STUDIES OF THE CANNABINOID RECEPTOR 1 IN MANTLE CELL LYMPHOMA

Kristin Gustafsson
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ABSTRACT

Mantle cell lymphoma (MCL) is a malignant B-cell lymphoma that affects older individuals and has a male predominance. MCL has one of the worst prognoses among lymphomas and currently there is a search for a curative therapy. This thesis focuses on the possibility to induce cannabinoid receptor mediated cell death in MCL and other malignant lymphomas.

- The effects of cannabinoids on cell fate were investigated in MCL cell lines and patient samples. Nanomolar doses of cannabinoids did not induce growth of MCL cells. Instead, micromolar doses induced cell death and decreased viability and growth of MCL cells expressing the cannabinoid receptors CB1 and CB2 while control cells lacking expression of CB1 remained unaffected. Interestingly, at micromolar doses the cannabinoid receptor agonist anandamide and the antagonist SR141716 additively decreased viability.

- Signaling in MCL after treatment with the cannabinoids Win55 and methanandamide was investigated. The signaling was mediated via both CB1 and CB2 since blocking with nanomolar doses of antagonists prevented apoptosis. The signaling was mediated via de novo synthesis of the second messenger ceramide which caused phosphorylation of the MAP-kinase p38. This was followed by a disruption of the mitochondrial membrane potential and subsequently apoptosis. Signaling via the CB1 and CB2 receptors in MCL led to cell death while normal B-cells were spared.

- The expression of cannabinoid receptors in other B-cell lymphomas was investigated. Using quantitative real-time PCR it was found that 80% of the selected lymphoma samples expressed CB1 and/or CB2. The expression was confirmed at protein level by Western Blot and immunohistochemistry. Further, methanandamide treatment induced cell death in B-CLL cell lines. Importantly, we showed that methanandamide treatment reduced tumor burden in a MCL xenograft mouse model. Thus the cannabinoid system could be a potential target in malignant lymphoma.

- The ceramide metabolism in connection to cannabinoid treatment was studied. Pharmacological inhibition of the enzymes serine palmitoyl transferase, ceramide synthase or dihydroceramide desaturase prior to cannabinoid treatment led to disruption of ceramide synthesis and cell death in MCL. An upregulation of ceramide synthase 3 and 6 mRNA was observed after treatment with cannabinoids. Moreover, the effect of cannabinoid treatment on viability and cell death was potentiated by inhibiting the ceramide metabolizing enzymes sphingosine kinase-1 and glucosylceramide synthase.

In conclusion, MCL and a large percentage of other B-cell lymphomas express functional cannabinoid receptors that can mediate cell death specifically in malignant cells. Cannabinoid receptor mediated cell death in MCL could be potentiated by modulation of ceramide metabolism.
<table>
<thead>
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<th>Description</th>
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<tr>
<td>2-AG</td>
<td>2-arachidonoyl glycerol</td>
</tr>
<tr>
<td>B-CLL</td>
<td>B-cell chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>BCR</td>
<td>B-cell receptor</td>
</tr>
<tr>
<td>BL</td>
<td>Burkitt lymphoma</td>
</tr>
<tr>
<td>CB1</td>
<td>Cannabinoid receptor 1</td>
</tr>
<tr>
<td>CB2</td>
<td>Cannabinoid receptor 2</td>
</tr>
<tr>
<td>CB</td>
<td>Cannabinoid</td>
</tr>
<tr>
<td>CerS</td>
<td>Ceramide synthase</td>
</tr>
<tr>
<td>DEGS</td>
<td>Dihydroceramide desaturase</td>
</tr>
<tr>
<td>DLBCL</td>
<td>Diffuse large B-cell lymphoma</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular regulated kinase</td>
</tr>
<tr>
<td>FAAH</td>
<td>Fatty acid amide hydrolase</td>
</tr>
<tr>
<td>FACS</td>
<td>Fluorescence-activated cell sorting</td>
</tr>
<tr>
<td>FL</td>
<td>Follicular lymphoma</td>
</tr>
<tr>
<td>GCS</td>
<td>Glucosylceramide synthase</td>
</tr>
<tr>
<td>GPR55</td>
<td>G-protein coupled receptor 55</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IS</td>
<td>Internal standard</td>
</tr>
<tr>
<td>JNK</td>
<td>C-jun N-terminal kinase</td>
</tr>
<tr>
<td>MCL</td>
<td>Mantle cell lymphoma</td>
</tr>
<tr>
<td>MZL</td>
<td>Marginal zone lymphoma</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>P-gp</td>
<td>P-glycoprotein</td>
</tr>
<tr>
<td>R-MA</td>
<td>R(+)-Methanandamide</td>
</tr>
<tr>
<td>SK-1</td>
<td>Sphingosine Kinase-1</td>
</tr>
<tr>
<td>S-1-P</td>
<td>Sphingosine-1-Phosphate</td>
</tr>
<tr>
<td>SPT</td>
<td>Serine palmitoyl transferase</td>
</tr>
<tr>
<td>THC</td>
<td>Tetrahydrocannabinol</td>
</tr>
<tr>
<td>Win55</td>
<td>(R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)]</td>
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LIST OF PUBLICATIONS

I. J. Flygare, K. Gustafsson, E. Kimby, B. Christensson, B. Sander
Cannabinoid receptor ligands mediate growth inhibition and cell death in mantle cell lymphoma
FEBS Letters, 2005 Dec 19;579(30):6885-9

II. K. Gustafsson, B. Christensson, B. Sander, J. Flygare
Cannabinoid receptor-mediated apoptosis induced by R(+)-methanandamide and Win55,212 is associated with ceramide accumulation and p38 activation in Mantle Cell Lymphoma

III. K. Gustafsson, X. Wang, D. Severa, M. Eriksson, E. Kimby, M. Merup, B. Christensson, J. Flygare, B. Sander
Expression of Cannabinoid Receptors type 1 and type 2 in Non Hodgkin Lymphoma: Growth Inhibition by Receptor Activation.

IV. K. Gustafsson, B. Sander, J. Bielawski, Y.A. Hannun and J. Flygare
Role for de novo synthesis of ceramide in cannabinoid-induced cytotoxicity in Mantle Cell Lymphoma
Manuscript submitted to Journal of Biological Chemistry
INTRODUCTION

Mantle cell lymphoma (MCL) is an essentially incurable B cell malignancy with poor clinical outcome. MCL is currently treated with combination therapies containing multiple cytotoxic drugs and this causes remission of the cancer but the remission time is short. Based on gene expression analysis in the Affymetrix system we found an upregulation of cannabinoid receptors 1 (CB1) and 2 (CB2) (1). These G-protein coupled receptors had previously been studied in brain and on immune cells respectively (2,3). This thesis focuses on the possibility to induce cell death in MCL via the CB-receptors.

1. B-CELL NEOPLASMS

B-cell neoplasms comprise 95% of all lymphomas. According to the WHO classification (WHO classification 2001) there are 15 different entities of B-cell lymphomas. The classification of the lymphoma subtype is crucial for determining the optimal treatment for each patient.

The major known risk factors for B-cell lymphomas are immunodeficiencies such as autoimmune diseases, HIV infection, Epstein-Barr virus (EBV) infection or immunodeficiencies induced by medical treatment. In some subgroups of lymphomas the development of disease is driven by infections such as EBV or Helicobacter pylori(4,5).

1.1 The origin of B-cell neoplasms

B-cell lymphomas tend to mimic the normal differentiation stages of maturing B-cells from which they are thought to origin (Fig 1.). Similar to normal B-cells the tumor cells undergo selection for a functional B-cell receptor (BCR) (6,7). The first stage occurs in the bone marrow where the BCR receptor genes should be successfully rearranged. Further, maturation occurs in the germinal center by somatic hypermutation, immunoglobulin class switch and clonal expansion. Gene expression profile studies show that B-cell malignancies seem to be frozen at a particular stage of differentiation (6). The
two tumor entities MCL and B-CLL resemble naïve B-cells (8) but could also be described as cells that have not encountered antigen in the germinal center. In FL a pattern of ongoing somatic mutations has been observed which is similar to normal B-cells in the germinal center (GC) and this suggest that the transforming event leading to development of FL takes place in the GC (9,10). However, the (14;18) translocation characteristic for FL occurs during the VDJ recombination in the bone marrow which would correspond to naïve B-cells (11). The translocation is the first event in the development of FL but further mutations are necessary for progression. Gene expression studies have shown that DLBCL is a heterogeneous group with distinct subtypes. It can occur de novo or as a further progression (transformation) of another lymphoma such as FL or B-CLL. One subgroup of DLBCL corresponded well with the expression profile of normal GC cells. Another subgroup showed correspondence to activated B-cells (9).

Burkitt lymphoma (BL) expresses the GC marker BCL6 and has mutated immunoglobulin genes which are typical features for GC blast cells (12). After maturation in the GC a B-cell can become an antibody secreting plasma cell or a memory B-cell. Gene expression profiling has shown large similarities between B-CLL with unmutated Ig-genes and B-CLL that has undergone somatic mutations and their genetic profile matches that of memory B-cells (13). However, some B-cell neoplasms such as hairy cell leukemia cannot be related to normal B-cell development.

Similar to normal B-cells the malignant cells also depend of a specific microenvironment to proliferate. Follicular lymphoma cells need to interact with follicular dendritic cells and T-cells in the germinal center similar to normal germinal center B-cells.

It has been observed (14) that B-cell neoplasms (FL, MCL) require CD40 stimulation in vitro for survival as do normal germinal center cells(6).

1.2 Transforming events

The characteristics of the cancer cells are evasion of apoptosis, limitless replicative potential and insensitivity to anti-growth signals. This is a result of genetic alterations. (15) In lymphoma the first event is often a translocation but as in most cancers additional events are required for the full development of a lymphoma. Some of the initial
Translocations are very characteristic for the lymphoma entities and are used as diagnostic markers. MCL is characterized by the CCND1-IgH translocation (16-18). BL has a cMYC-IgH translocation (19). Most cases of FL have a BCL2-IgH translocation (11). The above mentioned translocations place a proto-oncogene under the control of immunoglobulin enhancer which leads to constitutive activation of the gene. In DLBCL the transcription factors BCL6 or MUM1 can be overexpressed with effects on cell proliferation and differentiation (12,20). In CLL a deletion in the 17p chromosome leads to a loss of the tumor suppressor gene p53 and other tumor suppressor genes (21). The knowledge of genetic changes in mature B-cell neoplasms can be improved by using gene expression profiling, CGH-arrays and DNA-microarrays (22).

Fig1. The origin of B-cell neoplasms
2. MANTLE CELL LYMPHOMA

MCL comprises 3-10% (17,18) of all non Hodgkin lymphomas and affects older individuals and has a marked male predominance (74%). MCL is a B-cell neoplasm thought to be derived from the mantle zone of the germinal centre in secondary follicles of lymph nodes. Most MCL cases are considered to be aggressive but a subset of cases are indolent and proliferate at a slow rate.

The growth pattern of MCL can be either vaguely nodular, diffuse or resembling an expanded mantle zone. A subset of MCL comprises a blastic subgroup with aggressive progression associated with shorter survival. In 2003 Rosenwald et al built a gene expression based predictor of survival for MCL cases (23). By precise measurements of the expression of genes associated with proliferation it was shown that the degree of tumor cell proliferation is a powerful predictor of survival for MCL (23). This is also supported by studies where less sensitive immunohistochemistry staining of the proliferation marker Ki67 was correlated with survival (24-26). This demonstrates the importance of deregulation of the cell cycle in driving the progression of MCL.

The survival time is approximately 3 years and MCL has one of the worst prognosis among all lymphomas. At the initial treatment, MCL is responsive to therapy but most patients relapse rapidly after treatment and cannot be cured unless heavily treated with immunochemotherapy and autologous stem cell transplantation (27).

2.1 Oncogenic mechanisms of MCL

The genetic marker of MCL is the chromosomal translocation t(11;14)(q13;q32) (28,29) that can be detected in most cases of MCL. The proto-oncogene CCND1 comes under control of the strong IgH enhancer at chromosome14q32. The translocation leads to a constitutive overexpression of the CCND1 (BCL1, PRAD1) gene which encodes for the Cyclin D1 protein. Cyclin D1, which promotes cell cycle progression from G1- to S-phase is normally not expressed in B-cells. This translocation is considered to be the first genetic alteration in MCL. Some cases negative for Cyclin D1 instead express Cyclin D2
or D3 (30). Cyclin D1 can also have two transcript lengths referred to as the short and the long form (31). The short form does not express the 3' end AU-rich elements and is therefore insensitive to degradation. Expression of the short Cyclin D1 transcript is associated with high proliferation (23,28,32).

The translocation of Cyclin D1 is the first event but not sufficient to drive the tumor progression and aggressive course of MCL. The most common secondary cytogenic events in the development of MCL are deletions in the 11q22-23 chromosome region where the ataxia-telangiectasia mutated (ATM) gene is encoded. The ATM gene encodes for a protein kinase which belongs to the phosphatidylinositol kinase 3 superfamily and has a central role in the response to DNA damage in the cell (33). The mutation causes a defect in DNA damage response, which results in genomic instability in MCL including genetic changes such as aberrations, losses, gains and high copy numbers of certain chromosome regions. Oncogenic events among them INK4a/ARF locus deletion and p53 mutations or protein overexpression can be predictors of survival for MCL (34-38).

Global gene expression array is a powerful tool to obtain more knowledge of the molecular pathogenesis in MCL. Our group among many others has found alterations in genes involved in proliferation, apoptosis, signaling pathways and differentiation (1,23,39-42). In our group we have focused on genes overexpressed in MCL compared to reactive lymph nodes, among them the neuronal transcription factor SOX11, the key enzyme in leukotriene synthesis 5-lipoxygenase and cannabinoid receptor type 1 and 2 (1).
3. THE CANNABINOID RECEPTORS

3.1 The endocannabinoid system

The endocannabinoid system is comprised of the two cannabinoid receptors CB1 and CB2. These two G-protein coupled receptors are found on the cell surface in various cell types. The human body produces endogenous ligands, endocannabinoids that bind to these receptors. The two most studied endocannabinoids are anandamide and 2-arachidonoyl glycerol (2-AG) which are synthesized from phospholipid precursors. The endocannabinoids can act on target cells via autocrine or paracrine signaling (Fig 2.). Anandamide was first discovered in tissues in the laboratory of Raphael Mecholam (43). How the uptake of anandamide into the cell occurs is still unknown (44-46). There is a theory about an anandamide transporter but the protein is not yet isolated (45). In contrast to this suggestion, there are studies supporting transport via endocytosis (47,48). It has been established that FAAH plays a role in transport by maintaining an inward concentration gradient of anandamide thereby promoting transport via simple diffusion (49). As anandamide has been internalized in the cell it is rapidly degraded by fatty acid amide hydrolase (FAAH) (50). In contrast, the metabolism of 2-AG is not only performed by FAAH but also by monoacylglycerol lipase (51).
Fig 2. The endocannabinoid system
3.2 Cannabinoids

Before the endocannabinoids were first discovered the derivate from the marijuana plant, *cannabis sativa* Δ-9-tetrahydrocannabinol (THC) was described. THC is a psychotropic agent and was observed to bind to brain tissue. Subsequently, several phytocannabinoids were discovered and pharmaceutical companies started to develop chemical analogues to both phytocannabinoids and endogenous cannabinoids. Win55 which belongs to the substance group aminoalkylindoles is a non-selective agonist for CB1 and CB2 receptors. There is also a group of compounds classified as eicosanoids which anandamide and analogues of anandamide such as R-(+)-methanandamide belong to. Properties such as high stability after administration and four-fold higher binding affinity for the CB1 receptor than anandamide are the advantages of this agonist (52). However, in most pharmacological studies, agonists that bind equally well to both CB1 and CB2 such as THC, HU-210 and Win55 have been used (53).

Several antagonists to the two cannabinoid receptors have been developed. For the CB1 receptor the antagonist SR141716 (54) and the analogues AM251 and AM281 are equally efficient (53) SR141716 effectively reverse effects mediated via CB1. However, recently it was observed that SR141716 can block the newly identified GPR55 receptor (55,56). In several in vivo studies with obese rodents it has shown to have appetite-reducing effects by blocking the gratification signals via CB1 in the brain (57,58). SR141716 (Acomplia or Rimonabant) has shown successful results in obese patients in clinical trials (59). At higher micromolar doses SR141716 can act as an inverse agonist (60,61) reversing the constitutive activity of the CB1 receptor. It seems SR141716 has a low concentration range where it works as a “neutral” agonist and at higher concentrations it exhibits inverse agonist properties. For CB2, SR144528 is a potent antagonist (54,62) which can also act as an inverse agonist.
Working with cannabinoids is a challenge because of their lipophilic properties. Studies have demonstrated that anandamide readily bind to albumine in fetal bovine serum (63). Therefore studies are often performed in media containing lower serum concentrations.

### 3.3 Cannabinoid receptors

The first cannabinoid receptor was visualized by radioactive labeling in 1989 and later it was named Cannabinoid receptor type 1 (CB1) (2). Before the receptor was visualized Howlett et al had already discovered that adenylyl cyclase could be inhibited by cannabinoids and observed that these effects were coupled to G-proteins (64). Further, Howlett showed that the Gi subunit was involved in this process. The fact that the receptor was part of a G-protein superfamily was clear (65). The CB1 receptor was cloned (2) and because of its pre-dominant expression in the central nervous system it was denoted the central cannabinoid receptor. Later it has been shown that the CB1 receptor is expressed in other tissues such as pituary gland, immune cells, vascular endothelium, the gastrointestinal tract and reproductive tissues (66,67).

Subsequently the second cannabinoid receptor (CB2) was discovered (3). Initially and consequently it was denoted the peripheral cannabinoid receptor because expression was detected in macrophages and the marginal zone of spleen (3). However in 2005 CB2 was found on brain stem cells (68). The CB2 receptor has a 44% homology of the total protein with CB1 (69). There are also two known splice variants to the CB1 receptor CB1a and CB1b (70). Pharmacological studies have shown that both splice variants have increased binding affinity for Win55 and HU-210 compared to the full-length CB1 receptor (70).

In CB1 knockout mice a significant binding of cannabinoids in rat brain was observed which suggested that there was a yet uncloned novel cannabinoid receptor (71). Thereafter came several reports that suggested a non-CB1 non-CB2 receptor in brain (72-74). In addition the presence of a novel CB receptor was described in vascular
endothelium, vascular smooth muscle and in the immune system. Ryberg et al cloned the GPR55 receptor (55) which is expressed in all the tissues mentioned above providing new possibilities for researchers working with CB receptors. Further evidence for GPR55 as a novel cannabinoid receptor was provided by treating cells with several cannabinoids and detecting signaling through the Go13 subunit of G-proteins (55). This indicates that GPR55 should be recognized as the long-awaited cannabinoid receptor 3.

4. CANNABINOID RECEPTOR SIGNALING

The CB1 receptor is classically associated with signaling in neuronal synapses(75). When Ca²⁺ levels are elevated, biosynthesis of endocannabinoids is triggered. Cannabinoid signaling has been studied in great detail in brain. Here the focus has been cannabinoid signaling involved in cell fate.

4.1 Regulation of mitogen activated protein kinase

There are several MAP kinases among them p38, c-jun N-terminal kinase (JNK) and extra cellular regulated kinases (ERK) which can determine cell fate. MAP kinases studied in relation to the cannabinoid system often induce pro-apoptotic or anti-proliferative effects (76). It is clearly established that ligation of CB receptors regulates MAP kinases but the response is specific for the system studied (Fig 3).

Activation of MAP kinases via the CB1 receptor has mostly been studied in neuronal cells, tissue and in vivo models. ERK signaling usually induces proliferation while a prolonged ERK activation induces apoptosis. In neuronal cells regulation of ERK and JNK via CB1 was induced by cannabinoid treatment (77,78). Studies in ex vivo brain tissue have shown that the cannabinoids CP55,940, anandamide, 2-AG and Win55 can induce ERK phosphorylation (79,80). There are also studies showing that CB1 stimuli can induce p38 activation in brain tissue (79). In contrast to the observations above
cannabinoid induced downregulation of phosphorylated ERK and/or Akt led to activation of Bad and subsequently apoptosis in glioma cells (81).

The mechanism of induction of MAP kinases via CB1 is not fully elucidated. PI3K signaling via PKB to trigger MAP kinases (82,83). Sanchez et al observed that sphingomyelin activation and generation of ceramide could activate MAP kinases (84).

MAP kinase signaling via CB2 has been investigated in immune cells and in lymphatic/leukemic cells. MAP kinases ERK or p38 are regulated via CB2 in leukemia cells and splenocytes (85) (86). MAP kinases have also been implicated in immune responses in lymphocytes from spleen and blood cells (85,87). The mechanisms observed for MAP kinase activation via CB2 are also PI3K signaling and ceramide accumulation. In dendritic and lymphoma cells both CB-receptors are necessary to regulate MAP kinases (88,89).
Fig 3. Cannabinoids and cell signaling
5. CANNABINOIDS AND CANNABINOID RECEPTORS IN CANCER

In some countries cannabinoids are used as palliative treatment for cachexia, emesis, pain and appetite stimulation (90). The two cannabinoids nabilone and dronabinol are licensed for clinical use for these purposes. In 1975 anti-proliferative effects on tumors were observed after in vivo treatment of Lewis lung carcinoma with tetrahydrocannabinol (91), but it was not until 10-15 years any real interest for the field was observed. Since the early 1990’s great efforts have been put into investigating anti-cancer effects of cannabinoids (92). Cannabinoids can act directly on the malignant cells via cell signaling resulting in cell cycle arrest, induction of apoptosis, anti-angiogenic effects and reduced migration. Cannabinoids have also been shown to act indirectly through other receptor systems or molecules to prevent cancer progression (93-95).

5.1 Cannabinoid receptor expression in cancer compared to normal cells

Increased expression of the CB1 and/or CB2 receptors have been reported in malignant lymphoma, acute myeloid leukemia, breast cancer, hepatocellular carcinoma, thyroid cancer, melanoma, pancreas and prostate cancer compared to their normal counterparts (39,96-101). High expression levels of the CB1 receptor is related to poor prognosis in prostate cancer (Fowler et al, CB meeting abstract). The higher expression of CB-receptors in malignant tissue makes it possible to target the cancer cells while sparing the normal cells making cannabinoid therapy attractive. Reports have also shown that cancers lacking expression of the CB-receptors are not affected by cannabinoid treatment (102).

5.2 Direct effects of cannabinoids in cancer

In colon cancer, glioma, T-cell lymphoma and leukemia ligation of THC to CB1 activated BAD via de-phosphorylation of ERK and Akt which led to apoptosis (81,103,104). Cannabinoid administration in pancreatic cancer led to an activation of p8 and induction of ER stress subsequently resulting in apoptosis (98). In lymphoid cancers of T-cell origin e.g. T-lymphoblastic leukemia cannabinoid action is dependent of CB2 (105). In the T-cell line Jurkat signaling via the CB2 receptor induced p38 activation and consequently cell death (86).
5.3 Indirect effects of cannabinoids in cancer

Cannabinoids can act on the CB1 receptor to regulate other receptors or growth factors which in turn lead to cancer inhibition. Breast cancers express the prolactin receptor and are dependent on prolactin for their growth. In breast cancer cell lines that express the prolactin receptor, cannabinoids were shown to downregulate the receptors which, in turn induce growth-arrest (93). Cannabinoids have been observed to downregulate the EGFR receptor in prostate cancer (94) as well as the androgen receptor and prostate specific antigen (PSA) which in turn induces apoptosis (95).

Tumor development and growth is dependent on angiogenesis (106). Angiogenesis is induced by pro-angiogenic factors such as vascular endothelial growth factor (VEGF). Many cancer types produce VEGF as a response to hypoxia. Cannabinoids have been shown to be able to suppress angiogenesis and formation of new vessels. In thyroid, glioma and skin cancer cell lines cannabinoids inhibit the production of VEGF (107,108). In vivo treatment with cannabinoids can directly inhibit vessel formation.

Cannabinoids can also exert their anti-cancer effects independently of receptor interaction. It has been suggested that anandamide can interact with lipid-rafts (cholesterol/lipid rich microdomains) in cholangiocarcinoma (109). Independent of receptor binding methanandamide induced upregulation of COX-2 which in turn resulted in cell death in neuroglioma cells (110,111).

In contrast to these findings there is evidence that lower doses (nM) of cannabinoids can induce pro-proliferative effects in cell lines derived from bladder carcinoma, glioblastoma, astrocytoma, kidney cancer and lung cancer (112). The effects were induced by THC, anandamide and Win55, and TACE mediated transactivation of the EGF receptor which subsequently led to growth factor cleavage.

The first human clinical study has been performed by Guzman et al (113). Here THC was administered intra-cranially in glioma multiforme patients (113). The data showed that administration of THC to the patients had a fair safety profile and that THC exerted anti-
proliferative effects. In summary, there is an intense exploration of the cannabinoid system as a potential target in cancer treatment.

6. SPHINGOLIPIDS

Sphingolipids are composed of an amide-linked fatty acid and a sphingosine base. During the 1940’s when fatty acids were first discovered, stearic acid (C:18) was thought to be the major fatty acid in sphingolipids. With the development of new technologies it has become clear that the fatty acid can have varying chain length ranging from C14-C32 (114). Until recent years they were regarded as regulators of the fluidity of membranes and especially of the microdomains lipid rafts. During the last two decades it has been discovered that sphingolipids are involved in cell signaling, inflammatory responses, cell fate and cancer biology.

6.1 Sphingolipid metabolism

Ceramide is a minor sphingolipid and comprises approximately 2% of the total sphingolipid content in the cell. Despite this ceramide is the central molecule in sphingolipid metabolism. Ceramide can be produced through two major pathways de novo ceramide synthesis and sphingomyelin degradation (Fig 4.) (115). Sphingomyelin degradation is hydrolysis of a sphingomyelin by the enzyme SMase which yields ceramide.

In the de novo ceramide pathway several enzymes are involved the first being serine palmitoyl transferase (SPT) which catalyses the conversion of L-serine and palmitoyl-CoA into 3-ketosphinganine (116). The 3-keto group is cleaved off by the action of ketosphinganine reductase. Thereafter, sphinganine is acylated to dihydroceramide by dihydroceramide synthases (dihydro CerS). The dihydroceramide is converted to ceramide through desaturation by dihydroceramide desaturase (DES) (117). The subspecies ceramide C:16 has shown selectivity for formation through activation of the de novo synthesis pathway while other species are not selective in their formation (115).
SPT has been regarded as the key enzyme regulating the de novo ceramide synthesis (120,121). But a recent publication is pointing towards a new regulating role for ceramide synthases (CerS) (122). There are six different ceramide synthases (CerS), CerS 1-6 (with isoforms) which regulates the carbon length of the ceramide synthesized (114). To date this enzyme system is not thoroughly investigated.

Ceramide can be metabolized into sphingosine by ceramidases (CDases) followed by phosphorylation executed by sphingosine kinases (SK’s) to sphingosine-1-phosphate (S-1-P) and further into ethanolamine-1-phosphate and C-16 fatty acids by S-1-P lyase. This pathway can be reversed when complex sphingolipids are recycled through the action of CerS and is known as the salvage pathway (123).

Ceramide can also be metabolized by glucosylceramide synthase (GCS) into glucosylceramide and further into lactosylceramides and complex glycosphingolipids. The work with the ceramide metabolism has been simplified by pharmacological inhibitors developed towards the enzymes involved in sphingolipid metabolism (Figure 4). The specificity of the inhibitors has to be regarded when interpreting data since some of them can act on more than one enzyme. Moreover, investigating the ceramide system can be complex since some enzymes are active in more than one pathway.
Fig 4. Pathways in ceramide metabolism
6.2 Sphingolipids in cancer

The biological functions of sphingolipids are specific and some subspecies can contribute to tumor regression or tumor progression. Ceramide can regulate cancer growth and development by induction of cell death, cell cycle arrest and senescence. A recent study has also shown that dihydroceramide accumulation after fenretinide treatment can regulate the proliferation of neuroblastoma cells (124). In contrast, the enzymes S-1-P and glucosylceramide synthase are associated with proliferation of tumor cells and multidrug resistance respectively.

There is evidence of altered endogenous levels of sphingolipids and their synthesizing enzymes in cancer cells and tissues. Ceramide levels are lower in ovarian cancer and lung cancer compared to their normal counterpart (125). Ceramide is a molecule strongly associated with apoptosis, in cancer. In the first studies where exogenous short chain ceramides or ceramide precursors such as sphingosine was observed to work anti-proliferative through cell cycle arrest or apoptosis (126). Various stressors can also promote ceramide accumulation in the cell.

When the BcR was cross-linked in lymphoma cells there was an upregulation of ceramide C16 which led to apoptosis (119). During androgen ablation in prostate cancer there was also an increase in ceramide C16 followed by cell death (127,128). Stimuli such as Fas ligand, daunorubicin, etoposide, irinothecan, Ara-C and irradiation also induce ceramide accumulation in cancer treatment (118,129,130) Cannabinoids can stimulate ceramide accumulation through both de novo synthesis and sphingolipid synthesis in glioma, pancreatic cancer and lymphoma which leads to apoptosis (89,98,131) and decreased invasiveness.

As ceramide is converted to S-1-P by the action of SK-1 the balance is shifted to promote tumor growth by inducing proliferation, angiogenesis, transformation and migration (Fig 5.). SK-1 and or S-1-P are upregulated in various human cancer tissues compared to their normal counterpart (132). In primary cancers from colon, breast, ovary, kidney and uterus there are higher levels of sphingosine kinase-1 compared with normal controls.
In parallel with these data breast cancer and glioblastoma with increased S-1-P formation show promoted invasiveness and tumorigenesis. Moreover, a recent study by Bayerl et al showed a correlation in non Hodgkin lymphoma between high SK1 mRNA and protein levels and clinicopathological features (133). In astrocytomas high expression of SK-1 correlates with shorter survival (134). Further, overexpression of SK-1 has been observed to promote expression of the ABC-transporter P-glycoprotein (Pgp) which is a member of the multidrug resistance (MDR) gene family and counteract ceramide formation (135). In MCF-7 cells upregulation of expression and activity of SK-1 via prolactin stimulated cell migration while inhibition of SK-1 by siRNA counteracted the effect. In an erythroleukemic mouse model SK-1 was observed to participate in the transformation of the leukemia from growth factor dependence to independence. Inhibitors to SK-1 have been developed with promising results. The inhibitors SKI I-V developed by French et al showed tumor growth reduction both in vivo (132) and in vitro (136). The competitive inhibitor DMS also inhibits SK-1 but has also shown activity against PKC. The SK-1 inhibitor Safingol has even had beneficial results in clinical trials (137).

In contrast to ceramide, glucosylceramide is associated with pro-proliferation and drug resistance in cancer. In leukemia and breast cancer an increased activity of GCS has shown to induce resistance to anti-cancer agents. Interestingly it has been shown that GCS can upregulate the expression of MDR1 and P-gp (138-142).

Conceptually, targeting sphingolipids to battle tumors could be conducted by inducing pro-apoptotic ceramide accumulation or by inhibiting the conversion to other sphingolipids that clear the system from ceramide and induce tumor progression effects.
Stress stimuli:
Chemotherapy
Cannabinoids
Radiation

Apoptosis, growth arrest

Ceramide synthesis → CERAMIDE
(SMase, de novo)

Glucosylceramide
Drug resistance
MDR-1 expression
P-gp expression

S-1-P
Proliferation
Transformation
Migration
Drug resistance

Fig 5. Sphingolipids and their influence in cancer
7. MATERIALS AND METHODS

7.1 Cell culture

In these studies of MCL, cell lines derived from MCL patients were used. The MCL cell lines Rec-1, JVM-2 and JeKo-1 cell line carry the t(11;14), (q13;q32) translocation (28,143) (144). The cell lines MEC1 and MEC2, both overexpressing Bcl-2 were derived from peripheral blood from a patient diagnosed with B-CLL (145). The cell lines Raji and Namalwa were derived from patients with BL (146,147). Namalwa overexpresses cMyc. U937, a myelomonocytic cell line (148) and SKMM-2, a plasma cell line (149) have been used as control cell lines due to lack of expression of both CB-receptors and the CB1 receptor respectively.
To confirm results in our studies cells from primary MCL tumors were used when available.

7.2 Mouse model

To be able to study MCL in an *in vivo* environment we established an animal model using immunodeficient NOD/SCID mice which lack B-cells, T-cells and NK-cells. The mice were bred at the animal facility at Karolinska campus south. Xenografts were established by subcutaneous injection of the MCL cell line JeKo. Tumors were visible after 3 weeks (*Paper III*).

7.3 Flow cytometry

Flow cytometry enables subpopulations of cells to be identified by labeling with probes or antibodies. We have used flow cytometry to measure apoptosis by double staining with AnnexinV FITC antibody and/or PI/7AAD (*Paper I, II and IV*).
Annexin V staining

As apoptosis is induced in cells the phospholipid organization is disrupted leading to exposure of phosphatidylserine (PS) on the cell surface. When the cells are healthy PS resides on the cytoplasmic side of the cell membrane. PS is recognized by phagocytes which remove the apoptotic cells.

To detect apoptosis in vitro the interaction between AnnexinV and PS can be utilized. To detect apoptosis Annexin V conjugated with fluorescein isothiocyanate (FITC) is used. Propidium Iodide is used to detect necrotic cells with damaged cell membranes. Necrotic cells with permabilized membranes can bind Annexin on the inside of the cell membrane. Necrotic cells stain with both Annexin V FITC and PI, which is excluded from viable and early apoptotic cells. In the absence of phagocytosis late apoptotic cells will label with both FITC and PI which makes it difficult to distinguish them from necrotic cells.

Mitochondrial membrane potential

Mitochondria play a critical role during apoptosis since release of Cytochrome C is necessary for the activation of caspase-9 and subsequently effector caspases are activated. Collapse of the mitochondrial membrane is associated with early apoptosis. However there are strong indications that the mitochondrial membrane potential can be restored for some time after release of Cytochrome C when caspases are activated in the cell.

To detect changes in the mitochondrial membrane potential a lipophilic cationic probe with a conjugated fluorochrome such as DiOC6 can be used. When live cells are incubated with the probe it accumulates in the mitochondria due to the attraction of the positive membrane potential. Measuring the intensity of staining by flow cytometry reflects the mitochondrial membrane potential.

7.4 Quantitative real time PCR

Real time PCR is a widely used method to quantify gene products. The method is a powerful tool with high sensitivity and demands little processing after amplification. We
have used the ΔCt-method ($2^{- \Delta \Delta C_t}$) measuring SYBR green incorporation. β-actin was used as the endogenous control (Paper III and IV).

7.5 Western Blot

Western Blot is a semi-quantitative technique used to detect proteins according to molecular size. The proteins are separated by electrophoresis on a SDS-polyacrylamide gel. Subsequently the proteins are transferred onto a PVDF-membrane. The membrane is exposed to a primary antibody which should bind to the protein of interest. Thereafter a secondary antibody (immunoglobulin) conjugated with horse radish peroxidase (HRP) is applied. The reaction with HRP-conjugated antibody and a chemiluminescent substrate produces luminescence that can easily be detected by photography film or an imaging instrument (Paper I and III).

7.6 High performance liquid chromatography/Mass Spectrometry HPLC/MSMS

HPLC /MSMS is a powerful technique to separate and detect lipids. Moreover, it can with accuracy be used to detect subspecies of the same lipid. The technique was used in collaboration with the core facility at the Medical University of South Carolina (MUSC), South Carolina to detect ceramide and other sphingolipids (Paper II and IV)

Liquid chromatography

In liquid chromatography the analytes are separated according to their polarity. The analytes are passed through a stationary phase where molecules which are unpolar will bind to the unpolar column whereas polar molecules will have difficulty to attach. A mobile phase is used to transport the analyte through the column, by changing the polarity in the mobile phase the analytes will be eluated by order of polarity.

Mass spectometry

The mass-spectrometer is used to identify the analytes eluated from the HPLC. The advantage is identification with simultaneous mass determination. The mass-spectrometer is built up by three components; an ion-source, an analyzer and a detector. In the ion-
source the molecules in the sample are ionized by electrospray ionization (ESI). The mobile phase containing the analyte is introduced to the ion-source through a capillary. In the ion-source an electrical field is applied to give current which enables the formation of a “steam cloud”. The vehicle that the analyte is dissolved in will evaporate in the ion source which brings the charged ions of the analyte closer to each other. The ions are subsequently transported into the analyzer where they are chosen by mass and charge (m/z). The analyzer contains three quadruples where each quadruple utilizes four rods with current attached to create an electrical field. The first quadruple has affinity for ions of the determined mass (scanning over a specific mass area). Varying the current over the electrical field will let ions with different m/z pass. The second quadruple is a collision cell where the analytes are fragmented. The third quadruple is calibrated to ions of selected m/z which increases selectivity.

Quantification of sphingolipids
The quantification is performed by using an internal standard (IS). The IS is the same as the molecule of interest but it is labeled with a radioactive substance. The IS is eluted simultaneously with the analyte and they should have the same chemical and physical characteristics while not being able to react together. The IS is used to find the connection between the response from the instrument and the analyte by using known concentrations of the IS. A calibration curve is set up and by using the IS and the amount of analyte can be determined.
8. AIM OF THE THESIS

The overall aim of the thesis was to study the cannabinoid receptors as potential targets for induction of cell death specifically in mantle cell lymphoma while sparing the normal immune cells.

1) To study the growth, viability and cell death response in MCL cells after short and long term exposure to cannabinoids (Paper I).

2) To delineate the signaling pathway induced by CB-receptor ligands at doses generating cell death in MCL (Paper II).

3) To study the expression and function of cannabinoid receptors on other malignant B-cell lymphomas. To determine the effect of cannabinoid treatment in a xenograft in vivo model (Paper III).

4) To study the ceramide metabolism in MCL after cannabinoid treatment and to potentiate the cytotoxicity of cannabinoids by preventing conversion of ceramide into non-toxic, growth promoting sphingolipids (Paper IV).
9. ETHICS

All Studies were performed in accordance with ethical permissions Dnr: 689/03 (Paper I and Paper II), Dnr: S69-04, Dnr:S81-03, Dnr: S222-04, Dnr: S107-06, Dnr:S79-03, Dnr:S80-03 (Paper III). In Paper IV no ethical permission applies since it is based solely on cell line results.
In this study we have investigated if treatment with cannabinoid receptor ligands caused decreased viability and cell death in MCL cells. First we determined that treatment with anandamide or Win55 resulted in decreased viability in both primary MCL and in the MCL cell line Rec-1. The effect of cannabinoids was increased as the serum concentration was reduced. This could be explained by the lipophilic nature of the cannabinoids, which readily associate with albumin a component in serum. We further studied the effects of simultaneous treatment with the CB1 specific antagonist SR141716 and the CB1/CB2 agonist anandamide in MCL cells. At a low dose SR141716 slightly reversed the decreased viability induced by anandamide. In contrast, at a higher dose SR141716 together with anandamide had an additive negative effect on viability. By using the plasma leukaemia cell line SK-MM2, which does not express CB1, we analyzed if the effects on viability were mediated via CB1. Anandamide significantly reduced the viability of MCL cells while SK-MM2 remained unaffected. This suggests that some parts of the immune system could be spared in a future therapy for MCL using cannabinoids.

Next the effect of cannabinoids was investigated on long-term growth in presence of serum in the MCL cell line Rec-1. The Rec-1 cells were treated with a low and a higher dose of Win55 and SR141716. One micromolar of Win55 reduced growth substantially, whereas a 10-fold higher dose of SR141716 was required to cause growth reduction. In
contrast to previous studies on other cancer types there was no increased growth rate in Rec-1 cells upon treatment with nM doses of Win55.

To elucidate whether the treatment with Win55 induced apoptosis, cells were treated for 24 hrs, labelled with Annexin V PI and analyzed by flow cytometry. Our results show a moderate induction of apoptosis in the treated cells compared to the untreated control. The rate of apoptotic cells increases if the cells are serum starved prior to treatment (data not shown). The induction of apoptosis can partly explain the growth inhibition, but other factors e.g. cell cycle arrest could also contribute. These results indicate that cannabinoids can effect viability, growth and induce apoptosis in MCL.
Cannabinoid receptor-mediated apoptosis induced by \( R(+) \)-methanandamide and Win55,212 is associated with ceramide accumulation and p38 activation in Mantle Cell Lymphoma


After determining that cannabinoid receptor agonists induce growth suppression and apoptosis in MCL cells (150) the aim of the second paper was to determine the role of the receptors and to investigate the signalling pathway leading to apoptosis after treatment with cannabinoids. We studied the mechanisms of induction of apoptosis by the endocannabinoid analogue R-MA and the synthetic cannabinoid Win55 in an MCL cell line and primary MCL. We used two different agonists, R-MA, which displays higher affinity for CB1 than for CB2 and Win55, which binds to CB1 and CB2 with similar affinities. To confirm the role of the receptors we treated with nM doses of SR141716 or SR144528, specific inhibitors of CB1 and CB2 respectively. The study showed that ligation of both receptors is needed to induce activation of caspase-3. Do et al has previously shown that ligation of both receptors is necessary to induce cell death in dendritic cells (88). Normal B-cells from buffycoats and tonsil that lack CB1 and the control cell line U937, which lacks CB1 and CB2, were unaffected by the treatment with R-MA and Win55.

Further, the signal transduction was investigated in the MCL cell line Rec-1. Pre-incubation with inhibitors showed that phosphorylation of MAPK p38 was involved in the signal transduction leading to apoptosis. Most studies performed in neuronal cells show that growth inhibition signalling goes through ERK/Akt regulation (81). Treatment
with Win55 and R(+)-MA induced ceramide accumulation in Rec-1 but not in normal B-cells.

Pharmacological inhibition of the de novo pathway of ceramide synthesis led to a disruption of signalling, as p38 phosphorylation and depolarization of the mitochondrial membrane were abrogated. In contrast the pan-caspase inhibitor z-VAD did not protect against mitochondrial membrane depolarization. Taken together the results indicate a signalling pathway where ligation to both the CB1 and the CB2 receptor leads to ceramide accumulation, activation of p38, depolarization of mitochondrial membranes and finally caspase activation. Apoptosis was induced in primary MCL but not in normal B-cells, which suggests that targeting the cannabinoid receptors could be used as a possible future target in mantle cell lymphoma.
In the first two papers the expression and function of the cannabinoid receptors were restricted to MCL. We further wanted to determine if the CB receptors were expressed and functional in other B- cell lymphoma entities. This study was also extended by analysing the expression of the CB1 receptor splice variants. The project was taken to a new level when the results from in vitro studies were translated into in vivo by treating xenografted mice with a cannabinoid.

In order to investigate the expression of cannabinoid receptors 1 and 2 in B-cell malignancies a panel of patient samples (n=62) containing the entities B-CLL, MCL, FL, DLBCL, BL, MZL and pre –B ALL was compiled and analyses was performed using quantitative RT-PCR. The majority of the cases examined had higher expression of CB1 and/or CB2 compared to control tissue. The only entity that showed uniform upregulation of the CB receptors was MCL while the other entities showed high variability in expression. There was a moderate correlation between expression of CB1 and CB2 in our lymphoma panel. Gene expression data in the Omnibus database support our data showing a variable expression of CB1 and CB2 in FL and of CB2 in DLBCL. Further, investigations of the expression of the splice variants CB1a and CB1b was performed. CB1a could be detected in 44% of the cases while we did not succeed to detect CB1b.

Western Blot and immunohistochemistry was performed in samples representing different entities. Moreover, function of the receptors was investigated by using cell lines
derived from BL (Raji, Namalwa) B-CLL, (MEC1, MEC2) MCL, (Rec-1) and plasma cell leukemia (SKMM-2). The cell lines were pre-incubated with the antagonists SR141716 or SR144528 binding to CB1 and CB2 respectively before incubation with the stable endocannabinoid analogue R-MA. Interestingly, R-MA only induced cell death in MCL and B-CLL cell lines while BL cell lines remained unaffected.

Further, we xenografted mice with human MCL cells which was followed by in vivo treatment with R-MA. The mice treated with R-MA showed a 40% decrease in tumour burden and a 25% reduction in mitotic index compared to mock treated mice. Taken together, these data showed that treatment with cannabinoids induced cell death and tumour regression malignant lymphomas.
13. PAPER IV

Role for de novo synthesis of ceramide in cannabinoid-induced cytotoxicity in Mantle Cell Lymphoma

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The last manuscript is a continuation of paper II where ceramide accumulation was detected after cannabinoid treatment of MCL cells.

The MCL cell line Rec-1 was treated with the stable endocannabinoid analogue R-MA in order to investigate the pathways involved in ceramide accumulation further. After treatment with R-MA a time dependent increase of total ceramide was detected. Accumulation of ceramide subspecies were measured by HPLC MSMS after 12 hrs, and accumulation of ceramide C16, C18, C24 and C24:1 was observed. To prove that the accumulation of ceramide was mediated via ceramide synthesis de novo and not through the salvage pathway, three enzymes in the de novo pathway were pharmacologically inhibited. The enzymes serine palmitoyl transferase, ceramide synthase (CerS) and ceramide desaturase were inhibited with myriocin (151), fumonisin B1(152) and C₈-CPPC (153) respectively. Inhibition of either enzyme led to abrogation of the ceramide synthesis and cell death, which confirms that ceramide is produced via the de novo synthesis pathway. Further, when MCL cells were incubated with R-MA and the expression of CerSs 1-6 was examined an upregulation of CerS3 and CerS6 was observed.

To potentiate the cell death induced by R-MA we further inhibited the conversion of ceramide into the tumor promoting sphingolipids sphingosine-1-phosphate (S-1-P) and glucosylceramide. Following inhibition of the enzymes sphingosine kinase-1 and glucosylceramide synthase, that convert ceramide to S-1-P and glucosylceramide respectively, the effects of R-MA on cell death and viability were enhanced. These
results were confirmed by RNA interference against SK-1 or GCS in combination with R-MA. This is the first successful combination of cannabinoid treatment with ceramide metabolism inhibitors.
14. CONCLUSIONS
This thesis work investigated the expression of CB-receptors on MCL and other B cell malignancies and explored the possibility to selectively induce cell death via receptor ligation with cannabinoids. Further the signaling and the ceramide metabolism after treatment with cannabinoids was explored.

Cannabinoids bound to both the CB1 and the CB2 receptor on MCL cells and induced growth inhibition and cell death. Signaling leading to apoptosis was mediated via the second messenger ceramide which induced phosphorylation of p38. Downstream of p38, a loss of the mitochondrial membrane potential was induced leading to caspase activation and finally cell death. Importantly, normal B-cells remained unaffected by cannabinoid treatment. Moreover, xenograft MCL tumors were responsive to treatment with the cannabinoid R-MA. In extension we found that the majority of mature B-cell neoplasms expressed CB1 and/or CB2.

Cannabinoids induced ceramide accumulation via the de novo ceramide synthesis, and ceramide synthases within this pathway were upregulated. Modulation of ceramide-metabolizing enzymes by pharmacological inhibitors or siRNA led to a potentiation of R-MA treatment in MCL.

Cannabinoids could be used as part of a possible future therapy in MCL, perhaps in combination with chemotherapy or ceramide metabolism inhibitors. However, there are still many aspects of the effects of cannabinoids in MCL that need to be investigated before the transition from bench to bedside is possible. This thesis is the beginning of investigations regarding the cannabinoid system in MCL.
15. FUTURE PERSPECTIVES

During the last two decades a great deal of effort has been put into investigating the effect of cannabinoids in cancer. The collected data show that cannabinoids can induce cell death, induce growth inhibition and tumor regression. Most cancer treatments consist of multiple drugs working together to eradicate the malignant cells. It would be interesting to study combinations of cannabinoids with other chemotherapeutic drugs.

Cannabinoid-induced signaling in MCL could be investigated in further detail. Which G-proteins are involved? How come both receptors are active during signaling? It would be very interesting to determine if the cannabinoid receptors exert different signals that cooperate at a downstream signaling target. Experimentally this could be done by using an inducible transfection system inducing expression of both receptors in MCL cells.

Based on the results presented in these studies it would be interesting to study the cannabinoid system in more depth in malignant lymphoma in relation to the microenvironment. Do leukemic and lymphatic MCL and B-CLL express similar levels of CB-receptors? Further investigating the function of cannabinoid system in the tumor-microenvironment might provide insight to why cannabinoid receptors are upregulated in malignant lymphoma.

One intriguing result obtained during these studies is the possible regulation of ceramide synthases in cannabinoid-induced ceramide accumulation. The knowledge about the regulating role of ceramide synthases is limited. To investigate their role further, artificial transfection systems could be used.

In view of the observed in vitro potentiation of cannabinoid treatment when adding inhibitors to ceramide metabolism to the regimen similar studies in vivo using our MCL model are warranted. Hopefully a combination of cannabinoids that specifically target the malignant cells and ceramide metabolism inhibitors could be turned into a patient therapy.
Finally, the question remains: Why does mantle cell lymphoma express the CB-receptors? What is the advantage for the lymphoma? Can the cancer cells produce cannabinoids and utilize paracrine or autocrine signaling to promote growth? To find the answers for these questions thorough investigations of microenvironment and measurements of cannabinoid production should be performed.

16. POPULÄRVETENSKAPLIG SAMMANFATTNING

Till min familj och mina vänner som ofta har undrat vad jag egentligen gjort om dagarna under dom här åren.....


Genom att undersöka förändringar hos MCL tumörer och jämföra med normal lymfatisk vävnad hittade vi molekyler som förändrats i cancervävnaden i jämförelse med frisk vävnad. Två av dessa molekyler som förkom i ökade nivåer hos MCL var cannabinoid receptor 1 och 2. Cannabinoid receptor 1 förekommer vanligtvis i hjärnvävnad där dess funktion är att skicka signaler om välbefinnande till kroppen. Cannabinoid receptor 2 återfinns ofta på immunceller. Cannabinoid receptorer fungerar som mottagare av signalmolekyler som kallas cannabinoider. Den mest kända cannabinoiden är den aktiva
komponenten i marijuana som heter tetrahydrocannabinol. Efter dess upptäckt blev många läkemedelsföretag intresserade av att tillverka syntetiska cannabinoider pga deras effekter mot smärta, illamående och viktökning.

När vi upptäckte att dessa mottagar molekyler för cannabinoider fanns i höga nivåer på MCL vävnad uppkom iden att utnyttja detta signalssystem för att behandla MCL. Genom att behandla MCL celler med cannabinoider som binder till mottagarmolekylerna – cannabinoidreceptorerna kan man få cancer cellerna att genomgå en programmerad död (Fig.6). Eftersom mottagar molekylerna bara finns på de sjuka cellerna och inte den friska vävnaden blir behandlingen selektiv för cancer cellerna. Vårt mål med denna forskning är att i slutändan kunna behandla patienter med den här typen av cancer med goda resultat.
Fig 6. Döds signal i mantel cells lymfom
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18. REFERENCES


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