 and CB1 receptor binding studies[46–48] with CBD enantiomers and their derivatives revealed that (–)-CBD (*natural* enantiomer) and its 7-OH and 7-COOH metabolites were devoid of receptor affinity; synthetic (+)-CBD was a modest receptor ligand (K_i = 842 and 203 nM for the CB1 and CB2 receptor, resp.), but its 7-OH and 7-COOH derivatives had high affinity to the CB1 receptor with respective K_i values of 5.3 and 13.2 nM; these two compounds also bound to the CB2 receptor (respective K_i values: 322 and 156 nM). Furthermore, while both CBD isomers proved to be agonists of the type-1 vanilloid receptor, the 7-OH and 7-COOH metabolites of (–)-CBD were inactive. Also, (–)-CBD and (+)-CBD as well as 7-OH-(–)-CBD inhibited FAAH with IC_{50} values of 27.5, 63.5 and 34.0 nM, resp.; anandamide uptake was inhibited also by (–)-CBD and its 7-OH metabolite with IC_{50} values of 22.0 and ~50 nM, resp.) as well as by (+)-CBD (IC_{50} = 17.0 nM). Not only CBD but its 6-OH, 6-oxo and 10-OH metabolites were found to inhibit mouse liver microsomal CYP450 2C and 3A.[49] The key role of the resorcinol moiety of CBD (and of its metabolites) in CYP450 enzyme-inhibition has been established.[50] A patent[51] mentioned 7-OH- and 7-COOH-CBD dose-dependently inhibiting *in vitro* the generations of nitric oxide and reactive oxygen species as well as the production of TNF-α.

Studies in animals

In the standard mouse 'tetrade' test with (+)-CBD (*unnatural* isomer) and its analogues (20 mg/kg, i.p. administration) only peripheral pharmacological action was noted:[47,52] in spite of being CB-receptor ligands, (+)-CBD and its 7-OH and 7-COOH derivatives lacked central activity; however, the (+)-CBD-derived 7-OH and 7-COOH compounds potently inhibited intestinal motility (defecation) indicating peripheral activity perhaps via a CB-receptor independent mechanism; (–)-CBD and its 7-COOH metabolite failed to inhibit defecation; weak antinociceptive effects for 7-OH-(+)-CBD were also noted. Anti-inflammatory and antinociceptive effects in mice of the 7-OH and 7-COOH metabolites of CBD have been mentioned in a patent.[51]

In mice, four metabolite-like oxygenated CBD-diacetates proved to be equipotent with CBD as anticonvulsants, in prolonging pentobarbital sleep time and in reducing spontaneous motor activity.[53] Cannabielsoin (***Fig. 3***), obtained first from CBD photochemically,[54] has not been isolated from humans but recently identified as a CBD metabolite in guinea pigs.[55,56] Cannabielsoin (at ≤10 mg/kg, i.v.) "showed no CNS activity" in rodents[57] and did not affect body temperature or pentobarbital-induced sleep time in mice.[55,56] In rabbits, a 5 mg/kg i.v. dose of cannabielsoin reduced intraocular pressure; CBD was inactive at 10 mg/kg while THC was active at 1 mg/kg.[58] A hydroxyquinone derivative of CBD (***Fig. 3***), also known as HU-331, obtained first by synthesis,[59] has been postulated to be a short-lived (re)active oxidative metabolite of CBD with CYP450 inhibitory properties.[60,61] This atypical quinone is a selective inhibitor of topoisomerase II[62,63] and is being investigated as a potential anticancer agent.[62,64] The synthetic 6-oxo-CBD-diacetate, which *in vivo* could hydrolyze to the corresponding 6-oxo-CBD, recently identified in dogs as a glycoside,[65] was found to be a potent anticonvulsant in the mouse.[53]

Human studies

There are no publications describing the biological activity of CBD metabolites in humans.

Interaction with other drugs

The pharmacological actions of CBD on receptors, ion channels, cellular uptake processes and enzymes have recently been reviewed[16,18] and are not reiterated here. What follows is a brief summary of effects with possible relevance in the clinical use of CBD. It is well known that CBD is not only a substrate but also an inhibitor of CYP450 enzymes thus could interfere with the metabolism other xenobiotics, including THC and medicinal products.[16,20,66–68] No relevant data are available for genuine CBD metabolites. In healthy humans, the antibiotic rifampicin, an inducer of CYP3A4 involved in the metabolism of CBD, significantly reduced the peak plasma concentration of CBD (Sativex® spray), while the antifungal ketoconazole, a CYP3A4 inhibitor, nearly doubled the peak plasma concentration of CBD.[69] Furthermore, CBD interacts with P-glycoprotein efflux transporters involved in multidrug resistance, may affect placental permeability and the pharmacokinetics of other drugs.[70–73]

SYNTHESIS OF CBD METABOLITES

Basically all single-site modified CBD metabolites have been prepared. Syntheses of metabolites hydroxylated at the pentyl side chain were described already in 1972.[38,74,75] Side-chain hydroxylated derivatives have also been produced by microbial oxidation.[76] The syntheses of the epimeric 6α- and 6β-OH-CBD[77] as well as the syntheses of the 7-OH-CBD[48,77–79] and metabolite species 10-OH[77,80] have been reported. The rabbit-metabolite 8,9-diOH-CBD was obtained by incubating 8,9-epoxy-CBD[81] with guinea-pig hepatic microsomes.[82] The synthesis of the 7-COOH metabolite of CBD has been described.[48,83] Microbial conversions of CBD to 5"-COOH and 3"-COOH have also been reported.[76] Synthesis of the side-chain terminal carboxylic acid (5"-COOH, identified only in animals[84–87]) has also been described.[88] A patent describes the methyl ester of 2-COOH-3",4",5"-trinor-CBD.[89] The glucuronide of CBD has been prepared using a glucuronyltransferase.[90] Cannabielsoin, a putative CBD metabolite may be obtained from CBD by several routes.[57,91,92] Chemical syntheses of metabolites oxidized at multiple sites have apparently not been published.

SUMMARY

Many drugs used in therapy are metabolically converted into active metabolites and interindividual variations in the generation and pharmacokinetics of such active drug metabolites may cause variability in the response to treatment by different individuals.[94] Pharmacological studies on CBD metabolites are scarce yet suggest interesting biological activities which are unrelated to the cannabinoid receptors and are worth exploring. Intriguing questions also arise:

Could any of the pharmacological effects observed for CBD be attributed to its metabolites?

Could any of the CBD metabolites be used as templates for the development of novel therapeutic agents?

It is hoped that further studies will reveal any potential involvement of some of these abundant metabolites in the complex pharmacology and in the proven therapeutic effects of CBD-containing preparations.

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