

European Industrial Hemp Association (EIHA) review on: Safety and Side Effects of Cannabidiol – A review of clinical data and relevant animal studies

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Abstract

This literature survey aims to provide an update of the extensive survey performed by Bergamaschi and colleagues in 2011. Apart from updating the literature, this paper focuses on clinical studies and CBD's potential interactions with other drugs. In general, the often described excellent safety profile of CBD in humans was confirmed and extended by the reviewed research. The majority of studies were performed for treatment of epilepsy and psychotic disorders. Here, the most commonly reported side effects were tiredness, diarrhea and changes of appetite/weight. In comparison with other medicinal drugs used for the treatment of these medical conditions, CBD has a very favorable side effect profile, which may improve compliance and adherence to treatment. Up until now, CBD was mainly used as adjunct therapy. Therefore, more clinical research is warranted in CBD's action on hepatic enzymes and drug-transporters and interactions with other drugs. This can have positive or negative effects, e.g. reducing the needed clobazam doses in epilepsy and therefore this drug's side effects. A third result of this survey was, that some of the parameters summarized by Bergamaschi et al. (2011), which were observed in animal experiments, have not been studied in humans, yet. This is the case, for example, for several hormones (e.g. luteinizing hormone). Given that the endocannabinoid system also plays an important role in endocrine regulation, further research of CBD's off-target effects in this area is needed, e.g. by including the measurement of endocrine parameters in upcoming clinical trials with CBD.

Introduction

Since several years other pharmacologically relevant constituents of the Cannabis plant, apart from THC, have come into the focus of research and legislation. The most prominent of those is Cannabidiol (CBD). In contrast to THC, it is non-psychotropic, but exerts a number of pharmacological effects, which may be of therapeutic interest. For instance, it is anxiolytic, anti-inflammatory, anti-emetic and anti-psychotic. Moreover, neuroprotective

properties have been shown. Consequently, it could be used at high doses for the treatment of a variety of conditions ranging from psychiatric disorders such as schizophrenia and dementia, as well as diabetes and nausea (Bergamaschi et al., 2011; for a complete and up-to-date review please refer to Grotenhermen et al., 2016). At lower doses it has beneficial physiological effects which promote and maintain health (anti-oxidative, anti-inflammatory, neuroprotection). For instance, CBD is more effective than vitamin C and E as a neuroprotective antioxidant and can ameliorate skin conditions such as acne (Hampson et al., 1998; Olah et al., 2014).

The comprehensive review by Bergamaschi and colleagues describes the safety profile of CBD, mentioning several properties: catalepsy is not induced and physiological parameters are not altered (heart rate, blood pressure and body temperature). Psychological and psychomotor functions are not adversely affected. The same holds true for gastrointestinal transit, food intake, and absence of toxicity for non-transformed cells. Chronic use and high doses of up to 1,500 mg per day have been repeatedly shown to be well tolerated by humans. Nonetheless, some side effects have been reported for CBD, but mainly *in vitro* or in animal studies. They include alterations of cell viability, reduced fertilization capacity, inhibition of hepatic drug metabolism and drug transporters (e.g. p-glycoprotein; Bergamaschi et al., 2011).

On May 3rd, 2016, the German Federal Institute for Drugs and Medical Devices (BfArM) advised that CBD should be legislated as a prescription-only drug (Sachverständigenausschuss Verschreibungspflicht, 2016). They argued, that the safety and side effects of CBD were not studied enough. On October 1st, 2016 CBD became a prescription-only drug in Germany. This paper will demonstrate, that numerous clinical studies have been performed on CBD and that its safety, even at high doses, has been proven in the context of various medical conditions. Already in 2011, Bergamaschi and colleagues reviewed 132 original studies on CBD's safety and side effects. This review will build on the clinical studies mentioned there and update it with new studies published until September 2016.

CBD-drug interactions

Effects on P-Glycoprotein Activity and Other Drug Transporters

In vitro studies have shown that CBD inhibits the ABC transporters P-gp (3-100 μM CBD) and Bcrp (Bih et al., 2015). After 3 days, the P-gp protein expression was altered in leukemia cells. This can have several implications, because various anti-cancer drugs also bind to these membrane-bound, energy-dependent efflux transporters (Bergamaschi et al., 2011). It has to be pointed out here, that the used CBD concentrations can be considered supraphysiological. 3 μM CBD approximately corresponds to plasma concentrations of 1 $\mu\text{g}/\text{ml}$. On the other hand, a 700 mg CBD oral dose reached a plasma level of 10 ng/ml (Consroe et al., 1991). This means that to reach a 1 $\mu\text{g}/\text{ml}$, one would need to administer considerably higher doses of CBD. The highest ever applied CBD dose was 1,500 mg (Bergamaschi et al., 2011 and references therein). Consequently, more research is warranted, where the CBD effect on ABC transporters is analyzed using CBD concentration of e.g. 0.03 – 0.06 μM . Studies summarized by Bih et al., 2015 of CBD's effect on ABCB1 and ABCG1 in SF9 human cells showed that the first effect was with CBD concentrations of 0.08 μM . Using the pharmacokinetic relationships mentioned above, one would need to administer an oral CBD dose of 2.100mg CBD to affect ABCB1 and ABCG1.

We used 10 ng/ml for the above calculations and in table 1, based on a 6-week trial using daily oral administration of 700 mg CBD, leading to mean plasma levels of 6-11 ng/ml, which reflects the most realistic scenario of CBD administration in patients (Consroe et al., 1991). That these levels seem to be reproducible, and that chronic CBD administration does not lead to elevated mean blood concentrations, was shown by another study where a single dose of 600 mg lead to reduced anxiety and mean CBD blood concentrations of 4.7 – 17 ng/ml (Fusar-Poli et al., 2009). It also seems warranted to assume that the mean plasma concentration exerts the total of observed CBD effects, compared to using peak plasma levels which only prevail for a short amount of time. This is not withstanding that a recent study measured C_{max} values for CBD of 221 ng/ml, 3h after administration of 1 mg/kg fentanyl concomitantly with a single oral dose of 800 mg CBD (Manini et al., 2015).

A recent study with P-gp, Bcrp and P-gp/Bcrp knockout mice, where 10 mg/kg was injected subcutaneously, showed that CBD is not a substrate of these transporters itself. This means that they do not reduce CBD's transport to the brain. This phenomenon also occurs in paracetamol and haloperidol, which both inhibit P-gp, but are not actively transported substrates. The same goes for gefitinib inhibition of Bcrp. Seen as these proteins are also expressed at the blood brain barrier (BBB) effectively pumping out drugs such as risperidone, they are seen as a cause of resistance to treatment. In addition, polymorphisms in these genes, making transport more efficient, have been implied in interindividual differences in

pharmacoresistance (Brzozowska et al., 2016). In theory, it is thinkable, that even though clobazam levels are increased via CBD cytochrome inhibition, this effect is alleviated by upregulation of P-gp. This hypothesis has to be tested in a co-administration study in humans though. Moreover, the CBD metabolite 7-OH CBD, which has recently been shown to be a more potent anticonvulsant should also be evaluated if it is a P-gp substrate and alters pharmacokinetics of co-administered P-gp-substrate drugs.

An *in vitro* study using 3 types of trophoblast cell lines and *ex vivo* placenta perfused with 15 μM CBD found BCRP inhibition leading to accumulation of xenobiotics in the fetal compartment. BCRP is expressed at the apical side of the syncytiotrophoblast and removes a wide variety of compounds forming a part of the placental barrier. 72h of chronic CBD incubation also showed that 25 μM CBD led to morphological changes in the cell lines but not to a directly cytotoxic effect, whereas 1 μM did neither affect cell nor placenta viability. The authors consider this effect cytostatic. Nicardipine was used as the BCRP-substrate in the *in vitro* studies, where the Jar cell line showed the biggest increase in BCRP expression correlating with the highest level of transport. The *ex vivo* study used the anti-diabetic drug and BCRP-substrate glyburide. After 2h of CBD-perfusion the largest difference between the CBD and the placebo-placentas ($n = 8$ each) was observed. CBD's inhibition of the BCRP-efflux function in the placental cotyledon warrants further research of co-administration of CBD with known BCRP-substrates like nitrofurantoin, cimetidine, and sulfasalazine. Here, a dose-response curve should be established in male and female subjects (CBD absorption was shown to be higher in women) because the concentrations used here, are normally not reached by oral or inhaled CBD administration. Nonetheless, CBD could accumulate in organs physiologically restricted via a blood barrier (Feinshtein et al., 2015).

Additionally, CBD can be a substrate of UDP-glucosyltransferase (Ujvary and Hanus, 2016). To our knowledge no studies exist so far on the question, whether this can also cause clinically relevant drug interactions in humans.

Cytochrome P450-complex enzymes

This paragraph describes CBD's interaction with general (drug)-metabolizing enzymes, such as those belonging to the cytochrome P450 family. The suggested molecular actions of CBD are mostly based on *in vitro* studies and still need to be verified in clinical studies (Bih et al., 2015). Nonetheless, we already mention potential targets that might have an effect for co-administration of CBD with other drugs. For instance, CBD is metabolized, amongst others, via the CYP3A4 enzyme. Various drugs such as ketoconazol, itraconazol, ritonavir and clarithromycin inhibit this enzyme. This leads to slower CBD degradation and can consequently lead to higher CBD doses, that are longer pharmaceutically active. In contrast, phenobarbital, rifampicin, carbamazepine and phenytoin induce CYP3A4, causing reduced CBD bioavailability (DAC/NRF 2015/02). It was estimated that 60% of clinically prescribed drugs are

metabolized via CYP3A4 (Bergamaschi et al., 2011). Table 1 shows an overview of the cytochrome inhibiting potential of CBD. It is interesting to see that the *in vitro* studies used supraphysiological CBD concentrations. Consequently human studies which monitor CBD-drug interactions are needed. In the following paragraphs we describe the only studies performed with CBD and either clobazam or hexobarbital to date. It has to be pointed out, that the used daily CBD doses of either 500 mg (assuming an average weight for the children of 20 kg) and 600 mg are already in the high-dose range for CBD administration.

CBD can also inhibit CYP2D6, which is also targeted by omeprazole and risperidone. There are also indications, that CBD inhibits the hepatic enzyme CYP2C9, reducing the metabolism of warfarin and diclofenac (Grotenhermen et al., 2016 and references therein; Ujvary and Hanus, 2016). More clinical studies are needed, to check in how far this interaction warrants an adaptation of the used doses of the co-administered drugs.

The antibiotic rifampicin induces CYP3A4, leading to reduced CBD peak plasma concentrations. In contrast, the CYP3A4 inhibitor ketoconazole, an anti-fungal

Table 1: Inhibition of human metabolic enzymes by exogenous cannabinoids *in vitro* and the extrapolated levels of oral daily CBD administration in humans needed to reach these *in vitro* concentrations (adapted from Consroe et al., 1991; Stout and Cimino, 2014).

CYP-450 isoform	1A1	1A2	1B1	2A6	2B6	2C9	2D6	3A4	3A5	3A7
CBD (in μM)	0.2	2.7	3.6	55.0	0.7	0.9–9.9	1.2 – 2.7	1.0	0.2	12.3
*Extrapolated oral daily CBD doses to reach the levels above (in mg)	4,900	63,000	84,000	1.28 Mio.	Ca. 16,000	21,000 – 231,000	28,000 – 63,000	Ca. 23,000	4,900	0.29 Mio.

*The calculations made here are based on the assumption that the CBD distribution in the blood follows the pharmacokinetics of a hydrophilic substance like alcohol. The reality is more complex, because CBD is lipophilic and e.g. will consequently accumulate in fat tissue. These calculations were made with the intention to give the reader an impression and an approximation of the supraphysiological levels used in *in vitro* studies.

It has also been shown *in vitro* that CBD inhibits CYP2C19 and CYP2C9 of the cytochrome P450 complex. Many proton pump inhibitors and antiepileptic drugs (e.g. risperidone, pantoprazol, clobazam) are also degraded by the mentioned enzymes. Co-administration can therefore lead to lower degradation of anti-epileptic/psychotic drugs leading to their increased bioavailability). This was shown in an 8-week long clinical study including 13 children who were treated for epilepsy with clobazam (initial average dose of 1 mg/kg b.w.) and CBD (oral; starting dose of 5 mg/kg b.w. raised to maximum of 25 mg/kg b.w.). The CBD interaction with isozymes CYP3A4 and CYP2C19 caused increased clobazam bioavailability, making it possible to reduce the dose of the anti-epileptic drug, which in turn reduced its side effects (Geffrey et al., 2015).

These results are supported by another study described in the review by Grotenhermen, Gebhardt and Berger (2016). Here 33 children were treated with a daily dose of 5 mg/kg CBD which was increased every week by 5mg/kg up to a maximum level of 25 mg/kg. CBD was administered on average with 3 other drugs including clobazam (54.5 percent), valproic acid (36.4 percent), levetiracetam (30.3 percent), felbamat (21.2 percent), lamotrigin (18.2 percent) und zonisamid (18.2 percent). The co-administration lead to an alteration of blood levels of several anti-epileptic drugs. In the case of clobazam this led to sedation, and its levels were subsequently lowered in the course of the study.

drug, almost doubles CBD's peak plasma concentration. Interestingly, the CYP2C19 inhibitor omeprazole, used to treat gastroesophageal reflux, could not significantly affect the pharmacokinetics of CBD (Ujvary and Hanus, 2016).

A study, where a regimen of 6 x 100 mg CBD daily, was co-administered with hexobarbital in 10 subjects, found that CBD increased the bioavailability and elimination half-time of the latter. Unfortunately, it was not mentioned whether this effect was mediated via the cytochrome P450 complex (Benowitz et al., 1980). But other literature indicates that hexobarbital is a CYP2C19 substrate. This is in line with the enzymes being inhibited by CBD (Pelkonen et al., 1998; Karlgren and Bergström, 2015). Studies also propose that this effect might be caused *in vivo* by one of CBD's metabolites. Generally, the metabolite 6a-OH-CBD was already demonstrated to be an inducer of CYP2B10. Recorcinol was also found to be involved in CYP450 induction. The enzymes CYP3A and CYP2B10 were induced after prolonged CBD administration in mice livers, as well as for human CYP1A1 *in vitro* (Bornheim et al., 1994; Ujvary and Hanus, 2016). On the other hand, CBD induces CYP1A1 which is responsible for degradation of cancerogenic substances such as benzopyrene. CYP1A1 can be found in the intestine and CBD-induced higher activity could therefore prevent absorption of cancerogenic substances into the bloodstream and therefore could help to protect DNA (Grotenhermen et al., 2016 and references therein).

Narimatsu et al. (1990) showed interaction of CBD with vitamin A metabolism via cytochrome P450 2C inhibition. Bergamaschi (2011) assumed that this might cause problems with high vitamin A supplementation. To our knowledge and literature survey, no research has been performed, as of September 2016, describing the effects of concomitant CBD and vitamin A treatment in humans.

Another aspect, which has not been thoroughly looked at, to our knowledge, is that several cytochrome isozymes are not only expressed in the liver but also in the brain. It might be interesting to research organ-specific differences in the level of CBD-inhibition of various isozymes. Apart from altering the bioavailability in the overall plasma of the patient, this interaction might alter therapeutic outcomes on another level. Dopamine and tyramine are metabolized by CYP2D6 and neurosteroid metabolism also occurs via the isozymes of the CYP3A subgroup (Persson and Ingelman-Sundberg, 2014; Ghosh et al., 2016). Studying CBD's interaction with neurovascular cytochrome P450 enzymes might also offer new mechanisms of action. It is, for instance, thinkable that CBD-mediated CYP2D6 inhibition increases dopamine levels in the brain, which could help in explaining the positive CBD effects in addiction/withdrawal scenarios and might support its 5HT (=serotonin) elevating effect in depression.

Acute Clinical Data

Bergamaschi and colleagues (2011) already list an impressive number of acute and chronic studies in animals and humans, showing CBD's safety for a wide array of side effects. They also conclude from their survey, that none of the studies reported tolerance to CBD. Already in the 1970s, it was shown that oral CBD (15 – 160 mg), iv injection (5 – 30 mg) and inhalation of 0.15 mg/kg b.w. CBD did not lead to adverse effects. In addition, psychomotor function and psychological functions were not disturbed. Treatment with up to 600 mg CBD neither influenced physiological parameters (blood pressure, heart rate) nor performance on a verbal paired-associate learning test.

As a mouse model for angiogenesis, a subcutaneously injected matrigel pellet together with the angiogenic factors VEGF, TNF-alpha and heparin was used. It was shown that CBD administration (0.0625 mg, 0.125 mg, 0.25mg 0.5 mg per pellet) could reduce vascularization 4 days after injection, which is an important component of tumor growth. Health status was monitored daily and the study does not describe adverse effects of CBD treatment (Solinas et al., 2012).

Fasino and colleagues (2016) created an overview of clinical studies being conducted as of early 2016 in table form. In the following chapter we describe several clinical studies with CBD, which further lend evidence to the claim of CBD's excellent safety profile in more detail:

Effects on cognition, mood and addiction

The review of Bergamaschi et al. (2011) also cites a study using CBD at doses between 0.75 and 2 mg/kg b.w. (i.p.) CBD in rats, on a delayed match to sample task. CBD did not inhibit hippocampal discharges, whereas THC did in this study. Consequently, no delay and dose-dependent behavioral deficit in the tested task could be observed for CBD (Heyser et al., 1993).

A Dutch study compared subjective adverse effects of 3 different strains of medicinal cannabis, distributed via pharmacies, using visual analog scales (VAS). "Visual analog scale is one of the most frequently used psychometric instruments to measure the extent and nature of subjective effects and adverse effects. The 12 adjectives used for this study were as follows: alertness, tranquility, confidence, dejection, dizziness, confusion/disorientation, fatigue, anxiety, irritability, appetite, creative stimulation, and sociability." The high-CBD strain contained the following concentrations: 6% THC/7.5% CBD (n = 25). This strain showed significantly lower levels of anxiety and dejection. Moreover, appetite was increased less in the high-CBD strain. The highest noted adverse effect was "fatigue" with a score of 7 (out of 10), which did not differ for the 3 available strains (Brunt et al., 2014).

Co-administration with opioids and effects on vital signs, pharmacokinetics and affect

In a double-blind, placebo-controlled cross-over study, CBD was co-administered with intravenous fentanyl to a total of 17 subjects. Blood samples were obtained before and after 400 (previously demonstrated to decrease blood flow to (para)limbic areas related to drug craving) or 800 mg CBD pretreatment, followed by a single 0.5 (Session 1) or 1.0 mcg/kg (Session 2, after 1 week of first administration to allow for sufficient drug washout) intravenous fentanyl dose. Adverse effects and safety were evaluated with the Systematic Assessment for Treatment Emergent Events (SAFTEE). This tool has two forms, a General Inquiry (GI) and a Specific Inquiry (SI). The GI is an open-ended inquiry about any physical or health problems and its impact on functioning. The SI is a detailed and systematic inquiry regarding 78 adverse effects divided into 23 categories corresponding to organ systems or body parts. "SAFTEE data were similar between groups without respiratory depression or cardiovascular complications during any test session."

The results of the evaluation of pharmacokinetics were as follows: Peak CBD plasma concentrations of 400 and 800 mg group were measured after 4 h in the first session (CBD administration 2h after light breakfast). Peak urinary CBD and its metabolites concentrations occurred after 6h in the low-CBD-group and after 4 h in the high-CBD-group. No effect was evident for urinary CBD and metabolite excretion except at the higher fentanyl dose, in which CBD clearance was reduced. Importantly, fentanyl co-administration did not produce respiratory depression or

cardiovascular complications during the test sessions and CBD did not potentiate fentanyl effects. No correlation was found between CBD dose and plasma cortisol levels.

Various vital signs were also measured (blood pressure, respiratory/heart rate, oxygen saturation, EKG, respiratory function): CBD did not worsen the adverse effects (e.g. cardiovascular compromise, respiratory depression) of iv fentanyl and co-administration was safe and well tolerated, paving the way to use CBD as a potential treatment for opioid addiction.

The validated subjective measures scales Anxiety (VAS), PANAS (positive and negative subscores), and OVAS (specific opiate VAS) were administered across 8 time points for each session without any significant main effects for CBD for any of the subjective effects on mood (Manini et al., 2015).

Cannabis withdrawal

A case study describes a patient treated for Cannabis withdrawal according to the following CBD-regimen: “treated with oral 300 mg on Day 1; CBD 600 mg on Days 2–10 (divided into two doses of 300 mg) and CBD 300 mg on Day 11.” CBD treatment resulted in a fast and progressive reduction in withdrawal, dissociative and anxiety symptoms measured with the Withdrawal Discomfort Score, Marijuana Withdrawal Symptom Checklist, Beck Anxiety Inventory and Beck Depression Inventory. Hepatic enzymes were also measured daily, but no effect was reported (Crippa et al., 2013).

Studies about reinstatement and salience influencing drug relapse caused by craving

Naturalistic studies with smokers inhaling cannabis with varying amounts of CBD showed, that the CBD levels were not altering psychomimetic symptoms (Bergamaschi et al., 2011 and references therein). Interestingly, CBD was able to reduce the “wanting/liking” = implicit attentional bias caused by exposure to cannabis and food related stimuli. By altering the attentive salience of drug cues, CBD might work to alleviate disorders of addiction. The study did not further measure side effects (Morgan et al., 2010).

That the non-hedonic CBD can also reduce heroin-seeking behaviors (e.g. induced by a conditioned cue) was shown in an animal heroin self-administration study, where mice received 5mg/kg CBD i.p. injections. The observed effect lasted for 2 weeks after CBD administration and could normalize the changes seen after stimulus cue-induced heroin seeking (expression of AMPA GluR1 and CB1R). In addition, the described study was able to replicate previous findings showing no CBD side effects on locomotor behavior (Ren et al., 2009 and references therein). The described results could be replicated in a small double-blind pilot study with individuals addicted to opioids, who have been abstinent for 7 days. They either received placebo or 400 or 800 mg oral CBD on 3 consecutive days. Craving was induced with a cue-induced reinstatement

paradigm (1h after CBD administration). 1 h after the video session, subjective craving was reduced in the CBD groups, already after a single CBD administration. The effect persisted for 7 days after the last treatment. Interestingly, anxiety measures were also reduced after CBD treatment whereas no adverse effects were described (Hurd et al., 2015).

Mnemonic CBD effects

48 Participants in total, received sub-anxiolytic levels (32 mg) of CBD, either before or after the extinction-phase in a double-blind, placebo-controlled design of a Pavlovian fear-conditioning experiment (recall with conditioned stimulus and context after 48 h and exposure to unconditioned stimulus after reinstatement). Skin conductance (= autonomic response to conditioning) and shock expectancy measures (=explicit aspects) of conditioned responding were recorded throughout. Amongst other scales, the Mood Rating Scale MRS (Bond and Bodily Symptoms Scale (BSS) were used to assess anxiety, current mood and physical symptoms. “CBD given post extinction (active after consolidation-phase) enhanced consolidation of extinction learning as assessed by shock expectancy.” Apart from the extinction-enhancing effects of CBD in human aversive conditioned memory, CBD showed a trend towards some protection against reinstatement of contextual memory. No side/adverse effects were reported (Das et al., 2013).

CBD reduces number of cigarettes smoked in persons trying to quit smoking

A pilot study with 24 subjects was conducted in a randomized double blind placebo controlled design to evaluate the impact of the ad-hoc use of cannabidiol (CBD) in smokers, who wished to stop smoking. Pre- and post-testing for mood and craving of the participants was executed. These tests included the BIS (Behaviour Impulsivity Scale), BDI (Beck depression inventory), STAI (Spielberger Trait Anxiety Inventory) and the SDS (severity of dependence scale). During the week of inhalator-use, subjects used a diary to log their craving (on a scale from 1 to 100 = VAS measuring momentary subjective craving), the cigarettes smoked and the number of times they used the inhaler. Craving was assessed using the Tiffany Craving Questionnaire (TCQ: II). On day 1 and 7 exhaled CO was measured to test smoking status. Sedation, depression and anxiety were evaluated with the MRS (Mood Rating Scale). Over the course of 1 week, participants used the inhaler when they felt the urge to smoke and received a dose of 400 µg CBD via the inhaler (leading to >65% bioavailability), this significantly reduced the number of cigarettes smoked by ca. 40%, while craving was not significantly different in the groups post-test. At day 7, the anxiety levels for placebo and CBD-group did not differ. CBD did not produce an increase in depression compared to the selective CB1 antagonist rimonabant. CBD might weaken the attentional bias to smoking cues or could have disrupted reconsolidation, thereby destabilizing drug-related memories (Morgan et al., 2013).

Effects on hormones

High CBD concentrations (1 mM) inhibited progesterone 17-hydroxylase, which creates precursors for sex steroid and glucocorticoid synthesis, whereas 100 μ M CBD did not in an *in vitro* experiment with primary testis microsomes (Watanabe et al., 2005). Rats treated with either 10 mg/kg i.p. b.w. CBD showed inhibition of testosterone oxidation in the liver (Narimatsu et al., 1990).

We looked for studies with humans, analyzing CBD's effect on the above-mentioned hormones, but we could only find one study by Zuardi and colleagues from 1993: 11 healthy volunteers were treated with 300 mg (7 patients) and 600mg (4 patients) oral CBD in a double-blind placebo-controlled study. Growth hormone and prolactin levels were unchanged. In contrast, the normal decrease of cortisol levels in the morning (basal measurement = 11.0 +/- 3.7 μ g/dl; 120 min after placebo = 7.1 +/- 3.9 μ g/dl), was inhibited by CBD treatment (basal measurement = 10.5 +/- 4.9 μ g/dl; 120 min after 300 mg CBD = 9.9 +/- 6.2 μ g/dl; 120 min after 600 mg CBD = 11.6 +/- 11.6 μ g/dl).

A more recent study also used 600 mg oral CBD for a week and compared 24 healthy subjects to people at risk for psychosis (n=32; 16 received placebo and 16 CBD). Serum cortisol levels were taken before the TSST (Trier Social Stress Test), immediately after, as well as 10 and 20 min after the test. Compared to the healthy individuals the cortisol levels increased less after TSST in the 32 at-risk individuals. The CBD-group showed less reduced cortisol levels but differences were not significant (Appiah-Kusi et al., 2016).

Embryogenesis

According to our literature survey, there currently are no studies about CBD's role in embryogenesis neither in animals nor in humans, even though cell migration does play a role in embryogenesis and CBD was shown to be able to at least influence migratory behavior in cancer (Bergamaschi et al., 2011). For instance, it was recently shown in studies that CBD was able to inhibit Id-1. Helix-loop-helix Id proteins play a role in embryogenesis and normal development via regulation of cell differentiation (Murase et al., 2012). High Id1-levels were also found in breast, prostate, brain and head and neck tumor cells which were highly aggressive. In contrast, Id1 expression was low in non-invasive tumor cells. Id1 seems to influence the tumor cell phenotype by regulation of invasion, epithelial to mesenchymal transition, angiogenesis and cell proliferation. Two studies showed in various cell lines and in tumor-bearing mice that CBD was able to reduce tumor metastasis (Benhamou et al., 2012; Murase et al., 2012). Unfortunately, the *in vivo* study was only described in a conference abstract and no route of administration or CBD doses were mentioned (Murase et al., 2012). But an earlier study used 0.1, 1.0, or 1.5 μ mol/L CBD for 3 days in the aggressive breast cancer cells MDA-MB231. CBD downregulated Id1 at promoter level and reduced tumor aggressiveness (McAllister et al., 2007).

It remains to be shown if CBD also regulates Id1 in embryos and if this might have an adverse effect on embryo development. There only seems to exist one study which could not show an adverse CBD effect on embryogenesis: An *in vitro* study could show that the development of two-cell embryos was not arrested at CBD concentrations of 6.4, 32 and 160 nM (Paria et al., 1995).

Food intake and glycemic effects

Animal studies summarized by Bergamaschi and colleagues (2011) showed inconclusive effects of CBD on food intake: i.p. administration of 3 – 100 mg/kg b.w. had no effect on food intake in mice and rats. On the other hand, the induction of hyperphagia by CB1- and 5HT1A-agonists in rats could be decreased with CBD (20 mg/kg b.w. i.p.). Chronic administration (14 days, 2.5 mg/kg or 5 mg/kg i.p.) reduced the weight gain in rats. This effect could be inhibited by co-administration of a CB2R antagonist.

Judging from the clinical studies we reviewed in this paper, the effect of CBD in human studies is not straight forward because when weight and appetite were measured as part of a measurement-battery for side effects, appetite was either increased or decreased, the same goes for weight. This could be either due to the fact that CBD only has a small effect on these factors or appetite and weight are complex endpoints influenced by multiple factors starting with diet and genetic predisposition and both these broad factors were not controlled for in the reviewed studies.

The positive effects of CBD on hyperglycemia seem to be mainly mediated via CBD's antiinflammatory and antioxidant effects. For instance, in ob/ob mice (an animal model of obesity) 4-week treatment with 3mg/kg (route of administration was not mentioned) increased the HDL-C concentration by 55% and reduced total cholesterol levels by more than 25%. Additionally, the treatment increased adiponectin and liver glycogen concentrations (Jadoon et al., 2016 and references therein).

In a placebo-controlled study, randomized, double-blind with 62 subjects with noninsulin-treated type 2 diabetes, 13 were treated with twice daily oral doses of 100mg CBD for 13 weeks. This resulted in lower resistin levels compared to baseline. The hormone resistin is associated with obesity and insulin resistance. Compared to baseline glucose-dependent insulinotropic peptide (GIP) levels were elevated after CBD treatment. This incretin hormone is produced in the proximal duodenum by K cells and has insulinotropic and pancreatic b-cell preserving effects. CBD was well tolerated in the patients but with the comparatively low CBD concentrations used in this phase-2-trial no overall improvement of glycemic control was observed (Jadoon et al., 2016).

Chronic CBD studies in humans

A first pilot study in healthy volunteers in 1973 by Mincis and colleagues administering 10mg oral CBD for 21 days and did not find any neurological and clinical changes (EEG; EKG). The same holds true for psychiatry, blood and urine examinations. A similar testing battery was performed in 1980, at weekly intervals for 30 days with daily oral CBD administration of 3 mg/kg b.w., which had the same result (Cunha et al., 1980).

Parkinson's disease

A study with a total of 21 Parkinson's patients (without comorbid psychiatric conditions or dementia) were treated with either placebo, 75 mg/day CBD or 300 mg/day in an exploratory double-blind trial for 6 weeks. The higher dose showed significant improvement of quality of life as measured with PDQ-39. This rating instrument is comprised of the following factors: mobility, activities of daily living (ADL), emotional well-being, stigma, social support, cognition, communication and bodily discomfort. For the factor "activities of daily living", a possible dose-dependent relationship could exist between the low and high CBD group - the two CBD-groups scored significantly different for them. Side effects were evaluated with the UKU (Udvalg for kliniske undersøgelser). This assessment instrument analyzes adverse medication effects including psychic, neurologic, autonomic and other manifestations. Using the UKU and verbal reports, no significant side effects were recognized in any of the CBD-groups (Chagas et al., 2014).

Huntington's Disease (HD)

15 Patients with HD, who were neuroleptic-free, were treated with either placebo or oral CBD (10 mg/kg b.w. per day) for 6 weeks in a double-blind, randomized crossover design. Using various safety outcome variables, clinical tests and the Cannabis side effect inventory, it was shown, that there were no differences between the placebo group and the CBD-group (Consroe et al., 1991).

Anti-psychotic, cataleptic and motor change effects

The review by Bergamaschi et al. (2011) mentions three acute human studies, which have demonstrated CBD's anti-psychotic effect without any adverse effects being observed. This holds especially true for the extrapyramidal motor side effects elicited by classical anti-psychotic medication. Moreover, the review describes a chronic study, where a teenager with severe side effects of traditional anti-psychotics was treated with up to 1500mg/day of CBD for 4 weeks. No adverse effects were observed and her symptoms improved. The same positive outcome was registered in another study described by Bergamaschi and colleagues (2011) where 3 patients were treated with a starting dose of CBD of 40mg which was ramped up to 1280mg/day for 4 weeks.

In a 4 week open trial, CBD was tested on Parkinson's patients with psychotic symptoms. Oral doses of 150 mg/day – 400 mg/day CBD (in the last week) were

administered. This led to a reduction of the psychotic symptoms. Moreover, no serious side effects or cognitive and motor symptoms were reported (Zuardi et al., 2009).

Comparison of anti-psychotic and side effects of CBD with amisulpride

A double-blind, randomized clinical trial of cannabidiol vs amisulpride, a potent anti-psychotic in acute schizophrenia, was performed on a total of 42 subjects, which were treated for 28 days starting with 200 mg per day each and increased stepwise by 200 mg per day to a daily dose of 200 mg four times daily (total 800mg per day) each within the first week. The respective treatment was maintained for another 3 weeks. A reduction of each treatment to 600 mg per day was allowed for clinical reasons, such as unwanted side effects after week 2, which was the case for three patients in the cannabidiol and five patients in the amisulpride treatment. While both treatments were effective (no significant difference in PANSS total score), CBD showed the better side effect profile. Amisulpride, working as a dopamine D2/D3-receptor antagonist, is one of the most effective treatment options for schizophrenia. In contrast, cannabidiol treatment was accompanied by a significant increase in serum anandamide levels, which was significantly associated with clinical improvement, suggesting inhibition of anandamide deactivation via reduced FAAH activity. Additionally, the FAAH-substrates palmitoylethanolamide and andoleoyl-ethanolamide (both lipid mediators) were also elevated in the CBD-group. CBD showed less serum prolactin increase (predictor of galactorrhoea and sexual dysfunction), fewer extrapyramidal symptoms measured with the Extrapyramidal Symptom Scale (EPS) and less weight gain. Moreover, electrocardiograms as well as routine blood parameters were other parameters whose effects were measured but not reported in the study. The better safety profile might improve acute compliance and long-term treatment adherence (Leweke et al., 2005; Leweke et al., 2012).

CBD's effect on treatment-resistant schizophrenic psychosis

A press release by GW Pharmaceuticals of September 15th, 2015 described 88 patients with treatment-resistant schizophrenic psychosis, treated either with CBD (in addition to their regular medication) or placebo. Important clinical parameters improved in the CBD-group and the number of mild side effects was comparable to the placebo group (Grotenhermen et al., 2016).

Table 2 shows an overview of studies with CBD for the treatment of psychotic symptoms and its positive effect on symptomatology and the absence of side effects (Iseger and Bossong, 2015).

CBD alleviates THC's pro-psychotic effects

15 male, healthy subjects with minimal prior THC exposure (<15 times) were tested for CBD affecting THC's pro-psychotic effects using fMRI and various questionnaires on three occasions, at 1-month intervals, following administration of either 10 mg delta-9-THC, 600 mg CBD

or placebo. Order of drug administration was pseudo-randomised across subjects, so that an equal number of subjects received any of the drugs during the first, second, or third session in a double-blind, repeated measures, within-subject design (Bhattacharya et al., 2010).

No CBD effect on psychotic symptoms as measured with PANSS positive symptoms subscale, anxiety as indexed by the State Trait Anxiety Inventory (STAI) state and VAMS tranquilization or calming subscale, compared to the placebo group. The same is true for a verbal learning task (=behavioral performance of the verbal memory). Moreover, pretreatment with CBD and THC-administration could reduce its psychotic and anxiety symptoms using a standardized scale, caused by opposite neural activation of relevant brain areas. Additionally, no effects on peripheral cardiovascular measures like heart rate and blood pressure were measured (Bhattacharya et al., 2010).

Physiological measures and symptomatic effects were assessed before, and at 1, 2 and 3 hours post drug administration using PANSS (a 30-item rating instrument was used to assess psychotic symptoms, with ratings based on a semi-structured clinical interview yielding sub-scores for positive, negative, and general psychopathology domains), the self-administered Visual Analogue Mood Scale (VAMS) with 16 items (e.g. mental sedation or intellectual impairment, physical sedation or bodily impairments, anxiety effects and other types of feelings or attitudes), the ARCI (Addiction Research Centre Inventory; containing empirically derived for drug-induced euphoria; stimulant-like effects; intellectual efficiency and energy; sedation; dysphoria and somatic effects) was used to assess drug effects and the Spielberger State Anxiety Inventory (STAI-T/S), where subjects evaluated on their current mood and their feelings in general.

Table 2: Studies with CBD with patients with psychotic symptoms (adapted from Iseger and Bossong, 2015).

Assessment	Oral CBD administration	Total number of study participants	Main findings
BPRS (brief psychiatric rating scale)	Up to 1,500 mg/day for 26 days	1	Improvement of symptomatology, no side effects
BPRS	Up to 1,280 mg/day for 4 weeks	3	Mild improvement of symptomatology of 1 patient, no side effects
BPRS, Parkinson Psychosis Questionnaire (PPQ)	Up to 600 mg/day for 4 weeks	6	Improvement of symptomatology, no side effects
Stroop Colour Word Test, BPRS, PANSS (positive and negative symptom scale)	Single doses of 300 or 600 mg	28	Performance ↑ after placebo and CBD 300 mg compared to CBD 600 mg; no effects on symptomatology
BPRS, PANSS	Up to 800 mg/day for 4 weeks	39	CBD as effective as amisulpride in terms of improvement of symptomatology; CBD displayed superior side-effect profile

Effects on cognition and mood

CBD has no physiological or symptomatic effects (compared to THC).

A randomized, double-blind, cross-over, placebo-controlled trial was conducted in 16 healthy non-anxious subjects using a within-subject design. Oral THC = 10 mg, CBD = 600 mg or placebo were administered in three consecutive sessions at one-month intervals. The doses were selected to only evoke neurocognitive effects without causing severe toxic, physical or psychiatric reactions. The 600mg CBD corresponded to mean (standard deviation) whole blood levels of 0.36 (0.64) ng/mL, 1.62 (2.98) ng/mL and 3.4 (6.42) ng/mL, 1h, 2h and 3h after administration, respectively.

There were no significant differences between the effects of CBD and placebo on positive and negative psychotic symptoms, general psychopathology (PANSS), anxiety (STAI-S), dysphoria (ARCI), sedation (VAMS, ARCI), and the level of subjective intoxication (ASI, ARCI), where THC did have a pronounced effect. The physiological parameters, heart rate and blood pressure, were also monitored and no significant difference between the placebo and the CBD-group was observed (Martin-Santos et al., 2012).

Bipolar Disorder (BD)

Seen as anticonvulsant and antipsychotic drugs are also often used to treat manic episodes in bipolar disorder and since CBD has positive effects on the symptoms of

psychosis and epilepsy a few studies have evaluated CBDs effect on BD.

Treatment of two patients for 24 days with 600 – 1,200 mg/day CBD, who were suffering from bipolar disorder did not lead to side effects (Zuardi et al., 2010).

One of the theories trying to explain the etiology of BD is that oxidative stress can play a role in BD development. Valvassori and colleagues (2011) therefore used an animal model of amphetamine induced hyperactivity to model one of the symptoms of mania. Rats were treated for 14 days with various CBD concentrations (15, 30, 60 mg/kg daily i.p.). Whereas CBD did not have an effect on locomotion, it did increase BDNF levels and could protect against amphetamine-induced oxidative damage of protein in the hippocampus and striatum. No adverse effects were recorded in this study.

Another model for BD and also schizophrenia is prepulse inhibition (PPI) of the startle reflex both in humans and animals, which is disrupted in these diseases. Peres et al., (2016) lists 5 animal studies, where mostly 30mg/kg CBD was administered and had a positive effect on PPI.

Apart from the study with 2 patients mentioned above CBD was not yet tested systematically in acute or chronic administration scenarios in humans for BD (Braga et al., 2015; and own literature search).

Epilepsy

Epileptic patients were treated for 135 days with 200 – 300 mg oral CBD daily and evaluated every week for changes in urine and blood. Moreover, neurological and physiological examinations were performed which did not show signs of CBD toxicity or severe side effects. The study also illustrated that CBD was well tolerated (Cunha et al., 1980).

A review by Grotenhermen and colleagues (2016) describes several clinical studies with CBD. In a study with 23 patients with therapy-resistant epilepsy (e.g. Dravet syndrome) were treated for 3 months with increasing doses of up to 25 mg/kg b.w. CBD in addition to their regular epilepsy medication. Apart from reducing the seizure frequency in 39% of the patients, the side effects were only mild to moderate and included reduced/increased appetite, weight gain/loss and tiredness.

Another clinical study lasting at least 3 months with 137 children and young adults with various forms of epilepsy treated with the CBD-drug Epidiolex, was presented at the American Academy for Neurology in 2015. The patients were suffering from Dravet syndrome (16%), Lennox-Gestaut-syndrome (16%) and 10 other forms of epilepsy (some amongst them were very rare conditions).

Here almost 50% of the patients experienced a reduction of seizure frequency. The reported side effects were: 21% experienced tiredness, 17% diarrhea, and 16% reduced appetite. In a few cases severe side effects occurred, but it is not clear, if these were caused by Epidiolex: status epilepticus (n=10), diarrhea (n=3), weight loss (n=2) and liver damage in 1 case.

The largest CBD study was an open-label study with Epidiolex in 261 patients (mainly children, the average age of the participants was 11) suffering from severe epilepsy, who could not be treated sufficiently with standard medication. After 3 months of treatment, where patients received CBD together with their regular medication, a median reduction of seizure frequency of 45% was observed. 10% of the patients reported side effects (tiredness, diarrhea, exhaustion; Grotenhermen et al. 2016 and references therein).

After extensive literature study of the available trials performed until September 2016, CBD's side effects were generally mild and infrequent. The only exception seems to be a multi-center open label study with a total of 162 patients aged 1-30 years, with treatment-resistant epilepsy. Subjects were treated for one year with a maximum of 25 mg/kg (in some clinics 50 mg/kg) oral CBD in addition to their standard medication which led to a reduction in seizure frequency. In this study, 79% of the cohort experienced side effects. The 3 most common adverse effects were somnolence (n=41 [25%]), decreased appetite (n = 31 [19%]), diarrhea (n = 31 [19%]; Devinsky et al., 2016). It has to be pointed out that no control group existed in this study (e.g. placebo or other drug) to be able to put the side effect frequency into perspective. Another point is, that proving that CBD caused the side effects is particularly difficult in severely sick patients. Thus, it is not possible to draw reliable conclusions on side effects of CBD from this study.

Graft versus Host disease (GVHD)

48 patients were treated with 300 mg/kg oral CBD 7 days before and until 30 days after the transplantation of allogeneic hematopoietic cells from an unrelated donor to treat acute leukemia or myelodysplastic syndrome in combination with standard measures to avoid GVHD (cyclosporine and short course of MTX). The occurrence of various degrees of GVHD was compared with historical data from 108 patients who only received the standard treatment. Patients treated with CBD did not develop acute GVHD. In the 16 months after transplantation the incidence of GHVD was significantly reduced in the CBD-group. Side effects were graded using the Common Terminology Criteria for Adverse Events (CTCAE v4.0) classification, which did not detect severe adverse effects (Yeshurun et al., 2015).

Animal experiments suggesting CBD side effects in humans

Before we will discuss relevant animal research, which studies CBDs possible effects on various parameters, several important differences between route of administration and pharmacokinetics between human and animal studies have to be mentioned. Firstly, CBD has been studied in humans using oral administration or inhalation. In mice, which are often used to test CBD, administration occurred either via intraperitoneal injection or via the oral route. Secondly, the plasma levels reached via oral administration in mice and humans can differ. Both these observations can lead to differing active blood concentrations of CBD. The following study, which showed a positive effect of CBD on obsessive compulsive behavior in mice and reported no side effects, illustrates the differences pointed out above. Deiana and colleagues (2012) administered 120 mg/kg CBD either orally or intraperitoneally and measured peak plasma levels. The group of mice, which received oral CBD, had plasma levels of 2.2 µg/ml CBD. In contrast, ip injections resulted in peak plasma levels of 14.3 µg/ml. As mentioned earlier, administering 10 mg/kg CBD orally to humans lead to blood levels of 0.01 µg/ml (Consroe et al., 1991). This corresponds to human blood levels of 0.12 µg/ml when 120 mg/kg CBD would be given to humans. This calculation was performed assuming the pharmacokinetics of a hydrophilic compound, as mentioned earlier. We are aware, that actual levels of the lipophilic CBD will vary, but we used this approximation to give the reader a rough idea of the interspecies pharmacokinetic differences one has to be aware of when trying to extrapolate results gathered through animal experimentation to humans.

These numbers mean that if mice and humans are given the same CBD dose, more of the compound becomes actually available in the mouse organism. This higher bioavailability in turn can cause larger CBD-effects.

Physiological effects

In chronic (up to 14 days of treatment) studies with rodents, it was shown that CBD (3 - 30 mg/kg b.w. i.p.) did not affect blood pressure, heart rate, body temperature, glucose levels, pH, pCO₂, pO₂, hematocrit, K⁺ or Na⁺ levels, gastrointestinal transit, emesis or rectal temperature (Bergamaschi et al., 2011 and references therein).

Mice treated with 60 mg/kg b.w. CBD i.p. for 12 weeks (3 times per week) did not show ataxia, kyphosis, generalized tremor, swaying gait, tail stiffness, changes in vocalization behavior or open-field physiological activity (urination, defecation; Bergamaschi et al., 2011 and references therein).

Hepatic drug metabolism

Studies in mice have shown that CBD inactivates cytochrome P450 isozymes in the short term but can induce them after repeated administration similar to their induction by phenobarbital. This suggests that a mechanism which works via the 2b subfamily. Another study showed this effect to be mediated by upregulation

of mRNA for CYP3A, 2C and 2B10 after repeated CBD administration (Bergamaschi et al., 2011 and references therein). It will be interesting to research by which process this effect is mediated, as it has been recently shown that CBD can alter the expression of several genes related to skin cell differentiation via epigenetic DNA methylation mediated via DNMT1 (Pucci et al., 2013).

Effects on affect (anxiety, depression etc.)

Some studies indicate that under certain circumstances the acute anxiolytic effects observed in rats could be reversed after repeated 14-day administration of CBD (Grotenhermen et al., 2016). Clinical chronic studies in humans are crucial here, because these experimental inconsistencies (sometimes the anxiolytic effect prevails after chronic treatment) can also be explained by the animal models for different symptoms of anxiety or depression being used. In case reports chronic CBD treatment proves to be consistently anxiolytic, so that it might be used in the treatment of e.g. PTSD (posttraumatic stress disorder).

The last point is demonstrated by another study which administered CBD in an acute and chronic (2 weeks) regimen and measured anxiolytic/antidepressant effects using behavioral and operative models (OBX=olfactory bulbectomy as model for depression). The only observed side effects were reduced sucrose preference, reduced food consumption and body weight in the non-operated animals treated with CBD (50 mg/kg). Nonetheless, the behavioral tests (for OBX-induced hyperactivity and anhedonia related to depression and open field test for anxiety) in the CBD-treated OBX-animals showed an improved emotional response. Using microdialysis, the researchers could also show elevated 5-HT and glutamate levels in the prefrontal cortex of OBX animals only. This area was previously described to be involved in maladaptive behavioral regulation in depressed patients and is a feature of the OBX-animal model of depression. The fact that serotonin levels were only elevated in the OBX mice is similar to CBD's differential action under physiological and pathological conditions. A similar effect was previously described in anxiety experiments, where CBD proved to be only anxiolytic in subjects where stress had been induced prior to CBD administration. Elevated glutamate levels have been proposed to be responsible for ketamine's fast anti-depressant function and its dysregulation has been described in OBX mice and depressed patients. Chronic CBD treatment did not elicit behavioral changes in the non-operated mice. In contrast, CBD was able to alleviate the affected functionality of 5HT1A-receptors in limbic brain areas of OBX-mice (Linge et al., 2016).

Schiavon and colleagues (2016) cite three studies which used chronic CBD administration to demonstrate its anxiolytic effects in chronically stressed rats, which were mostly mediated via hippocampal neurogenesis. One example is the study by Campos et al. (2013) which could also show involvement of 5HT1A-R in the dorsal periaqueductal gray area (DPAG) in panic-like responses of rats. The animals received daily i.p. injections of 5 mg/

kg CBD. Applying an 5HT1A-receptor antagonist in the DPAG, they could imply CBD in exerting its anti-panic effects via these serotonin receptors. No adverse effects were reported in this study.

Cancer

Various studies have been performed to study CBD's anti-cancer effects. CBD's anti-invasive actions seem to be mediated by its TRPV1 stimulation and its action on the CB-receptors. Intraperitoneal application of 5 mg/kg b.w. CBD every 3 days for a total of 28 weeks, almost completely reduced the development of metastatic nodules caused by injection of human lung carcinoma cells (A549) in nude mice (Leanza et al., 2016). This effect was mediated by upregulation of ICAM1 and TIMP1 caused by upstream regulation of p38 and p42/44 MAPK pathways. The typical side effects of traditional anti-cancer medication, emesis and collateral toxicity, were not described in these studies. Consequently, CBD could be an alternative to other MMP1-inhibitors like marimastat and prinomastat, which have shown disappointing clinical results due to musculoskeletal adverse effects (Ramer et al., 2010; Ramer et al., 2012 and references therein).

Another study also used xenografts to study the pro-apoptotic effect of CBD, this time in LNCaP prostate carcinoma cells (Leanza et al., 2016). In this 5-week study, 100 mg/kg CBD was administered daily i.p. Tumor volume was reduced by 60% and no adverse effects of treatment were described in the study. The authors assumed that the observed anti-tumor effects were mediated via TRPM8 together with ROS (reactive oxygen species) release and p53 activation (De Petrocellis et al., 2013). It has to be pointed out though, that xenograft studies only have limited predictive validity to results with humans. Moreover, to carry out these experiments, animals are often immunologically compromised in order to avoid immunogenic reactions as a result to implantation of human cells into the animals, which in turn can also affect the results (Fowler et al., 2015).

Another approach was chosen by Aviello et al. (2012). They used the cancerogen azoxymethane to induce colon cancer in mice. Treatment occurred using IP injections of 1 or 5 mg/kg CBD, 3 times a week for 3 weeks (including 1 week before carcinogen administration). After 3 months, the number of aberrant crypt foci, polyps and tumors was analysed. The high CBD concentration led to a significant decrease in polyps and a return to near normal levels of phosphorylated Akt (elevation caused by the cancerogen; Fowler, 2015). No adverse effects were mentioned in the described study (Aviello et al., 2012).

Neuroprotection and Neurogenesis

There are various mechanisms underlying neuroprotection, including e.g. energy metabolism (whose alteration has been implied in several other psychiatric disorders) and proper mitochondrial functioning (Valvasori et al., 2013). An early study from 1976 found no side effects and no effect of 0.3 – 300 µg/mg protein CBD after 1h of incubation on mitochondrial monoamine oxidase activity in porcine

brains (Schurr et al., 1976). In hypoischemic newborn pigs, CBD elicited a neuroprotective effect, no side effects and even led to beneficial effects on ventilatory, cardiac and hemodynamic functions (Alvarez et al., 2008).

A study comparing acute and chronic CBD administration in rats suggests another mechanism of neuroprotection. Animals received i.p. CBD (15, 30, 60 mg/kg b.w.) or vehicle daily for 14 days and mitochondrial activity was measured in striatum, hippocampus and the prefrontal cortex (Valvasori, et al., 2013). Acute and chronic CBD injections led to increased mitochondrial activity (complexes I-V) and creatine kinase, whereas no side effects were documented. Chronic CBD treatment and the higher CBD doses tended to affect more brain regions. The authors hypothesized that CBD changed intracellular Ca²⁺ flux in order to cause these effects. Since not only mitochondrial complex I and II have been implied in various neurodegenerative diseases, but also altered ROS levels, which have also been shown to be altered by CBD, this might be another possible mechanism of neuroprotection (Bergamaschi et al., 2011; Valvasori et al., 2013). Interestingly, it has recently been shown that the higher ROS levels after CBD treatment are concomitant with higher mRNA and protein levels of heat shock proteins (HSPs). In healthy cells this can be interpreted as a way to protect against the higher ROS levels resulting from more mitochondrial activity. Additionally, it was shown that HSP-inhibitors increase CBD's anti-cancer effect *in vitro* (Scott et al., 2015). This is in line with the studies described by Bergamaschi (2011), which also imply ROS in CBD's effect on (cancer) cell viability in addition to e.g. pro-apoptotic pathways such as via caspase-8/9 and inhibition of the pro-carcinogenic lipoxygenase pathway.

Another publication studied the difference of acute and chronic administration of 2 doses of CBD in non-stressed mice on anxiety. Already an acute i.p. administration of 3mg/kg was anxiolytic to a degree comparable to 20 mg/kg imipramine (an SSRI commonly prescribed for anxiety and depression). 15 days of repeated i.p. administration of 3 mg/kg CBD also increased cell proliferation and neurogenesis (using 3 different markers) in the subventricular zone and the hippocampal dentate gyrus. Interestingly, the repeated administration of 30 mg/kg also led to anxiolytic effects. But the higher dose caused a decrease in neurogenesis and cell proliferation, indicating dissociation of behavioral and proliferative effects of chronic CBD treatment. The study does not mention adverse effects (Schiavon et al., 2016).

Psychosis

Using the NMDAR antagonist MK-801, an animal model of psychosis can be created in mice. The behavioral changes (tested with the prepulse inhibition test) were concomitant with decreased mRNA expression of the NMDAR GluN1 subunit gene (GRN1) in the hippocampus, decreased parvalbumin expression (=a calcium-binding protein expressed in a subclass of GABAergic interneurons) and higher FosB/ ΔFosB expression (=markers for neuronal activity). After 6d of MK-801 treatment, various CBD doses were injected intraperitoneally (15, 30, 60 mg/kg) for 22

days. The two higher CBD doses had beneficial effects comparable to the atypical antipsychotic drug clozapine and also attenuated the MK-801-effects on the three markers mentioned above. The publication did not record any side effects (Gomes et al., 2015).

Immune system

Numerous studies show CBD's immunomodulatory role in various diseases such as multiple sclerosis, arthritis and diabetes. These animal and human *ex vivo* studies have been reviewed extensively elsewhere, but studies with pure CBD are still lacking, even though it would be interesting to see in which cases CBD is pro-inflammatory and under which circumstances it is anti-inflammatory and if this leads to side effects (Burstein, 2015: Table 1 shows a summary of its anti-inflammatory actions; McAllister et al., 2015: gives an extensive overview in Table 1 of the interplay between CBD's anti-cancer effects and inflammation signaling).

In case of Alzheimer's (AD), studies in mice and rats showed reduced amyloid beta neuroinflammation (linked to reduced interleukin-6 and microglial activation) after CBD treatment and amelioration of learning effects in a pharmacological model of AD. The chronic study we want to describe in more detail here used a transgenic mouse model of AD, where 2.5 month old mice were treated with either placebo or daily oral CBD doses of 20mg/kg for 8 months (mice are relatively old at this point). CBD was able to prevent the development of a social recognition deficit in AD transgenic mice. Moreover, the elevated IL-1beta and TNFalpha levels observed in the transgenic mice could be reduced to WT (wildtype) levels with CBD treatment. Using statistical analysis by ANOVA this was

shown to be only a trend, even though this might have been caused by the high variation in the transgenic mouse group. CBD increased the cholesterol levels in WT mice but not in CBD-treated transgenic mice, this might be due to the fact that its cholesterol levels were already elevated. This study observed no side effects (Cheng et al., 2014).

In non-obese diabetes-prone female mice (NOD), CBD was administered i.p. for 4 weeks (5 days a week) at a dose of 5 mg/kg per day. After CBD treatment was stopped, observation continued until the mice were 24 weeks old. CBD treatment lead to reduction of diabetes development (32% developed glucosuria in the CBD group compared to 100% in untreated controls) and to more intact islet of Langerhans cells. CBD increased IL-10 levels, which is thought to act as an anti-inflammatory cytokine in this context. The IL-12 production of splenocytes was reduced in the CBD group and no side effects were recorded (Weiss et al., 2008).

After inducing arthritis in rats using Freund's adjuvant, various CBD doses (0.6, 3.1, 6.2 or 62.3 mg/day) were applied daily in a gel for transdermal administration for 4 days. CBD reduced joint swelling, immune cell infiltration, thickening of the synovial membrane and nociceptive sensitization/spontaneous pain in a dose-dependent manner. Pro-inflammatory biomarkers were also reduced in a dose-dependent manner in the dorsal root ganglia (TNFalpha) and spinal cord (CGRP, OX42). No side effects were evident and exploratory behavior was not altered (in contrast to THC, which caused hypolocomotion; Hammell, et al., 2015).

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