

Endometriosis – an indication for cannabinoids ?

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ABSTRACT

Endometriosis is associated with imbalances of the endocannabinoid system (ECS) which correlate with symptoms: Whereas cannabinoid receptors CB1 and CB2 are downregulated, plasma levels of endocannabinoids (AEA, 2-AG and PEA) are elevated. In addition, levels of matrix metalloproteinases (MMPs) which are important for a wide range of pathological processes including spreading of cells and inflammation are also high in ectopic endometrium. Cannabinoids including cannabidiol (CBD) are known to interact with the ECS. Among other mechanisms, they decrease MMP production and therefore invasiveness of cells. As endometrial cells have a hyperproliferative phenotype and pro-angiogenic properties, it may be argued that the decrease of MMPs by CBD could also reduce endometriosis. At present, only population-based surveys suggest that cannabinoids reduce pain and other symptoms in women affected by endometriosis. Whereas a majority of women who have used cannabis confirm its efficacy, it is not without risks. Of the two main active compounds, Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD), CBD has the advantage of not being psychotomimetic, therefore not causing the typical "high". It is not a "scheduled drug", does not interfere with workplace testing or police controls when driving, and is well tolerated also when taken as long-term treatment. Pure CBD is devoid of potential impurities which are frequently found in "street cannabis". This could be a safe and novel approach for the treatment of endometriosis.

KEYWORDS: Endometriosis; Cannabinoids; Cannabis

INTRODUCTION

The Endometriosis is the presence of endometrial tissue outside the uterine cavity in sites such as the ovaries, pelvic peritoneum, and the rectovaginal septum. It is the most common cause of reduced fertility and chronic pelvic pain, with an inflammatory character. Worldwide, 5–15% of women of reproductive age have endometriosis with varying symptoms and intensity. Pain is commonly described as diffuse and poorly localized; it includes non-menstrual pelvic

pain, dysmenorrhoea (period pain), dyspareunia (pain during sexual intercourse), less commonly dyschezia (pain on bowel motions), and dysuria (pain on urination). Each patient's pain experience is different. Endometriosis has a tremendous impact on the quality of life of women and on socioeconomics. Annual health care costs were about 3.5 times higher in women with diagnosed endometriosis than for a matched group of women without [1].

Since long, retrograde menstruation is assumed as possible

mechanism but many pathogenetic aspects are still not completely understood. The mainstays of current non-surgical treatments are oral contraceptive pills, non-steroidal anti-inflammatory drugs (NSAIDs), opioids, and hormonal treatments. Their use is often limited by insufficient effectiveness and/or side effects, particularly after long-term use, with discontinuation rates between 25% and 50% [2]. Increased prostaglandins due to cyclooxygenase-2 (COX-2) activity and increased estrogens due to aromatase activity are common in women suffering from endometriosis; this has led to medications targeting respective pathways. Frustrated by insufficient symptom relieve, many women turn to self-management strategies such as heat, yoga/pilates, rest, exercises, dietary changes, alcohol, cannabis and extracts such as so called "Hemp-Oils" or "CBD-Oils".

Cannabis plays an increasing role in endometriosis

Cannabis has traditionally been used since more than 3000 years for painful gynaecological conditions [3]. Nonetheless, due to its illegal status in the past (and in many countries till today), there is a paucity of data on the treatment of endometriosis with cannabinoids. With an increasing number of countries that permit access to cannabis (often only to medical cannabis and only on prescription), the use of cannabis or medical cannabis is likely to increase. In two very recent surveys, cannabis and cannabis- /hemp-extracts ("CBD-oils") ranked among the most effective self-management strategies. According to investigations in Arizona, 75.9% of patients with a clinical diagnose of endometriosis found marijuana very or moderately effective in reducing pain [4]. Marijuana is the slang term for drug-type cannabis rich in delta 9-tetrahydrocannabinol (THC) in contrast to hemp (fibre-type cannabis) which is rich in cannabidiol (CBD) with THC-concentrations legally limited to 0.3% or less in most countries; cannabis is the overall term. CBD-oils, which are hemp extracts varying widely in their content of CBD and THC, and produced using various solvents and techniques, were also very effective but rated slightly lower (64.9%). In another recent Australian survey, 56% of women with surgically confirmed endometriosis were able to reduce concomitant medications by at least 50% by using cannabis, compared to a third of the patients that used CBD-oil [5]. However, it would be premature to conclude that CBD is inferior to THC-rich cultivars as dosages taken by women in these studies are unknown. CBD needs about 5 to 20 times higher doses than THC to demonstrate medical effects. In addition to these two

studies, numerous unconfirmed testimonials are found in the internet. An important limitation to the use of herbal cannabis as well as of derived products such as CBD-oils are however legal restrictions. Psychotropic effects ("high") caused by THC are unavoidable, as is the risk of positive workplace tests or of positive controls when driving a car. Eventually, the chronic use of drug-type cannabis may induce a problematic use/abuse and neuro-anatomical alterations. Reduced fertility has also been reported [6]. Urine can still be positive for THC-metabolites after three weeks or more following to the last intake [7]; as cannabinoids are lipophilic drugs they are slowly released from fatty tissues. In addition, cannabis contains hundreds of pharmacologically active phytocompounds such as β -caryophyllene or β -myrcene being the most prominent terpenes in a majority of cannabis cultivars [8]. Both have anti-inflammatory and analgesic properties [9,10]. Intriguingly, β -caryophyllene is a selective agonist of the cannabinoid receptor CB2 which brings it in line with other cannabinoids [11]. Known since long as flavour substance, β -caryophyllene is "GRAS", i.e. "generally recognised as safe" by the FDA (FEMA #2252). In a rat model of endometriosis, a relatively high dose of β -caryophyllene (10 mg/kg) demonstrated regression of endometrial implants [12] whereas decreased incidence of uterine endometrial hyperplasia was observed after myrcene [13]. However, the contribution of terpenes should not be overestimated as the anti-inflammatory activity of terpenoid-rich fractions of cannabis (essential oils) were inferior to purified CBD [14].

Most worrying however are quality aspects. Cannabis derived products range from beverages and liquids such as CBD-oils to edibles and highly refined extracts with various degrees of concentrations sold in shops or via the internet. They are frequently mislabelled; in up to 83% the declared content of cannabinoids differed by more than 10%. In some "CBD-oils" or "hemp oils" (not to be confused with hempseed oil!) no CBD at all could be detected [15-18]. Furthermore, in up to 45% of the samples the THC content exceeded the permitted limit. Therefore, it is impossible to know by consumers which substances and doses have been used. About 1200 cannabis varieties may exist, but most of them are poorly defined beyond their content of THC and CBD. The same is true for derived products. Other serious concerns are batch-to-batch variations and contaminations with residual solvents, pesticides, heavy metals, bacteria and fungi [19]. After consumption of such products there is a notable risk that patients are getting sick and need poison control for help.

In Switzerland, 11 of 12 herbal cannabis samples contained potentially toxic levels of bacteria and fungi, and pesticides were found in 8% of 151 samples [20]. A recent recall of marijuana in Denver, Colorado, for contamination by moulds and yeast (39 million yeast and mould colony-forming units per gram which is 3900 times the State limit) underlines this problem [21]. As the plant pulls heavy metals from the soil, chrome, cobalt, bismuth, aluminium, nickel, manganese, or lead among others have been found in herbal parts of cannabis. To note, metal impurities can also originate from e-cigarettes that are sometimes used as device for cannabis consumption [22]. Self-medication is therefore not free from serious risks.

More expensive but of higher quality is “medical cannabis” which is cultivated under authorised, controlled conditions and usually sold as dried, herbal material (cannabis flos). Examples are products such as Bedrobinol (12-14% THC, <1% CBD), Bediol (2.6-5.1% THC, 4.8-7.2% CBD) both originating from The Netherlands, FM2 (5-8 % THC, 7.5-12 % CBD) from Italy, M-1448 (0.6-0.9 % THC, 13-15 % CBD) from Switzerland or Avidel (<1 % THC, 16-19% CBD) from Israel. Major drawbacks of these products are their heterogeneity, varying concentrations of THC and CBD even in the same cultivar, and inconsistent effects. In contrast to pure CBD, these products are not free from THC, therefore illegal in many countries or needing a prescription.

CBD-oil is formally speaking an extract and legally a medicine if used for medical purposes; health claims related to, make it a medicine. In Europe, health authorities have regulated the grey market of CBD-oils manufactured for consumers by declaring CBD-oils as “Novel Food”. To note, a pharmacy may still prepare an extract from legally prescribed (medical) cannabis. The only extract in pharmaceutical quality that has received marketing authorisation so far is nabiximols (Sativex), a roughly 1:1 mixture of a THC- and CBD-rich extract, each containing about 65% to 70% of the respective cannabinoid. Basically, the problem specific for THC remains. No clinical studies on endometriosis or case series could be found with pure cannabinoids, nabiximols or other defined extracts. However, a number of reviews on cannabinoids concluded that these preparations have a low but significant efficacy in non-cancer pain [23-25]. Pure CBD, usually coadministered with other analgesics, is also effective [26] and can reduce the dosages of less well tolerated drugs including of opioids. Pharmacy products are in general well tolerated, and no cases

of overdose or death, specifically to THC or CBD have been reported. Compared to opioids and NSAIDs, cannabinoids have a good safety profile.

Endometriosis – a “clinical endocannabinoid deficiency syndrome” (CEDs)

The endocannabinoid system (ECS) is a complex, lipid-derived signalling system detected 30 years ago. It is vital for the homeostasis, i.e. the physiological balance, of a wide range of essential processes. Among others, the ECS controls energy-related processes in the organism such as cell growth, hunger and sleep. Among the best-studied components of the ECS are the cannabinoid-receptors CB1, CB2 and their endogenous ligands, the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG). Other less well studied receptors expressed in the endometrium are the orphan receptor GPR55 which may play a role in self-renewal and proliferation of cells and GPR18, but also the endocannabinoids N-palmitoylethanolamide (PEA) or virhodamine. The ECS regulates almost all levels of female physiological processes including reproduction, starting with oocyte production through to parturition; dysregulation of the ECS is associated with the development of many gynaecological disorders, from pelvic pain to infertility and cancer.

Endometriosis is associated with an imbalance of the ECS. It appears to be associated with down-regulation of (endo-) cannabinoid receptors and upregulation of TRPV1 receptors as well as of endocannabinoids. TRPV (standing for transient receptor potential vanilloid type) is a class of evolutionary very old ion channels that signalise to the organism physical changes of the environment. In case of TRPV1, this can be mechanical pressure and/or heat and/or chemical influences. CB1 and CB2 receptors were significantly lower, both in the eutopic and ectopic endometrium of adenomyosis, which strongly suggests that cannabinoid receptors participate in the pathogenesis [27-30]. CB1- and CB2 agonists induced apoptosis and reduced proliferation of Ishikawa cells (endometrial glandular cells) [27,31]. On the other hand, plasma levels of AEA, 2-AG and PEA were elevated in endometriosis whereby levels of endocannabinoids and TRPV1 expression correlated with pain symptoms in women with endometriosis [32,33]. In contrast to, and unsurprisingly, CB2 receptors were upregulated in the inflammatory tissues of endometritis [34]. CB2 receptors are commonly found on cells of the immune system and therefore in inflammation. In

leiomyomas, higher AEA levels but significantly lower levels of N-palmitoylethanolamide (PEA), lower expression of fatty acid amid hydrolase (FAAH), of CB1- and of GPR55-receptors were found in fibroid tissues [35]. Intriguingly, studies have demonstrated that cannabinoids including CBD decrease matrix metalloproteinase (MMP) production and therefore invasiveness of cancer cells [36]. MMPs are important for a wide range of pathological processes including spreading of cells and inflammation. Given the high levels of MMPs in ectopic endometrium, it can be argued that the decrease of MMPs by CBD could also reduce endometriosis. Although not binding directly to CB1 or CB2 and therefore not being a “canonical” (endo-) cannabinoid, PEA shares some properties with AEA but also with CBD. It was originally discovered in egg yolks, many decades before the ECS was detected, and is considered as nutraceutical. Indeed, PEA has been found to improve chronic pelvic pain caused by endometriosis [33,37]. Despite of its long history, pharmacokinetics of PEA are still insufficiently known, and experiences in man are limited [38].

Overall, this demonstrates that endometriosis and possibly many other diseases of the endometrium are characterised by an imbalance of the ECS.

Cannabidiol – a potential treatment for endometriosis?

More generally speaking, exo-cannabinoids such as THC and CBD are direct (THC) or indirect (CBD) cannabinoid agonists and mimic to some extent actions of endocannabinoids which are reported to increase apoptosis mechanisms in endometriosis and adenomyosis [27]. Cannabinoid agonists exert anti-proliferative effects on stromal endometriotic cells, linked to the inhibition of the Akt pathway, in vitro and in vivo [32]. The Akt pathway mediates downstream responses, including cell survival, growth, proliferation, cell migration and angiogenesis by phosphorylating a range of intracellular proteins. This pathway is highly conserved and present in all cells of higher organisms.

Apart from the psychotomimetic THC, cannabidiol (CBD) is the second most prominent and best studied phytocannabinoid. It has become very popular in recent years. The mechanism of CBD is very complex and still incompletely understood. CBD is a negative allosteric modulator of CB1 and CB2, an antagonist of GPR55 and a potent TPV1 agonist / activator (similar to capsaicin which is used locally for pain treatment). It increases indirectly, via inhibiting FAAH, the availability of AEA and of other endocannabinoids such as PEA which are natural

ligands to a number of targets that are also modulated by CBD. PEA which has demonstrated efficacy in endometriosis, shares many of its activities with CBD. Like CBD, PEA does not bind directly to CB1 and CB2 receptors but modulates GPR55 and inhibits the nuclear factor NF- κ B which regulates the transcription of genes involved in inflammation and pain, i.e. the expression of genes of pro-inflammatory cytokines such as IL-1b, IL-6, TNF α , expression of inflammatory mediators such as COX-2, inducible nitric oxide synthase (iNOS) but also genes involved in lipid transport / storage and cell proliferation. Like CBD, it targets also TRPV1. Similar to AEA, PEA levels are elevated in chronic inflammation (38), and also similar to AEA, PEA is degraded by FAAH. Thus, CBD modulates not only a physiological balance of AEA but also of PEA and is able to increase the level of both. CBD could therefore affect endometriosis either via similar mechanisms as described for PEA or via increasing the availability of PEA and AEA. Furthermore, CBD reduces inflammation, vascularisation, angiogenesis, cell proliferation and cell migration. As endometrial cells have a hyperproliferative phenotype and pro-angiogenic properties [31], treatment with CBD would represent a completely new strategy to control endometriosis. In addition, CBD reduces anxiety, is wake-promoting and neuroprotective [39,40]. Pure CBD is legal, is not a “controlled substance”, and does not induce a “high” in contrast to THC. Administered “add-on”, CBD allows to reduce the dose of less well tolerated analgesics, and reduces abuse behaviour in cases of drug dependence.

It goes without further saying that an appropriate immune response requires a regulated balance between reactions against non-self, and limited or no reactions against self. CBD follows the principle of hormesis, i.e. reactions to CBD can be biphasic/bidirectional, depending on conditions. CBD either enhanced or suppressed cytokine production (IL-2 and IFN γ) in response to relatively low or high degree of immune stimulation, respectively [43]. Among other anti-inflammatory actions, CBD suppresses the formation of TNF α , nitric oxide (NO), reduces the production of pro-inflammatory IL-6 and induces the formation of regulatory T-cells (Treg-cells) [43,44]. CBD also activates PPAR γ , suppressing oxidative stress and pro-inflammatory signalling [45]. As mentioned above, CBD has been shown to induce apoptosis whereby the upregulation of COX-2 and PPAR γ plays a prominent role [40]. This demonstrates that CBD prevents the release of pro-inflammatory mediators not by one, but by

multiple mechanisms. Many of these mechanisms play, to a varying extent, also a role in endometriosis.

Indeed, preclinical experiments, although still very limited, suggest that phytocannabinoids may be effective in endometriosis. In a mouse model of surgically-induced endometriosis, THC (2 mg/kg) alleviated pelvic hypersensitivity and inhibited the development of endometrial cysts [41]. In an in vitro model using Ishikawa cells, Hec50co endometrial cancer cells and normal HFF-1 fibroblast cells, endocannabinoids (AEA, 2-AG) and CBD (5 mcM) induced a significant reduction in cell viability in both Ishikawa and Hec50co cells, but not in normal fibroblast cells, whereas THC did not cause any effect [42]. This suggests that phytocannabinoids have differential effects on the proliferation of endometrial cells, leaving normal cells unaffected.

To sum it up, recent surveys report that cannabis alleviates pain of endometriosis, confirming the historic use for pelvic pain. The use of cannabis is, however, not without risks, on one hand because of quality concerns related to “street” cannabis and derivatives, on the other because of the inherent psychotropic and other side effects related to THC. Treatment experiences with CBD-rich products (“CBD-oils”) and preclinical investigations with pure CBD suggest a possible efficacy in endometriosis. Pure herbal CBD received recently marketing authorisation, and is available in some European countries as crystalline, active pharmaceutical ingredient for pharmacy preparations in a purity exceeding 99.8% (THC below limit of detection). This could be an alternative to cannabis for treatment of endometriosis.

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