In vitro assessment of drug-drug and herb-drug interactions of Cannabis-based medicinal products with anticoagulant drugs

DEPARTMENT OF PHARMACEUTICAL SCIENCES

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AIM

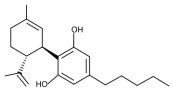
The aim of this study is to assess the risk of CYP450-mediated interaction of Cannabis extract based products, pure tetrahydrocannabinol (THC, dronabinol), and pure cannabidiol (CBD) with coumarin-derived vitamin K antagonists.

INTRODUCTION

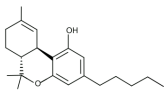
Cannabis-based products for medicinal use

Tetrahydrocannabinol (THC) acts on the endocannabinoid system and has effects in the central and peripheral nervous system. Medicinal indications for Cannabis-based products include chronic pain, spasticity in multiple sclerosis, treatment resistant epilepsy, and nausea and vomiting due to chemotherapy.[1] CBD has multiple targets and is also widely sold in non-regulated herbal products. While reports of drug interactions with purified CBD and THC are available to a limited extent, interaction studies with whole extracts containing many cannabinoids are very scarce.

Cannabis plant



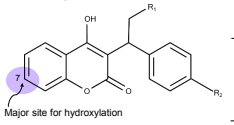
Cannabidiol (CBD)



Tetrahydrocannabinol (THC)

Metabolism of vitamin K antagonists

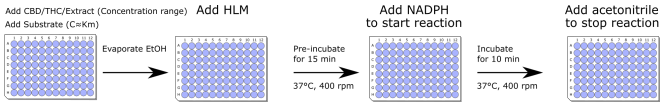
Coumarin derived vitamin K antagonists (phenprocoumon, acenocoumarol and warfarin) are used for long-term anticoagulation therapy and belong to the group of most frequently used drugs worldwide.[2] These drugs are susceptible to metabolic drug-drug or herb-drug interaction due to their narrow therapeutic window. Observations of clinically relevant Cannabis-warfarin interactions have been reported.[3] All three drugs are mainly excreted in their hydroxylated form. Hydroxylation can occur at various sites, whereof the *S*-7-OH form is the dominant form in all three drugs. Up to ten different minor metabolic forms, depending on various metabolic pathways, have been described.



	R1	R2	Major OH-metabolite[2]	Major pathway[2,4]
Phenprocoumon	$-CH_3$	-H	*S*-7-OH (44%)	CYP2C9, CYP3A4 (65 vs. 35%)
Acenocoumarol	$-COCH_3$	$-NO_2$	*S*-7-OH (56%)	CYP2C9
Warfarin	$-COCH_3$	-H	*S*-7-OH (52%)	CYP2C9, CYP2C19

METHODS

CYP-inhibition assay with human liver microsomes (HLM)



Pooled human liver microsomes (HLM) were incubated with Cannabis extracts (commercially available Sativex and normalized cannabis tincture), pure CBD or THC. Sulfaphenazole and ketoconazole were used as CYP2C9 and CYP3A4/5 isoform specific inhibitors, respectively.

Importantly, the ethanol included in the formulations was evaporated before incubation with the HLM. Ethanol has been demonstrated to have particularly strong inhibitory effects on CYP2C9 and CYP2C19 at concentrations as low as 0.3%.[5]

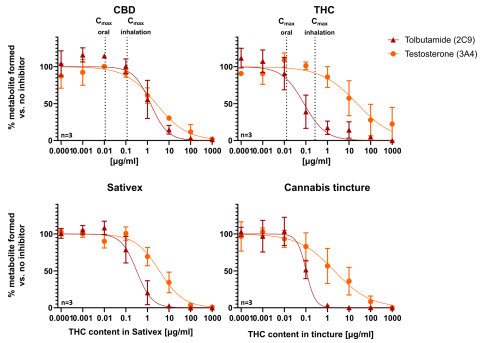
The substrates warfarin, acenocoumarol and phenprocoumon were added at concentrations approximating their reported Km. Tolbutamide and testosterone were used as CYP2C9 and CYP3A4/5 isoform specific substrates, respectively.

The formation of hydroxylated metabolites was followed using UPLC-MS/MS quantification with electrospray ionization in positive mode.

	Parent m/z	Daughter m/z	Internal Standard
OH-Phenprocoumon	281.1	203.0, 175.0	Phenprocoumon-D5
OH-Acenocoumarol	370.1	179.0, 312.0	Acenocoumarol-D5
OH-Warfarin	325.1	179.0, 267.0	Warfarin-D5

RESULTS

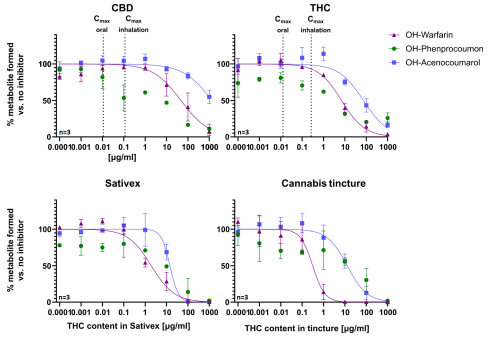
Inhibition curves with CYP-specific substrates



The inhibitory dose-response curves were fitted using the equation Y=100/(1+(IC50/X)^HillSlope), inhibor vs. normalized response function, GraphPad Prism 8.0.2

The control experiments with CYP isoform-specific substrates indicated that both THC and CBD inhibit CYP2C9 and 3A4. THC was a stronger inhibitor than CBD for CYP2C9 (IC_{50}=0.08 vs. 1.4 µg/ml). On the other hand, THC was a weaker inhibitor than CBD for CYP3A4 (IC_{50}=23.6 vs. 2.5 µg/ml). All IC_{50} values were above the maximal plasma concentration (C_{max}) observed after oral uptake of these substances (indicated as a dotted line).
The Cannabis extracts, normalized for their THC content, presented comparable inhibition profiles, indicating that CYP2C9- or CYP3A4-mediated interactions after oral uptake are not expected based on the *in vitro* IC_{50}, unless very high doses are ingested.

Inhibition curves with coumarin derived vitamin K antagonists



Large differences in IC_{50} were observed between the three vitamin K antagonists. All observed *in vitro* IC_{50} values were higher than reported plasma concentrations of CBD and THC (dotted lines). Additionally, interactions with acenocoumarol seem to be less likely than with warfarin. Inhibition of phenprocoumon did not show a classical sigmoidal inhibition-profile which can potentially be explained by the more complex inhibition pathways involving several CYP isoforms.

CONCLUSION AND OUTLOOK

Generally, the observed IC_{50} values with CYP-specific substrates were close to the highest reported CBD and THC plasma concentrations after inhalation of Cannabis-based products, indicating that interactions are possible at high doses. Herb-drug interaction after oral dosing seems to be less likely. However, reports of clinically achieved plasma concentrations after multiple dosing are scarce, and the resulting C_{max} could be higher. Reports of *in vivo* CYP-induction by cannabinoids after long-term use could counteract the inhibition.[6] Other factors, such as protein binding or accumulation of the highly lipophilic cannabinoids in tissues will need to be taken into account to better assess the relevance of these *in vitro* findings for clinical settings.

ACKNOWLEDGEMENT/REFERENCES

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