Menthol receptor and menthol use

in the treatment of pain

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Keywords: Menthol receptor, TRPM8 receptor, menthol, pain, neuropathic pain

The menthol receptor (TRPM8) (transient receptor potential melastatin 8) is an excitatory ion channel receptor that is part of the larger TRP receptor family. It is a non-selective ion channel that permeates ions upon activation. The menthol receptor is a polymodal receptor as it is activated by chemical agonists at cool and cold temperatures (8-28°C) and voltage changes. The menthol receptor is expressed in many tissues. It is most abundant in thin peripheral sensory neurons. The TRPM8 receptor is the major receptor for mediating cool and cold sensations in humans.

Menthol is one of the many agonists of the TRPM8 receptor. Menthol is a natural compound obtained from mint plants or can be prepared synthetically. It has many biological effects, some of which have been observed for a long time. Today, menthol is a common ingredient in many daily products such as food, cosmetics and self-care products. Menthol causes a cool sensation on the skin and mucosa through the menthol receptor.

Pain is a subjective, unpleasant feeling that can manifest in many ways. Pain conditions are very common. Pain is divided into acute, subacute, and chronic pain according to duration. It can be classified according to the mechanism of birth into nociceptive (tissue damage pain), neuropathic (nerve pain), pain sensitization and idopathic pain (partly unknown). Pain can be treated with many medications and other means. Because TRPM8 receptors are present in the pain-sensing and mediating system and because menthol has been shown to have analgesic effects, its use in the treatment of pain has been investigated.

Menthol has been found to help with neuropathic pain, which is often difficult to treat. Menthol has also been shown to reduce pain in soft tissue injuries and inflammatory pain conditions. There is currently a conflicting view of the efficacy of menthol in the treatment of migraine.

Overall, menthol has been shown to be an effective and safe way to treat pain, but further research is needed.

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The menthol receptor, TRPM8 receptor (transient receptor potential melastatin 8) is an excitatory ion channel receptor and a member of the TRP family. Menthol receptor is a nonselective and polymodal ion channel which opens through chemical agonists, cool and cold temperatures (8-28°C) and voltage. Menthol receptors are expressed in several tissues. TRPM8 receptor acts as the main mediator of cool and cold sensation in human.

Menthol is a natural compound, monoterpene, found in oils of *Mentha* species plants but is mainly produced synthetically. Menthol has many biological properties and some of those have been discovered over one hundred years ago. Menthol is a common ingredient in several consumer products such as food supplies, cosmetics and pharmaceutical products.

Pain is an unpleasant subjective experience. Pain is a very common symptom and it can occur in several different ways. Acute pain is temporary while subacute and chronic pain last longer. Pain treatment includes both non-medical and medical options. The fact that TRPM8 receptors are expressed in nociceptors and other pathways that modulate pain and that menthol is an analgesic compound has aroused interest in investigating menthol use in pain relief.

Treating neuropathic pain with menthol has shown promising results considering that neuropathic pain conditions are often challenging to relieve. Menthol decreases pain also in soft tissue injuries and inflammatory pain conditions. The effect of menthol on migraine is still controversial. However, some data suggest that menthol can act as an antinociceptive agent in migraine.

On the whole, menthol has the potential to serve as a safe treatment for pain, yet further clinical studies are needed.

ABBREVIATIONS

ACC	Anterior singular cortex Icilin
AG-3-5	
CA.	Cinnamic aldehyde
cDNA	Complementary deoxyribonucleic acid Chemotherapy-induced
CIPN	peripheral neuropathy, nerve damage caused by chemotherapy
CRM1	Cold and menthol-sensitive receptor 1, menthol receptor Delayed
DOMS	Onset muscle soreness, endothelium-derived hyperpolarizing
EDHF	factor
IL	Interleukin
LT	Leukotriene
mGluR	Metabotropic glutamate receptor
WELL	Nitric oxide
NRS	Numerical rating scale, numerical scale for pain
NSAID	assessment Nonsteroidal anti-infammatory drug, NSAID
PD	Pore domain, porous area
PG	Prostaglandin
PIP_2i.e. PI (4,5) P_2	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C enzyme
SD	Sensing domain, tumor
TNF	necrosis factor
TRP	Transient receptor potential Transient
TRPA	receptor potential ankyrin
TRPM8	Transient receptor potential vanastatin 8 receptor, menthol receptor
TRPV	Transient receptor potential vanilloid
VAS	Visual analogue scale, painful
VRS	Verbal rating scale, verbal pain assessment by
WHO	the World Health Organization

Table of contents

1 INTRODUCTION

The menthol receptor, or transient receptor potential melastatin 8 (TRPM8) receptor cDNA, was first cloned in 2001 from prostate cancer (Tsavaler et al. 2001). A year later, its gene was cloned and its function was further characterized. At that time, the menthol receptor was identified from dorsal root and trigeminal sensory neurons (McKemy et al. 2002; Peier et al. 2002). TRPM8 belongs to the broader TRP receptor family and is the major receptor for detecting cold and cool in mammals (McKemy et al. 2002). The menthol receptor is thus a relatively young discovery and research data has refined its structure, location, and function.

In their study, McKemy et al. (2002) found that menthol acts as an effective activator or agonist of the menthol receptor. Menthol affects the so-called. counter-Irritant mechanism; initially, menthol activates nose-receptor TRPM8 receptors, but then desensitizes, i.e., reduces their sensitivity to stimuli (Pergolizzi et al. 2018). Menthol causes a cool sensation on the skin and mucous membranes through the menthol receptor. Menthol is a naturally occurring compound that can be isolated from mint plants. It has been used for hundreds of years for medical purposes, e.g. for the treatment of upper respiratory distress, cough and indigestion (Eccles 1994). Menthol is now widely used in everyday products such as confectionery, cosmetics and cleaning supplies (OARS 2014). Menthol can be added to tobacco to bring in the taste and smell of mint and to increase the comfort of smoking. The WHO has issued a recommendation to ban menthol in tobacco products (WHO TobReg 2016).

Pain is a very common phenomenon that is experienced as an uncomfortable feeling on different parts of the body. It can be divided into acute, subacute, and chronic pain according to duration (Pain KH Recommendation 2015). Based on the mechanism of birth, pain can be divided into nociceptive pain, neuropathic pain and idiopathic pain, pain caused by no tissue or nerve damage (Kosek et al. 2016). Many non-medicinal and medicinal methods are used to treat pain (Haanpää & Pohjolainen 2015, Kipu KH Recommendation 2015).

The analgesic effect of menthol was first observed in 1870 when Wright (1870) found that menthol-containing peppermint oil reduced the incidence of pain in patients with facial pain.

pain sensation. After a few years, menthol was used as a local anesthetic to replace cocaine (Rosenberg 1885). Today, menthol is defined as an analgesic as well as a topical anesthetic (Galeotti et al. 2002). Its effectiveness has been studied in many different pain conditions. In the future, menthol may be a potential and well-tolerated method in the clinical treatment of pain.

This dissertation is a review of the literature looking at the menthol receptor, menthol, pain in general, and the use of menthol in the treatment of various types of pain.

2 RESEARCH TASK

This dissertation is a literature review. The review consists of three different areas, of which a coherent whole is written. The first part deals with the menthol receptor, the second with pain in general, and the third with the use of menthol in the treatment of pain.

The aim is to look at all aspects comprehensively. The structure, location, and function of the menthol receptor are examined. The menthol list describes what kind of substance it is. Pain is treated in general and is described e.g. how pain arises and how it is broken down. The last section explains how menthol works in the treatment of pain and what is the role of menthol in it. The treatment of menthol receptor and pain in the review provides a preliminary section examining the use of menthol in the treatment of pain.

The research hypotheses of this dissertation are:

1) TRPM8 receptor activation relieves pain

2) Menthol relieves pain

3) The analgesic effect of menthol is mediated through the TRPM8 receptor.

Evidence for research hypotheses is sought on the basis of research material and the research task is answered on the basis of it.

3 THEORETICAL BACKGROUND

3.1 Menthol receptors

The menthol receptor belongs to the TRP receptor family, which consists of excitatory ion channel receptors. The TRP family includes more than 30 receptors, which are divided into seven subgroups based on amino acid sequence identity. The menthol receptor belongs to the TRPM (transient receptor potential melastatin) subgroup (Pedersen et al. 2005). It is abbreviated as CMR1 (cold- and mentholsensitive receptor) and TRPM8 (McKemy et al. 2002, Clapham et al. 2001, Peier et al. 2002). The most important role of the menthol receptor is to detect cool and cold temperatures in the environment (Bautista 2007). It is considered to belong to the thermo-TRP group, i.e., temperature-activated TRP receptors together with TRPV1–4 and TRPA1 (Cohen & Moiseenkova-Bell 2014).

When studying the expression of the menthol receptor, it has been found to be expressed in many different tissues. The menthol receptor is expressed in approximately 15% of the thin peripheral sensory nerves (C and Aδ strands) emanating from the skin and oral mucosa (McKemy et al. 2002). These nerves end up in the dorsal root and facial area of the spinal cord in the trigeminal ganglia, which also contain TRPM8 receptors (McKemy et al. 2002). Of these, the action potential is further transferred to the central nervous system. In the central nervous system, menthol receptors are present in the spinal cord, midbrain, and cerebrospinal fluid (Du et al. 2009). A recent finding of menthol receptor expression is from human tooth odontoblasts (Tazawa et al. 2017). There are menthol receptors in the nasal epithelium, mucous membranes and blood vessels, and afferent neurons on the surface of the lower airways (Xing et al. 2008, Liu S et al. 2017). In addition to skin neural structures, menthol receptors are expressed in keratinocytes (Park et al. 2013). TRPM8 has been found in the stomach at least in rats (Mustafa & Oriowo 2005).

The menthol receptor gene is expressed in the prostate and to a lesser extent in the mammary gland and thymus, and occasionally in the lungs. Expression is increased in prostate carcinoma and other malignancies such as melanoma (Tsavaler et al. 2001, Sabnis et al. 2008). In addition to the prostate, expression of TRPM8 has also been observed in the testes, scrotum and bladder urothelium.

(Stein et al. 2004). TRPM8 receptors have also been found in some of the primary afferent neurons in the colon (Harrington et al. 2011). It is also found in white and brown adipose tissue, blood vessels, and sensory neurons in joints (Johnson et al. 2009, Ma et al. 2012, Rossato et al. 2014, da Silva Serra et al. 2016).

Table 1. Tissues and cells expressing the TRPM8 receptor

Tissue	**Source**
C and Aδ nerve fibers in the skin and oral mucosa	McKemy et al., 2002
Dorsal root and trigeminal ganglia	McKemy et al., 2002
The central brain and brain bridge	Du et al., 2009
Ascending and descending pain system and especially sensitized skin	Facer et al., 2007.
Odontoblasts	Tazawa et al. 2017
Nasal epithelium and mucous glands	Liu S et al., 2017
Nasal mucosa	Keh et al., 2011
Afferent neurons of the lower respiratory tract	Xing et al., 2008
Keratinocytes of the skin	Park et al., 2013
Lung epithelial cells	Sabnis et al., 2008
Stomach	Mustafa & Oriowo 2005
Prostate tissue, testicles and scrotum skin	Tsavaler et al., 2001, Stein et al., 2004
Primary afferent neurons in the colon	Harrington et al., 2011
Brown adipose tissue	Ma et al. 2012
White adipose tissue	Rossato et al., 2014
Sensory neurons in the joints	Da Silva Serra et al. 2016
Blood vessels	Johnson et al., 2009
Malignancies (eg melanoma, prostate cancer)	Tsavaler et al., 2001

The menthol receptor is the most important receptor for sensing cool and cold temperatures in humans (Bautista et al. 2007). It is activated mainly at non-painful temperatures (15-28 ° C) but also at painful temperatures (8-15 ° C). Chemical agents, menthol and icilin (AG-3-5), also cause a cool sensation when binding to the receptor (McKemy et al. 2002). The TRPM8 receptor is also involved in body temperature

regulation and temperature-dependent behavior (Almeida et al. 2012). The menthol receptor is believed to be involved in adipose tissue metabolism and thus also in the regulation of energy balance in the body (Rossato et al. 2014). Further studies on the role of the TRPM8 receptor in adipose tissue have shown that activation of TRPM8 in white adipose tissue results in increased expression of thermogenic genes. The same study also found that oral menthol increased the conversion of white adipose tissue to brown fat, and menthol reduced obesity associated with a high-fat diet (Jiang et al. 2017).

Cold can cause analgesia. This pain relief is mediated by the menthol receptor. Menthol receptor-mediated analgesia is caused either by direct inhibition of the nociceptor or through an inhibitory median neuron in the dorsal horn of the spinal cord and trigeminal ganglia (Laing & Dhaka 2016). Decades ago, Bini et al. (1984) found that the reduction in pain induced by a cool stimulus is not only a reaction on the surface of the skin, but also involves central levels. TRPM8 receptors located in the midbrain and brain bridge are also involved in pain regulation at these upper central nervous system levels (Du et al. 2009).

3.1.1 Structure

Like other TRP receptors, the TRPM8 receptor is a homotetramer composed of four similar subunits. These units are further composed of six transmembrane domains (S1-S6) that are structurallya-helices. TRPM8 is a homotetramer, i.e. its monomers are similar. Both the amino and carboxyl termini are located outside the cell (Cohen & Moiseenkova-Bell 2014). The S1-S4 domains form a sensing domain (SD) and the S5-S6 porous domain (PD). SD contains binding sites for menthol and icilin and affects receptor regulation. PD forms a permeable site on the receptor and controls selective permeability (Latorre et al. 2007). At the carboxyl terminus of the menthol receptor is the TRP region (i.e., the TRP box), which is also found in some of the other TRP receptors, e.g. TRPV1 receptor (Laing & Dhaka 2015).

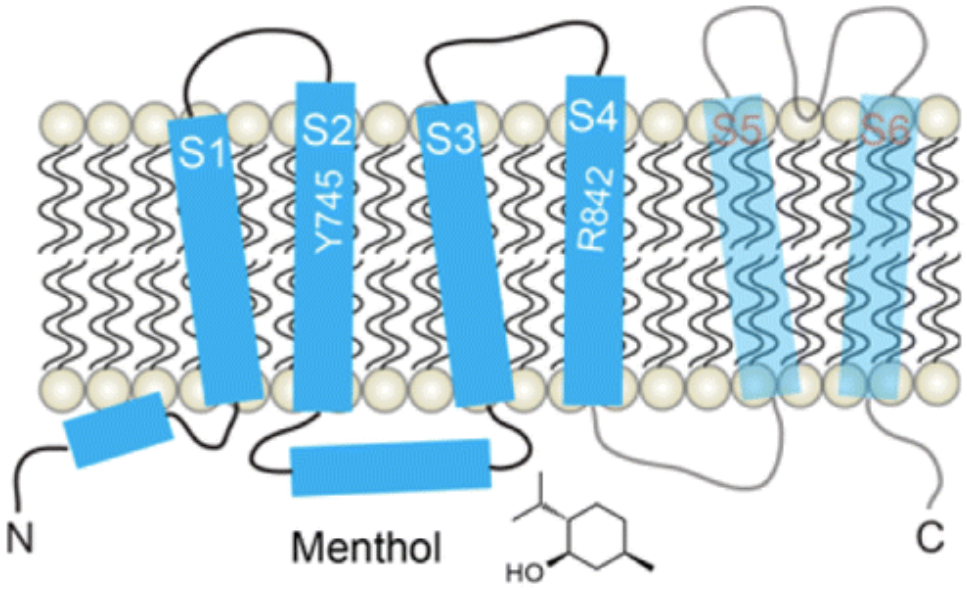

Figure 1. Menthol receptor structure. The cell membrane permeable domains (S1 – S6), amino terminus (N), carboxyl terminus (C), and menthol binding site are shown (Rath et al. 2016).

3.1.2 Operation and regulation

The menthol receptor is e.g. Ca^{2+}-an ion-permeable non-selective ion channel receptor that is activated at certain temperatures (8-28 ° C) as a result of chemical binding or tension. These stimuli cause ion channel opening and ion transport through the receptor (McKemy et al. 2002, Brauchi et al. 2004). There are indications that in addition to small cations, the menthol receptor would permeate even larger molecules when activated by an agonist (McCoy et al. 2017). The opening of a receptor is a complex event in which its conformation changes so that ions can pass through it. The bond between the TRP box and the S6 unit is believed to play an important role in allosteric activation (Taberner et al. 2014). Because the extracellular state of Ca^{2+} concentration is higher than intracellular, the opening of the ion channel causes the flow of ions into the cell. In this case, intracellular free Ca^{2+}concentration rises and causes depolarization in the cell. In the case of a neuron, the resulting action potential travels toward the central nervous system. Ca^{2+}ion also acts as a regulator of intracellular signaling chains in cells (Clapham et al. 2001).

Several agonists have been found for the TRPM8 receptor. Menthol and icilin activate the receptor, causing a strong ion flow through the receptor. Menton and eucalyptol also cause a small change in ion flow (Peier et al. 2002). Like menthol, terpene-borne borneol is a menthol receptor agonist (Wang et al. 2017). Testosterone has been found to activate the menthol receptor (Asuthkar et al. 2014). TRPM8 activator, 8-O-4′-neolignan, has been found in nutmeg (Shirai et al. 2017). Many other chemical compounds (e.g., carboxamide WS-12) have also been shown to act as receptor agonists (Bödding et al. 2007).

Menthol binds to the SD region of the menthol receptor, and the activation of the receptor is then dependent on at least two amino acids in the SD region based on biochemical structure and function studies. These are Y745 in the S2 domain and R842 in the S4 domain. Y745 and R842 are mutated amino acids that have been shown to associate these regions with receptor activation. They modulate the coupling of the SD region and the PD region, which is essential for receptor activation (Rath et al. 2016). It is possible that more than one menthol molecule must bind to the TRPM8 receptor for activation (McKemy et al. 2002). The temperature-responsive portion of the receptor is located at the carboxyl terminus (Laing & Dhaka 2016).

The TRPM8 receptor can be blocked by antagonists or inhibitors. Several such compounds have been found. For example, capsaicin, cannabidiol, and anandamide are menthol receptor antagonists. The receptor also becomes impermeable to ions through desensitization. It can be caused by continuous stimulation of the menthol receptor. $Ca^{2+}$the migration of ions into the cell probably acts as a mechanism for desensitization. Ca^{2+}increase in phospholipase C enzyme (PLC), which degrades PIP_2- molecule. $PIP_2$is essential for TRPM8 receptor function (Pérez de Vega et al. 2016). The menthol receptor can also be inhibited by ethanol and the effect is mediated by PIP_2through (Benedikt et al. 2006). Methylglyoxal inhibits the receptor by a mechanism that is still unclear (Ciobanu et al. 2016).

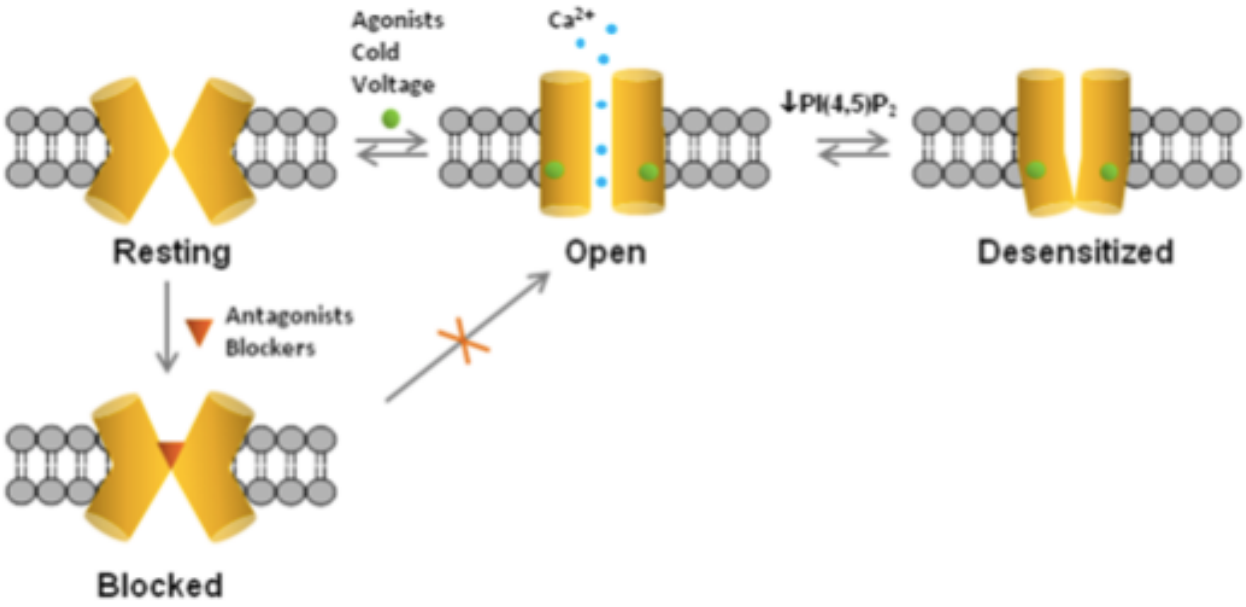

Figure 2. Different states of the menthol receptor. The receptor may be resting, blocked, open, or desensitized (Pérez de Vega et al. 2016).

Many factors regulate the menthol receptor and its activation by various agonists and stimuli. Such are e.g. pH changes and phosphoinositides such as PI (4,5) P_2or PIP_2. A decrease in intracellular pH within the physiological range prevents activation of the TRPM8 receptor by icilin and cold temperature. In the case of menthol, similar inactivation does not occur (Andersson et al. 2004). The menthol receptor is not activated if $PIP_2$is not free (Liu & Qin 2005, Hille et al. 2014).

3.2. Menthol

3.2.1 General list of menthol

Menthol ($C_{10}B_{20}O$, molecular weight 152.27 g / mol) is a cyclic monoterpene alcohol. It occurs mainly as a (-) menthol and less frequently as a (+) menthol. In addition, it has six other isomers. Menthol can be made either synthetically or produced from mint oils. Mint oils are made from mint plants by steam distillation.*Mentha arvensis*- beach mint oil isolated from the species contains 70-80% (-) menthol, while peppermint oil (*Mentha piperita*), it is

50%. Pure menthol is obtained by crystallization from oils. At room temperature, menthol is a crystalline, light or colorless solid. Menthol tastes and smells like peppermint due to small remnants of mint oil. (Eccles 1994)

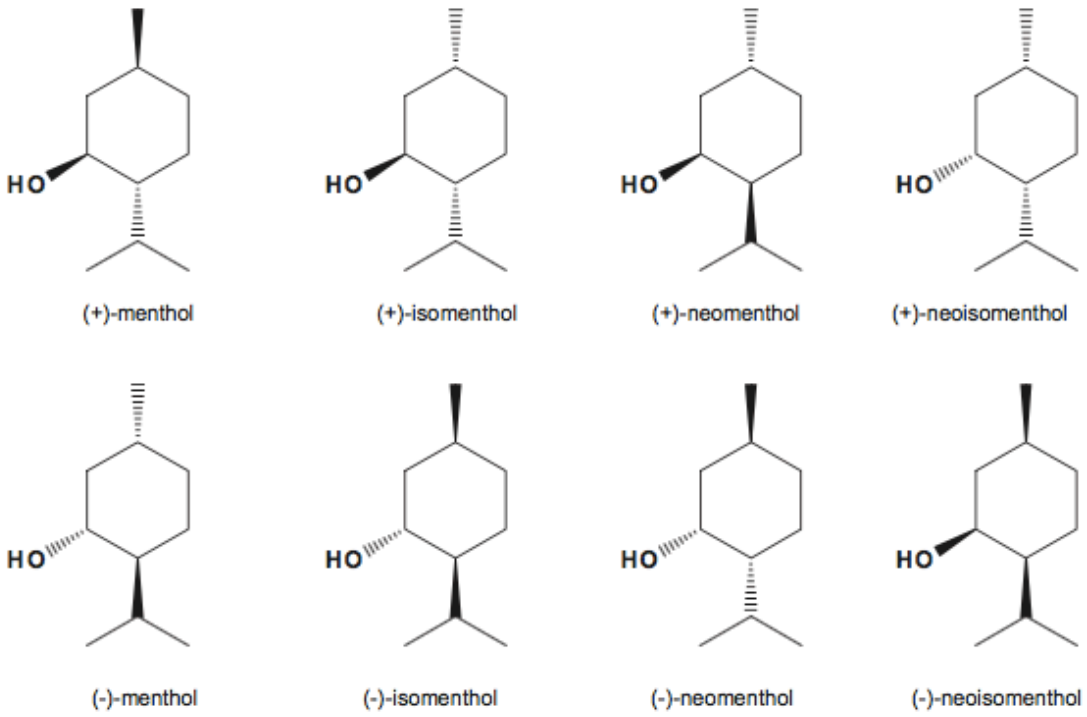

Figure 3. Isomers of menthol. Isomers include menthol, isomenthol, neomenthol and neoisomenthol with mirror image isomers. (Kamatou et al. 2013)

Figure 4. Mint (Mentha spicata).

Menthol is a fat-soluble substance that is thus highly soluble in fat-soluble solvents. Like fat-soluble compounds, menthol is absorbed into the bloodstream when administered orally or transdermally. Most of its metabolism occurs in the liver, where it is first hydroxylated and then incorporated into the glucuronide conjugate. It then becomes a more water-soluble metabolite and can be excreted via the kidneys in the urine. Some of the metabolites of menthol are also excreted in the faeces. (Eccles 1994) Menthol is excreted more rapidly if taken orally than after dermal exposure (OARS 2014).

Menthol has many sites of action in cells. In addition to the menthol receptor, it acts on many other receptors on the cell membrane and regulates intracellular Ca^{2+}concentration. Menthol inhibits e.g. As^{+}- and Ca^{2+}- channels. It activates the TRPV3 and TRPA1 receptors as well as the TRPM8 receptor. It also affects $GABA_A$, serotonin and nicotinic receptors and PLC activity. Through these effects, the electrical function and physiology of the cell change and continue to elicit responses (Oz et al. 2017).

3.2.2 Use and effects

Menthol has many uses and is used e.g. in the manufacture of confectionery, toothpaste, cigarettes, cold gels, detergents, animal care products and cosmetics. It is a common additive and flavoring and fragrance ingredient. The menthol contents of menthol-containing products range from 0.001-6%. Higher concentrations can be used in test setups (OARS 2014). Menthol is used in many products due to its taste and aroma properties, but other effects have been observed. In addition to taste and aroma, a significant property is the cooling effect of menthol (Eccles 1994). Its cooling effect is utilized in various cold gels and sprays, creams and confectionery. It is now known that the cooling effect on the skin and mucous membranes is mediated through the TRPM8 receptor (McKemy et al. 2002).

Menthol has been shown to have an analgesic effect. Of the isomers of menthol, (-) - menthol was found to be analgesic, whereas the same property was not observed for (+) - menthol (Galeotti et al. 2002). It should be noted, however, that the concentration of menthol affects the effect it produces. 40% of menthol applied to the skin caused pain and hyperalgesia to the cold and mechanical stimuli in the subjects (Binder et al. 2011). Menthol acts in part as a counter-Irritant, as it first activates the TRPM8 receptors on nociceptors and then reduces their sensitivity. Decreased sensitivity impairs the passage of the pain stimulus, and menthol applied to the skin by this mechanism acts as an analgesic (Pergolizzi et al. 2018).

The effect against bacteria, fungi and viruses has been studied a lot. Many studies have confirmed that menthol inhibits the growth of bacteria. It also reduces the growth of many different fungal species i.e. menthol is an antifungal agent (Kamatou et al. 2013).*In vitro*- a study examining the effect of peppermint oil on Herpes simplex-1 and Herpes simplex-2 viruses showed that it had a destructive effect on these viruses. It could be assumed that menthol plays a role in the antiviral effect, as peppermint oil contained 42.8% menthol and other isomers of menthol, menthol (14.6%) and isomentone (5.9%) (Schuhmacher et al. 2003). In addition to directly reducing and even killing microbial growth, menthol also suppresses inflammation. When examined for three inflammations

the neurotransmitter leukotriene (LT) B_4, prostglandin (PG) $E_2$and interleukin (IL) 1-b)concentrations, menthol reduced the synthesis of all of them (Juergens et al. 1998).

In 1995, Bromm et al. Demonstrated that histamine-induced pruritus is alleviated by the application of menthol to the skin. Similar results have been observed later (Andersen et al. 2017). Histamine is a neurotransmitter in the body that is involved in e.g. allergic and inflammatory reactions. Large amounts of histamine can also cause pain (Kalso & Kontinen 2009). TRPM8 receptors are essential for menthol to reduce pruritus (Palkar et al. 2018).

Menthol has been shown to have an antitussive effect (Laude et al. 1994). Evaporated menthol activates C-neuronal TRMP8 receptors in the nose and thus probably modulates the cough response in the central nervous system (Koskela & Naaranlahti 2016). In nasal epithelial tissue, menthol increases mucus secretion (Liu et al. 2017).

Menthol applied to the skin causes vasodilation in the blood vessels of the skin in a dose-responsive manner, resulting in increased blood flow through the skin (Craighead et al. 2016). Menthol-induced vasodilation in the skin is dependent on nitric oxide (NO), sensory nerves, and EDHF (Endothelium-Derived Hyperpolarizing Factor) (Craighead et al. 2017). The opposite effect was observed when examining upper arm arterial blood flow. Topically administered menthol reduced arterial blood flow, but did not change the diameter of the artery (Topp et al. 2013).

In addition to superficial blood vessels, the effect of menthol has also been studied in the aorta, coronary arteries and small intestinal arteries. In them, menthol induced dose-response relaxation mainly by inhibiting the Ca^{2+}channels. This effect is not believed to be associated with TRPM8 receptor activation (Cheang et al. 2013).

Menthol can act as a skin permeation enhancer, so it can be used in products to improve absorption or enhance the permeability of drug molecules (Liu et al. 2005, Patel et al. 2007, Kamatou et al. 2013). It has been found to improve e.g. permeability of diclofenac, ketoprofen, tetracaine, and cyclosporin A (Wu et al. 2001, Liu Y. et al. 2005, Liu H. et al. 2006, Moreira et al. 2017). The mechanism by which menthol is able to increase the outermost layer of the skin

permeability, is based on direct interaction with lipids, disrupting the order between lipids (Wang & Meng 2017).

3.2.3 Security

Menthol is considered a relatively safe substance and is widely used in many products. The WHO has set a maximum daily dose of 4 mg / kg for menthol (WHO 1999). The acute and chronic toxicity potential of menthol is low. It is not genotoxic, carcinogenic or harmful to development. Possible side effects of menthol include skin, eye, and respiratory tract irritation (OARS 2014). To date, only a few case reports of menthol overdose have been reported. Menthol caused severe neurological and renal impairment as a result of high acute exposure or long-term chronic use. However, after discontinuation of chronic use, the patient's ability to function returned to normal. Very high acute or chronic exposure is expected to occur, to make menthol life-threatening (Kumar 2016, Baibars et al. 2012). Safety is enhanced by the fact that in many products the concentration of menthol is relatively low and menthol is less absorbed when administered to the skin than when taken orally (OARS 2014).

3.3 Pain in general

Pain is a subjective, unpleasant experience. It can be caused by actual tissue damage or a threat thereof, or it can occur without noticeable damage. Indeed, based on the mechanism of birth, pain is divided into three groups; nociceptive (tissue damage pain), neuropathic (nerve pain) and idiopathic pain. In idiopathic pain, the mechanism of onset is not fully understood, but is thought to be related to altered or abnormal central nervous system function. In the future, idiopathic pain as a concept may be receding and it would be more accurate to speak of anomalous central pain processing (Kosek et al. 2016, Phillips & Clauw 2013). Pain can be divided into acute, subacute and chronic pain according to duration (Pain KH Recommendation, 2015). Location of pain,

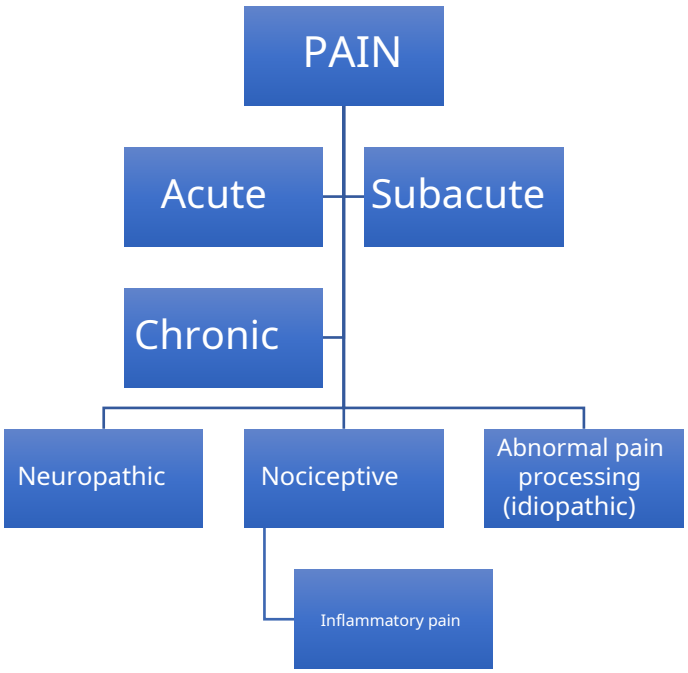

Figure 1. Pain classification

The duration, location, type, and intensity of pain can be carefully determined by interviewing and examining the patient. The intensity of the pain can be assessed by various measures. Commonly used pain measures include visual analogue scale (VAS), numerical rating scale (NRS), verbal rating scale (VRS), and facial images. (Pain KH Recommendation, 2015)

Figure 5. Gauges used to assess pain. As pain (VAS), numerical scale (NRS) and verbal assessment (VRS).

The pain system consists of four stages in which the pain signal travels from the periphery or tissue to the brain. The first stage, transduction, begins when the nerves (nociceptors) that carry the pain signal are activated. There are two types of nociceptors: Aδ and C nociceptors. Aδ nociceptors are myelin sheathed and thicker than C nose receptors, which are myelin sheathless (Vakkala 2016). Aδ and C nosoceptors respond to different stimuli. Aδ nosoceptors are sensitive to mechanical sharp stimuli and some to temperatures (mecanothermal). C-nosoceptors most often respond to mechanical, thermal, and chemical stimulation and are referred to as polymodal nosoceptors. Some of the C-causes do not respond at all to a mechanical stimulus (Kalso & Kontinen 2009). The action potential of nociceptors progresses toward the central nervous system. The frequency of the action potential depends on the intensity and duration of the stimulus.

A transmission is called an event in which the action potential arrives in the dorsal horn of the spinal cord and the projection neuron transfers the message to the dorsal horn on the opposite side and to the spinot trajectory. The spinach orbit carries a pain message toward the brain. In the facial area, pain is transmitted by the sensory branches of the trigeminal nerve. They end up in the trigeminal nucleus in the brainstem. The Pain Message from the facial area intersects the brainstem and enters the thalamus and eventually the cortex (Soinila 2015).

Modulation, or modification of pain, occurs in both the spinal cord and the brain. The pain message can be either strengthened or weakened. The final modulated pain sensation, or perception, occurs in the brain. In particular, the anterior singular cortex (ACC) and the somatosensory cortex are significant in the perception of pain. Thus, the transmission and perception of a nociceptive stimulus is regulated at many levels and the pain experience is subjective and individual. (Vakkala 2016)

Different types of pain are very common in the population and thus also cause a lot of costs (Haanpää & Pohjolainen 2015). According to a Finnish study, 40% (2237/5646) of patients went to the doctor because of pain. The study involved 25 primary health care centers. So the pain is one

the most common reasons for seeking medical attention. The three most common sites of pain were the lower back, abdomen, and head or face. The same study also looked at the duration and frequency of pain in patients. About 40% of pain patients experienced pain lasting one week or less, and 21% reported pain lasting more than 6 months. Approximately half of the patients experienced pain three times a month or less. One-fifth of patients suffered from persistent or multi-daily pain. (Mäntyselkä et al. 2001)

The prevalence of pain conditions was also observed in the Health 2011 study. The study asked patients about the occurrence of back, neck, shoulder and knee pain in the past 30 days. 41% of responding women and 35% of men suffered from back pain. The incidence of neck pain was almost equal to that of back pain (41% for women and 27% for men). The prevalence of shoulder pain was 26% in women and 29% in men. One-third of women and 29% of men had knee pain. Differences in the incidence of pain conditions were observed depending on age and gender. Compared to 2000, back, shoulder and knee pain became more common in the population. It is worrying that back, neck and knee pain have become more common, especially in those under 45 years of age. (Koskinen et al. 2011)

The goal of pain treatment is to relieve pain, but also to improve the patient's ability to function and quality of life. It is not always possible to completely eliminate the pain and the best possible way is to relieve the pain. Non-drug therapies and drug therapies and their combinations are used to treat pain, non-drug therapies are always preferred. (Pain KH Recommendation, 2015)

3.3.1 Acute, subacute and chronic pain

Pain is divided into acute, subacute, and chronic pain according to duration. Acute pain is one of the body’s defense mechanisms that warns of imminent tissue damage. Tissue damage can result from physical (eg pressure), chemical (eg corrosive substances) and microbiological (eg infection) factors. The acute pain resolves after the tissue damage has healed or with treatment within a few weeks and at the latest within a month. As acute pain persists, it can become first subacute and then chronic. Subacute pain ranges in duration from acute to chronic pain

between. It classifies pain conditions that last 1-3 months (Pain KH Recommendation, 2015). Acute and subacute pain should be treated as effectively as possible to prevent the pain from becoming chronic.

In chronic pain, the normal time required to heal tissue damage has been exceeded or the pain has lasted 3-6 months. Chronic pain can occur if tissue damage does not heal, peripheral nerves are damaged, or the pain-transmitting pathways of the pain system are damaged or the pain system is sensitized. Psychosocial factors also have an effect on the chronicization of pain. Chronic pain is challenging because it has a significant impact on a patient's life and psyche. It can be followed by e.g. depression, insomnia, exhaustion and social withdrawal. (Vakkala 2016)

3.3.2 Nociceptive pain

Nociceptive pain is caused by or threatened by tissue damage. It is referred to as tissue damage pain. The sensation of pain is caused by the actual tissue damage or threat thereof, e.g. as a result of inflammation or other damaging factor. Tissue damage releases neurotransmitters that sensitize to pain or can directly activate nociceptors (Kalso & Kontinen 2009). The pain mediating system described above is generally intact in nociceptive pain.

Nociceptive pain is divided into somatic and visceral on the basis of damaged tissue. Somatic pain originates from bones, tendons, blood vessels, and joints. An example of this is the pain caused by a surgical wound. In visceral pain, the damage is to the internal organs or the membranes surrounding them. Visceral nociceptive pain is, for example, pain caused by gallstones. (Vakkala 2016)

The cornerstones of the treatment of nociceptive pain are root cause treatment, non-drug treatments, and medication. Because tissue damage pain is the result of a damaging factor, treating the causative agent often works to relieve the pain. NSAIDs, paracetamol and opioids are used in drug treatment. Opioid use has focused on the treatment of more severe pain conditions. Many other methods can also be used to treat nociceptive pain. Such are e.g. antidepressants and antiepileptics, anesthetics, cold therapy, heat therapy, physiotherapy and exercise. (Heiskanen 2014)

3.3.3 Inflammatory pain

Inflammatory pain is one type of nociceptive pain. Pain is a typical sign of an inflammatory reaction. Inflammation releases many neurotransmitters that play an important role in causing inflammatory pain.

In the inflammatory reaction, eicosanoids are synthesized from arachidonic acid: prostanoids and leukotrienes. They sensitize nasal receptors, which can cause pain without the actual pain stimulus, e.g. from contact. The prostanoids released in inflammation are prostaglandin (PG) E_2, $PGD_2$and PGI_2. Leukotriene D_2 also affects other cells by enhancing the release of eicosanoids and substance P. Inflammation increases the acidity of the tissue, which lowers the pH. This stimulates nasal receptors and causes pain. (Kalso & Kontinen 2009)

Bradykinin has been shown to be a major mediator of inflammatory pain. It is formed from kininogen catalyzed by the enzyme kallikrein. Bradykinin stimulates nociceptors, causing pain and increases the synthesis of many other pain mediators (e.g., cytokines IL-1 and tumor necrosis factor (TNF)). Thus, bradykinin can also cause inflammatory pain indirectly when more analgesics are released (Moilanen & Vuolteenaho 2014).

Inflammatory pain is exacerbated by substances released from the nerve terminals of inflamed tissue, ie neuropeptides (eg substance P), which potentiate the inflammation and act as vasodilators (Heiskanen 2014). The anti-inflammatory effect is based on the ability of substance P to increase the release of cytokines, arachidonic acid, serotonin and histamine.

Inflammation activates platelets and mast cells to release serotonin, which can activate and sensitize nociceptors. In addition, mast cells release histamine under the influence of substance P, but only high concentrations of histamine cause pain. When the immune system is activated, cytokines are released, which include interferons, TNFs, and interferons. They are diverse with each other

effects and not all are related to the development of inflammatory pain. It is believed that their effect on the pain nerve is indirectly mediated. (Kalso & Kontinen 2009)

3.3.4 Neuropathic pain

Neuropathic, or nerve damage, pain is due to damage or changes in the functioning of the pain system, in contrast to nociceptive pain (Vakkala 2016). The pain is due to damage or disease affecting the somatosensory system. Based on the location of the lesion or disease, neuropathic pain is divided into peripheral (e.g., painful nerve compression), central (e.g., neuropathic pain in MS), and combined (e.g., shingles). (Haanpää & Vuorinen 2014, Treede et al. 2008)

The pathophysiological mechanisms of neuropathic pain are manifold. Peripherally, nerves are sensitized to neurotransmitters and their impulse output is enhanced. This increases their sensitivity to stimuli and also exposes them to spontaneous activation. Increased impulses sensitize the projection neurons in the dorsal horn of the spinal cord, resulting in increased release of glutamate and peptides. Glutamate and peptides may induce the destruction of inhibitory interneurons in the spinal cord. In addition, projection neurons can form new neural connections as a result of nerve damage. The aforementioned mechanisms that modulate the pain system may also occur in the brain. Damage to the spinatal pathway is also essential for the development of a central neuropathic pain state.

Facial neuropathic pain is due to neuralgia or traumatic trigeminal nerve damage (Forssell & Haanpää 2009). The most common neuralgias are trigeminus neuralgia and postherpetic neuralgia. Trigeminus neuralgia is likely to cause neuropathic facial pain through vascular nerve compression. Compression causes demyelination, i.e. damage to the myelin sheath surrounding the nerve cell, which exposes the nerve to spontaneous activation, i.e. it is a peripheral disorder (Haanpää et al. 2005). The cause of postherpetic neuralgia is a combination of both peripheral and central nerve disorders. Various traumas can damage the trigeminal nerve. Central lesions of the somatosensory system can also cause neuropathic pain in the facial area (Haanpää &

The most significant symptoms of neuropathic pain are persistent or intermittent pain and sensory abnormalities (weakening, sensitization, pain sensation to a normally non-painful stimulus). An event in which a painless stimulus usually causes pain is called allodynia. The term hyperalgesia is used for sensitization to pain. (Haanpää & Vuorinen 2014)

Neuropathic pain can be challenging to treat. In many patients, the pain cannot be completely treated and because of this, neuropathic pain limits the lives of many. Non-drug therapies are used primarily to treat neuropathic pain, but often medication, anesthesia, and stimulation treatments are also required. Pharmacotherapy includes e.g. antidepressants, antiepileptics, lidocaine, capsaicin and opioids. Capsaicin acts as an agonist of the TRPV1 receptor belonging to the same family as the TRPM8 receptor. (Vakkala 2016)

However, new solutions for the treatment of neuropathic pain are being developed. Research on the use of menthol in neuropathic pain has been positive (Proudfoot et al. 2006, Fallon 2015). This topic is explored in more detail in the next section.

3.4 Use of menthol in the treatment of pain

Of the isomers of menthol, l-menthol or (-) - menthol is analgesic (Galeotti et al. 2002). The analgesic effect of menthol has been studied in a variety of pain conditions and there are many pain products containing menthol on the market. Menthol is known to relieve acute and chronic pain, nociceptive, inflammatory, and neuropathic pain (Pergolizzi et al. 2018). The way in which the analgesic effect of menthol is mediated has also been studied. It has been hypothesized that menthol-activated sensory nerves inhibit the pain mediating system via the TRPM8 receptor and thus prevent the onset of pain sensation (Klein et al. 2010). Menthol activates the opioid system centrally (kappa) and is believed to play a significant role in analgesia (Galeotti et al. 2002). Menthol-induced analgesia is centrally dependent on group I and II metabotropic glutamate receptors (mGluR) (Proudfoot et al. 2006). In addition, inhibition of the Na channel has been proposed as a mechanism of action of menthol as an analgesic (Haeseler et al. 2002; Gaudioso et al. 2012).

Histamine is released in allergic and inflammatory conditions. It causes itching at low concentrations and at higher concentrations it can cause pain (Kalso & Kontinen 2009). In addition, it can cause hypersensitivity in the sensory nerves. Menthol relieves histamine-induced pruritus and potentially contributes to histamine-mediated pain relief (Luo et al. 2015).

In various combinations, menthol has been shown to enhance pain relief. When menthol was added to the tetracaine missing gel, the analgesic effect was enhanced. It has been hypothesized that this would be due, at least in part, to the improved skin permeability caused by menthol (Liu Y. et al. 2005, Fang et al. 2008). The cell membrane permeability of electrically charged lidocaine also increased as a result of TRPM8 receptor activation (McCoy et al. 2017). Thus, menthol has the potential to act in the treatment of pain both alone and in combination with other agents, such as local anesthetics and non-steroidal anti-inflammatory drugs (NSAIDs).

Menthol has no known interactions, so it can be safely combined with other non-pharmacological and pharmacological methods of pain management. Also, medications used by a patient to treat other conditions are not a contraindication to the use of menthol (Pergolizzi et al. 2018).

Menthol and neuropathic pain

The use of menthol in the treatment of neuropathic pain is an important area of research. Indeed, menthol has been shown to be useful in the peripheral and intrathecal administration of neuropathic pain. Superficial menthol administered to the spinal fluid induced behavioral changes in rats modeling neuropathic pain, suggesting pain relief (Proudfoot et al. 2006). In rats modeling chronic neuropathic pain, intrathecal menthol reduced mechanical allodynia and thermal hyperalgesia. Activation of the TRPM8 receptor at the level of the spinal cord reduces the typical symptoms of neuropathic pain due to nerve injury, i.e., allodynia and heat sensitization (Su et al. 2011).

A clinical study of menthol in the treatment of neuropathic pain caused by cancer treatment showed that 82% of the patients in the study received pain relief. The majority of patients had chemotherapy-induced peripheral nerve damage caused by chemotherapy.

neuropathy, CIPN), which caused pain. Menthol ointment (1%) was applied topically twice a day for 4-6 weeks. Pain was relieved in 82% of participants (31/38) and complete pain relief was achieved in 11% (4/38). The area of the distal parts of the limbs, where there was an abnormal sensation to various stimuli, shrank and its delimitation shifted towards the apical parts. Some patients also received relief from functional ailments caused by neuropathic pain, as well as anxiety and depression (Fallon et al. 2015). There have been a few patient case reports in which menthol has been shown to be useful in the treatment of neuropathic pain. In a single case, 1% menthol cream helped with chemotherapy-induced neuropathic pain and quality of life (Cortellini et al. 2017). In another case, in the treatment of CIPN-based pain, the patient benefited from 0,

Post-shingles neuropathic pain, or post-herpetic neuralgia, is often treatment-resistant. Peppermint oil containing 10% menthol has been tried to treat it. The patient's pain resolved rapidly with treatment and lasted for 4-6 hours. At two months of follow-up, the patient experienced that the oil continued to relieve pain well (Davies et al. 2002).

Menthol and nociceptive pain

Products containing menthol are used e.g. in acute soft tissue injuries to provide a cooling and analgesic effect and to improve recovery. A gel containing menthol (3.5%) and ethanol (cold gel) has been found to be more effective than placebo in reducing pain after soft tissue injury at rest and in motion (Airaksinen et al. 2003). The menthol content of the cold gels (0.5%, 4.6% and 10.0%) is not relevant for the cooling of the skin. All three concentrations cooled the skin for at least 60 minutes and no significant difference in temperature was observed. Perceived coolness is affected by the menthol content of the gel, as a gel containing 4.6% menthol caused a cooler sensation compared to gels containing 0.5% and 10.0% menthol (Lasanen et al. 2016). In addition to various gels and creams, menthol comes in patch forms that are applied to the skin. Patches containing methyl salicylate and 3% menthol alleviated the pain associated with mild to moderate muscle tension (Higashi et al. 2010).

When comparing menthol gel (3.5%) and ice in the treatment of delayed onset muscle soreness (DOMS), menthol was more effective in relieving pain. DOMS decreases muscle strength and menthol improved muscle strength more than ice (Johar et al. 2012). 4% menthol gel reduced chronic hand and wrist pain in slaughterhouse workers with carpal tunnel syndrome. A placebo gel was used as a reference (Sundstrup et al. 2014).

The combination product with the NSAID diclofenac and menthol (3%) was not significantly more effective in ankle sprains for ankle pain than the gel containing menthol alone. However, both treatments alleviated pain (Lai et al. 2017).

High levels of menthol (40%) have been shown to relieve neurogenic inflammation, pain, and mechanical and thermal hyperalgesia caused by local trans-cinnamic aldehyde (CA). CA is a TRPV1 receptor agonist. Such high concentrations of menthol alone can cause pain and hyperalgesia, but in the CA-mediated pain model, menthol is effective in analgesia and antihyperalgesia (Andersen et al. 2016).

Menthol relieves pain in the tongue area due to heat. In the study setup, the tongue was touched with an instrument with a temperature of 49°C. This thermal stimulus caused pain and the effect of menthol on pain was studied. Menthol significantly relieved pain, albeit rather poorly (Albin et al. 2008).

There is also evidence of an analgesic effect of menthol in inflammatory pain (Liu B. et al. 2013). An ointment containing fatty acids and menthol can be used to treat arthritis. On the knee, the cream increased the range of motion and ability to function. In the elbow and wrist, the ointment relieved severe pain associated with rheumatoid arthritis (Kraemer et al. 2005). An herbal cream in which menthol was one of the ingredients significantly alleviated the pain in the knees and hands caused by osteoarthritis more effectively than placebo (Gemmell et al. 2003). Also *spicata*-oil, in which menthol is one of the main ingredients, was effective in the pain caused by osteoarthritis. However, such preparations contain many other substances, so it is unclear whether menthol is the only active ingredient in pain (Mahboubi 2017). Topp et al. (2013) investigated differences in the effects of menthol and placebo on the performance of functional tests and on pain during the test in patients with osteoarthritis. The addition of menthol significantly improved the functional test

performed and reduced pain during their performance, however, no difference was observed between the test results or pain in the menthol ointment and placebo.

Neurogenic inflammation induced by histamine in volunteers was alleviated with 40% menthol applied to the skin. Histamine was injected intradermally at a concentration of 1%. The swelling and bumps of the skin that followed the inflammation also decreased after the application of menthol. (Andersen et al. 2017)

The anti-inflammatory effects of eucalyptol are most likely mediated through the TRPM8 receptor, so it is very possible that menthol also reduces inflammation and reduces pain in inflammatory conditions TRPM8-mediated (Caceres et al. 2017). Menthol has been shown to reduce the risk of certain inflammatory mediators (LTB4, PGE2 and IL-1).b)synthesis, which supports its role in the treatment of inflammatory pain (Juergens et al. 1998).

Ultraviolet radiation B from the sun causes an inflammatory reaction in the skin. Keratinocytes produce PGE2 under the influence of UV radiation, which causes the symptoms typical of inflammation. PGE2 production induced by UV-B radiation was inhibited when keratinocytes expressing TRPM8 receptors were treated with menthol or cooling. The results support the idea that activation of the TRPM8 receptor could reduce inflammatory reactions in the skin (Park et al. 2013).

Menthol and migraine / headache

Research data on the use of menthol in the treatment of migraines and headaches have been conflicting. Activation of the TRPM8 receptor has both caused and alleviated migraine (Dussor & Cao 2016). Activation of the TRPM8 receptor in the facial region inhibits migraine pain by inhibiting TRPV1 receptor activity at the level of TG neurons (trigeminal ganglion). The antinociceptive reaction occurred as if there was inflammation in the meninges (Kayama et al. 2017). Because menthol is an activator of the TRPM8 receptor, it is a potential treatment for migraine. There is research evidence for the use of menthol applied to the skin in the treatment of plague-free migraine. The painless time increased and the intensity of the pain also decreased (Borhani Haghighi et al. 2010).

The effect of menthol on migraine has also been studied in animal experiments by exposing the meninges to menthol. In a previous study, activation of TRPM8 receptors on the dura mater of the brain reduced pain behavior (Ren et al. 2015). In a more recent study, TRPM8 activation of the meninges caused migraine-suggestive behavior, suggesting that menthol would not be a pain reliever in all types of migraines but also a pain inducer in some patients (Burgos-Vega et al. 2016). For migraine receptor-targeted migraine treatments, more research is needed as it is not clear whether agonists (eg menthol) or antagonists are more effective. Research data are also needed on whether the response depends on the type of migraine (Dussor & Cao 2016).

4 MATERIALS AND METHODS

4.1 Research material

The material is compiled from PubMed's English-language articles and other related literature. The keywords for retrieving the material are TRPM8, pain and menthol +. If articles suitable for this review were found in the references of the articles found by the keywords, they were included in the material. Systematic data retrieval was performed with the keywords ((pain) AND menthol) AND (TRPM8 OR transient receptor potential melastatin 8) from the following databases: CENTRAL (Cochrane Library), CINAHL (EBSCOhost), EMBASE (Scopus), and MEDLINE (PubMed). Complete works were searched through the Cochrane Library, EBSCOhost and PudMed, and abstracts through Scopus.

4.2 Methods

Research material is collected as comprehensively and systematically as possible. The research material is carefully studied and a literature review is written on the basis of it. The text is processed and edited as the writing process progresses.

4.3 Ethical issues

There are no ethical aspects to this advanced study thesis. The material is publications retrieved from public databases and other literature that does not contain identifiable personal or patient information. Publications and other material are freely available under the logos of the University of Eastern Finland.

5 RESULTS

Evidence for the research hypotheses of this thesis is sought from the research material:

1) TRPM8 receptor activation relieves pain

2) Menthol relieves pain

3) The analgesic effect of menthol is mediated through the TRPM8 receptor.

A systematic search returned a total of 152 results, of which 32 were duplicates and were removed. There were thus 120 results after removing the duplicates. Based on the title, the abstracts were read from 43 articles. Based on the summaries, studies investigating the use of menthol in pain conditions were selected. Thus, 12 clinical trials were selected.

5.1 TRPM8 receptor activation relieves pain

In their study, Proudfoot et al. (2006) found that pain in animal models modeling neuropathic pain was alleviated when TRPM8 receptors were activated with chemical agonists or at cool temperatures, either from the skin surface or intrathecally. Another research group has also investigated the role of intrathecal menthol receptor activation in pain relief. It concluded that activation of the menthol receptor reduces mechanical allodynia and thermal hyperalgesia (Su et al. 2011).

5.2 Menthol relieves pain

Many research findings support that menthol relieves pain in both neuropathic and nociceptive pain conditions. A systematic search found 12 clinical studies investigating the ability of menthol to relieve pain (Appendix 1).

Green et al. (2000) investigated in an open-label study design how the administration of menthol-containing fluid to the tongue prior to the addition of capsaicin affects sensory sensation. The study found a reduction in capsaicin-induced irritation with prior use of menthol. The combination of menthol and capsaicin caused less irritation than capsaicin alone.

Airaksinen et al. (2003) investigated the efficacy of menthol gel in the relief of pain after soft tissue injury in a randomized, placebo-controlled clinical trial. Patients used the study product twice a day for two weeks. The subjects relieved pain statistically significantly within one week compared with placebo, and the difference was significant at both two and four weeks post-injury. Patients in the active group were more satisfied with the treatment they received and the functional result was better in the active group than in the placebo group.

Kraemer et al. (2005) investigated the efficacy of a menthol-containing ointment on arthritis pain in an open-label study design. Subjects used menthol ointment twice daily for one week. The study compared the intensity of pain and the subjects' performance in functional tasks before and after a week of treatment. The subjects' pain decreased statistically significantly and their functional performance in various tasks also improved.

Zhang et al. (2007) investigated the efficacy of a menthol-containing gel in the treatment of acute low back pain in a randomized controlled trial. Subjects used menthol gel three times daily for four weeks. Pain was statistically significantly reduced in the experimental group, but the results of the Roland Morris Disability Questionnaire did not improve.

Higashi et al. (2010) investigated the efficacy of menthol patch versus placebo in pain due to myocardial infarction in a randomized, double-blind, placebo-controlled clinical trial. Subjects received either a menthol or snow patch and used it for eight hours. The pain was reduced statistically significantly more with the menthol patch than with the placebo during the first seven hours, but no longer during the eighth hour. The pain was less both at rest and when moving. Satisfaction with treatment was statistically significantly better in the active group.

Borhani Haghighi et al. (2010) in a randomized comparative treatment study of the study group, migraine patients received two menthol solutions through the skin, of which less menthol-containing placebo. The solutions were applied to the forehead and temporis twice during a migraine attack and the intervals between additions were 30 minutes. Patients were assessed for pain two hours after the first addition. The active solution was more effective in achieving pain relief and reducing pain intensity and duration compared to placebo.

Johar et al. (2012) in a randomized open-label study design examined the efficacy of superficial menthol ointment and ice on delayed muscle pain after exercise. DOMS was induced by exercising according to the instructions given to the flexor muscles of the arm. Menthol or ice intervention was performed 48 hours after training. Pain and tenderness were statistically significantly reduced more in the menthol group than in the ice group. Muscle strength was also more than twice as good in those who used topical menthol cream as in those who had pretreated their muscles with ice pack.

Taylor et al. (2012) compared the efficacy of two menthol ointments (one containing oxidized triglycerides) in acute musculoskeletal pain in a double-blind, randomized study. Subjects used the ointment three times a day for eight days. The cream containing triglycerides reduced pain significantly more than the cream without them.

Topp et al. (2013) investigated the difference between the effects of menthol ointment and snow ointment on the performance of five functional tests and the pain experienced during them in patients with knee osteoarthritis in a randomized placebo-controlled comparative study. Patients performed functional tests twice during two separate visits and treatment was given between the two performances. The results of three functional tests were significantly improved, and in four tests, pain was significantly reduced with menthol treatment. However, no significant difference was observed between menthol and placebo.

Sundstrup et al. (2014) in a randomized placebo-controlled comparative study examined subjects with carpal tunnel syndrome and prolonged pain in the arm and arm area. On consecutive days, subjects applied creams to the wrist and hand so that menthol cream or placebo was used on different days. Subjects these days assess pain while doing slaughterhouse work. Subjects' pain was significantly reduced with menthol gel compared with placebo.

Fallon et al. (2015) investigated the efficacy of menthol gel in peripheral neuropathic pain induced by chemotherapy in an open-label clinical follow-up study. Patients used menthol ointment twice daily for 4 to 6 weeks. The average pain of the subjects decreased significantly and overall 82% of the subjects experienced a reduction in pain. Patients' mood also improved, catastrophe decreased, movement and limb sensation improved with the use of menthol ointment. The pain began to improve immediately after starting menthol ointment and no dose adjustment was required during the study.

Keshavarzian et al. (2017) investigated the effect of rosemary and menthol administered to the skin in the treatment of musculoskeletal pain in hemodialysis patients in a placebo-controlled clinical trial. The menthol, rosemary or snow product was applied to the pain area three times a day for three days. Pain was significantly relieved in both the menthol and rosemary groups, but not in the placebo group.

5.3 The analgesic effect of menthol is mediated through the TRPM8 receptor

The analgesic effect of menthol is at least partially dependent on the menthol receptor, and other mediating factors have been observed. Cold-induced analgesia is also mediated through the TRPM8 receptor (Laing & Dhaka 2016). The hypothesis is supported by an in vivo study investigating the effect of menthol on nociceptive pain in mice. Pain behavior was reduced in mice with TRPM8 receptors, but no response was obtained in TRPM8 knockout mice or when a TRPM8 antagonist was used (Liu B. et al. 2013). The location of TRPM8 receptors in peripheral sensory nerves, dorsal root of the spinal cord, and trigeminal ganglia, as well as in the descending and ascending pain system, also supports the claim that it plays an important role in pain relief (McKemy et al. 2002; Facer et al. 2007). These parts are essential for pain perception and thus also for pain relief. Other possible mechanisms of menthol-induced pain relief include regulation of the opioid system and metabotropic glutamate receptors in the central nervous system and inhibition of Na channels (Galeotti et al. 2002; Haeseler et al. 2002; Proudfoot et al. 2006; Gaudioso et al. 2012).

6 CONSIDERATION

Menthol is commonly known for its characteristic taste and aroma, but in addition it has been found to have many biological effects. The cooling effect of menthol on the skin and changes in sensory sensation have been observed early, it has been used since ancient times (Eccles 1994). It was not until the middle of this century that the menthol-activated TRPM8 receptor, through which effects are mediated, was identified and described (McKemy et al. 2002; Peier et al. 2002). Menthol has since been shown to be an analgesic (Galeotti et al. 2002). Many studies have shown that menthol has considerable potential in the treatment of pain. Different types of pain are very common and the aim is to use non-drug alternatives in their treatment (Kipu KHsuitusitus, 2015).

Menthol has the advantage of minor side effects compared to systemic drug therapy, such as opioids and NSAIDs (Highashi et al. 2010). Significant disadvantages of opioids include constipation, addiction, nausea, and respiratory depression (Kalso 2017). Disadvantages of NSAIDs include, for example, the risk of bleeding, damage to the gastrointestinal mucosa, kidney problems and skin reactions (Moilanen & Vuolteenaho 2014). In any case, menthol has been found to be a safe substance with few side effects and no significant known interactions with other pain treatments or other medications used by patients (OARS 2014, Pergolizzi et al. 2018). With creamy and gel-like products, menthol can be applied to the desired areas and, if necessary, several times a day. Menthol gels, creams, sprays and patches are currently on the market and transdermal menthol treatment is relatively inexpensive. In Finland, menthol preparations used for the treatment of pain are classified as technical preparations and must bear the Conformité Européenne (CE) mark (https://europa.eu/youreurope/business/product/cemark/index_en.htm .).

Menthol also has the advantage that it can also be applied to broken skin - but not as an alcohol-containing product. Preparations containing menthol should not be used if the person is hypersensitive to menthol or any of the other ingredients. The discomfort or inconvenience of treatment experienced by the patient may also complicate the use of menthol in the treatment of pain. Cold hyperalgesia can be prevented

use of menthol. It should also be borne in mind that high levels of menthol can irritate the skin and preparations containing alcohol dry the skin (Binder et al. 2011).

Neuropathic pain is often challenging to treat, so research findings on the benefits of menthol are welcome. The pain of peripheral nerve damage caused by chemotherapy is often troublesome, and the response to treatment with medications commonly used to treat pain is usually poor. Research on the use of menthol, especially for pain from cancer treatments, provides promising evidence for the benefits of menthol (Fallon et al. 2015). Neuropathic pain will be an interesting and useful research topic for the use of menthol in the future, as new ways to treat neuropathic pain are needed. For example, in regional pain syndrome, the central pathophysiological mechanism is vasospasm, and the skin in the sore area is touch-sensitive. Menthol dilates blood vessels and improves skin blood circulation, so it could alleviate the pain caused by this mechanism (Craighead et al. 2016, Craighead et al. 2017). TPRM8 receptors are particularly abundant in sensitized skin (Facer et al. 2007). Menthol cream could be a significant help in the treatment of such pain conditions.

Menthol is particularly well suited for the treatment of nociceptive pain conditions because it can only be applied to the required area and several times a day. The response is rapid and no dose adjustment is required. Menthol relieves pain e.g. in soft tissue injuries and also reduces inflammation. The use of menthol in soft tissue injuries can promote healing. (Airaksinen et al. 2003, Liu B et al. 2013)

In addition to the fact that menthol relieves pain on its own, its potential to act as an absorption enhancer for other substances must also be considered. Menthol increases the absorption of many substances on the skin and in the eye and may be a safer alternative to other permeation enhancers (Wang & Meng 2017). Menthol can be used to enhance the effect of superficial analgesics or local anesthetics (Kamatou et al. 2013). In addition, menthol may be used in combination with systemically administered drug alternatives if its efficacy alone does not produce an adequate response.

The effect of menthol would appear to be individual, as some have reduced pain and some have had no effect on pain. This is presumably influenced by various factors; eg intensity of pain experienced, mental factors, different functioning of the pain system and other individual

features. Therefore, it is advisable to try menthol in the treatment of the above-mentioned pain conditions and to evaluate its effectiveness individually.

The potential therapeutic uses of menthol are not limited to the treatment of pain. Menthol has been found to be effective in the treatment of histaminergic pruritus (Bromm et al. 1995; Andersen et al. 2017). In insect stings, menthol relieves itching within minutes (Bromm et al. 1995). Menthol reduces the risk of mosquito-borne infections by acting as an insect repellent and mortality agent (Samarasekera et al. 2008). Menthol has also been used in cancer studies. The TRMP8 receptor is expressed in many cancers, so menthol is able to activate these receptors and affect cancer cells. In prostate cancer and melanoma, menthol was found to reduce cell growth and migration and reduce cell viability (Liu et al. 2016).

Menthol is a drug-free method of pain management that is likely to have less potential harm than drug-based methods. A better understanding of the function of menthol has made it possible to exploit its properties in the treatment of pain and other uses. Menthol may potentially serve as a starting point for the development of new analgesics (Pergolizzi et al. 2018). Overall, menthol appears to be an effective and safe method of pain management that will hopefully benefit pain patients in the future. Effective pain management improves patients ’quality of life and reduces the cost to society. However, more research is needed on its use and research data is constantly increasing.

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Authors and reference	Cohort	Study design	Intervention	Findings
Green BG et al. 2000	12 subjects: 7 male	Open-label	Menthol sip before capsaicin application to tongue	The sensory response to capsaicin was significantly decreased by menthol
Airaksinen et al. 2003	74 patients, 47 male, with sport related soft tissue injuries	Randomized, prospective, double-blind trial	Application four times per day for 14 days Topical menthol, n = 37 Topical placebo, n = 37	Menthol: pain at rest decreased from 59 (SD 15) to 30 (16) at day 7, to 14 (13) at day 14 and to 7 (12) at day 28. Placebo: pain decreased from 59 (15) to 45 (15), 26 (18), 13 (14) in VAS 0-100
Kraemer WJ et al. 2005	28 patients diagnosed with arthritis (knee, elbow or wrist), 2 male	Open-label	Application of cream two times a day for 1 week	WOMAC pain reduced from 9.4 (SD 1.8) to 5.0 (3.8)
Zhang J et al. 2008	36 subjects, 25 male, acute low back pain	Randomized controlled study	Application three times per day for 4 weeks in experimental group (n = 18) Control group no topical treatment (n = 18)	Experimental group: pain decreased from 4.1 (SD 2.3) to 1.3 (1.7) Control group pain increased from 4.4 (2.4) to 5.2 (2.2) in VAS 0-10
Higashi Y et al. 2010	208 patients with mild to moderate muscle strain, 104 male	Randomized, double-blind, parallel group, placebo-controlled study	Application of patch and removal after 8 hours Menthol, n = 105 Placebo, n = 103	Menthol: Pain decreased 182 (SD 131) through 8 hours using SPID8. Placebo: pain reduced 130 (144)
Borhani Haghighi A et al. 2010	35 patients with migrant without aura, 7 male	Randomized, triple-blind, cross-over study	Menthol 10% -menthol 0.5% (2 initial attacks treated with menthol 10% and next 2 treated with menthol 0.5% (n = 17) or vice versa (n = 18)	Menthol10%: pain-free attacks 38% of treated attacks Menthol 0.5%: pain-free attacks 12%
Johar P et al. 2012	16 subjects, 12 male	Randomized, open-label	Application 48 hours or 2 days after DOMS inducing session Topical menthol, n = 8 Topical ice, n = 8	Menthol: soreness perception 1.1 (SD 0.4) Ice: soreness perception 3.1 (1.7)
Taylor R et al. 2012	69 subjects, 19 male, with acute musculoskeletal towards	Double-blind randomized trial	Application three times per day for 8 days Topical menthol, n = 34 Topical menthol + OGT, n = 35	Menthol: pain decreased from 42 (SD 18) to 29 (26) Menthol + OGT: from 47 (17) to 19 (18) in VAS 0-100
Topp R et al. 2013	20 subjects with osteoarthritis of the knee, 6 male	Randomized, cross-over, placebo-controlled trial	Application between assessments, topical menthol on the first visit and placebo on the second, n = 10 or	Menthol: pain significantly decreased during the performance of 4/5 functional tests Placebo: no significant

			vice versa, n = 10	decrease in pain in VAS 0-100 No differences in pain decrease between menthol and placebo
Sundstrup E et al. 2014	10 patients with carpal tunnel syndrome patients, 8 male	Triple-blind, randomized, cross-over, placebo-controlled trial	A single application day 1: topical menthol, day 2 placebo, n = 5 or vice versa, n = 5	Menthol: pain decreased - 1.2 (95% CI -1.7 to -0.6) compared to placebo in VAS 0-10
Fallon MT et al. 2015	51 patients with cancer treatment related neuropathic pain, 19 male	Open-label prospective clinical trial	Application twice per day for 4-6 weeks	Total media BPI decreased from 4.7 to 3.4 in 0-10 BPI
Keshavarzian S et al. 2017	105 patients undergoing hemodialysis, 51 male	Single-blind placebo-controlled clinical trial	Application three times per day for three days Menthol, n = 35 Rosemary, n = 35 Placebo: n = 35	Menthol: pain decreased from 7.3 (SD 1.9) to 4.81 (2.5) Rosemary: from 7.5 (2.0) to 4.2 (3.0) Placebo: from 7.2 (1.8) to 6.4 (2.3) in NRS 0-10

BPI brief pain Inventory

DOMS delayed Onset muscle soreness

NRS numeric rating scale

OGT oxygenated glycerol triesters

SPID8 summed pain intensity difference score through 8 hours

VAS visual analogue scale

WOMAC Western Ontario and McMaster University Osteoarthritis Index