氏　　名	髙石　雅之	
学位(専攻分野)	博士(理学)	
学 位 記 番 号	総研大乙第 245 号	
学位授与の日付	平成28年3月24日	
学位授与の要件	学位規則第6条第2項該当	
学 位 論 文 題 目	Analgesic mechanisms of essential oil components	
論 文 審 査 委 員	主　査	教授　久保　義弘
		教授　富永　真琴
		教授　鍋倉　淳一
		教授　水村　和枝　中部大学

(別紙様式 2)
(Separate Form 2)

論文内容の要旨
Summary of thesis contents

Transient receptor potential (TRP) channels respond to a wide variety of sensory stimuli, including temperature, nociceptive compounds, touch, osmolarity, and pheromones. TRPV1 can be activated by chemical ligands such as capsaicin, endocannabinoid, anandaminde and protons, and by physical stimuli such as heat, and acts as an integrator of multiple noxious stimuli. TRPA1 is an ion channel targeted by pungent irritants such as those from mustard oil and garlic and is thought to function in diverse sensory processes, including cold nociception and inflammatory pain. Therefore, TRPV1 and TRPA1 are ion channels involved in acute nociception and inflammatory pain and are considered to be promising targets for the development of analgesic agents. Most of the published TRPV1 and TRPA1 antagonists lack optimal properties for clinical development such as selectivity, solubility, oral bioavailability, and/or reasonable pharmacokinetics. Thus, naturally occurring antagonists of these channels which have been demonstrated to have a safety profile based on their long usage would be desirable.

Essential oils are often used in alternative medicine as analgesic and anti-inflammatory remedies. For example, menthol, the main ingredient of peppermint, is used for pain relief in daily life. Analgesic effects of menthol have been considered to occur through TRPM8 activation. However, molecular mechanisms for analgesic effects of these ingredients are largely unknown. It is reported that high doses of menthol caused sensory irritation through TRPA1 activation in humans. Camphor, another essential oil component, is now known to exert analgesic effects probably through inhibition of TRPA1 and activation of TRPM8. However, camphor is not suited for use as an analgesic compound because it causes a warm and hot sensation, probably through TRPV1 and TRPV3 activation. I thought that other effective analgesic compounds would activate TRPM8 and inhibit TRPA1, but not activate TRPV1.

Through the screening of essential oils, I found that eucalyptus oil exhibited a relatively high hTRPM8-activating ability with less activation of hTRPA1. Furthermore, 1,8-cineole, a main component of eucalyptus oil, inhibited hTRPA1 activated by several agonists with a half-maximal inhibitory concentration (IC_{50}) of 3.4 mM for AITC (20 μM)-evoked hTRPA1 currents. In addition, sensory irritation tests in vivo showed that 1,8-cineole conferred an analgesic effect on the sensory irritation produced by menthol and a TRPA1 agonist octanol. Moreover, 1,4-cineole, which is another component of eucalyptus oil, activated hTRPA1. Several compounds with similar structures exhibit different effects on hTRPA1. Menthol and 1,4-cineole activate hTRPA1, while camphor and 1,8-cineole inhibit hTRPA1.

Given these promiscuous effects on hTRPA1, more detailed analyses would lead to finding more effective compounds.

To identify more effective TRPA1 antagonists, I screened camphor analogs among monoterpenes which comprise a group of naturally occurring organic compounds derived from essential oils that have been used for anesthetic, analgesic, anti-inflammatory, and anti-pruritic applications. I found that borneol, 2-methylisoborneol and fenchyl alcohol inhibited hTRPA1 activity. The hTRPA1 currents induced by AITC (20 μM) were inhibited by borneol, 2-methylisoborneol or fenchyl alcohol in a dose-dependent manner, with IC_{50} values of 0.20, 0.12 and 0.32 mM, respectively, which are much lower than the IC_{50} values of 1,8-cineole (3.43 mM) and camphor (1.26 mM). In addition, sensory irritation tests in vivo showed that borneol conferred an analgesic effect on the sensory irritation produced by menthol. Furthermore, I found that the S873, T874, and Y812 residues of hTRPA1 were involved in the inhibitory effects, suggesting that the hydroxyl group in the cyclohexane of the inhibitors may interact with these amino acids.

Moreover, to understand analgesic mechanism of menthol, I examined the effects of menthol on hTRPV1. The hTRPV1 currents induced by capsaicin were inhibited by menthol in a dose-dependent manner, with an IC_{50} value of 1.17 mM. In addition, an in vivo sensory irritation test showed that menthol conferred an analgesic effect on the sensory irritation produced by VBE (vanillyl butyl ether), a TRPV1 agonist.

Furthermore, I found that Y511, S512 and T550 of hTRPV1, which are binding sites of capsaicin, were little involved in the inhibitory effects of menthol. These data suggest that menthol interacts with sites different from those of capsaicin. These results show that an analgesic effect of high-dose of menthol is derived from its inhibitory effect on TRPV1 to some extent.

In this study, I elucidated molecular mechanisms of analgesic effects of essential oil components. Further research of these compounds could lead to better understanding of the structural determinants for the action of these compounds on TRPV1 or TRPA1 and the development of anti-nociceptive agents through TRPA1 or TRPV1 inhibition.

(別紙様式 3)
(Separate Form 3)

博士論文の審査結果の要旨

Summary of the results of the doctoral thesis screening

申請者、高石雅之氏は、精油が清涼感を与え鎮痛作用を示すことの分子機構の解明、および清涼剤として優れた精油成分の探索を目的として、ヒト由来の HEK293 細胞を *in vitro* 発現系として用いた、パッチクランプ法と細胞内 Ca^{2+}イメージング法による Transient Receptor Potential (TRP) チャネルへの精油成分の作用の解析、およびヒトに精油成分を塗布した布をおしあてることによる感覚の解析等を行った。

これまでに、menthol が清涼感とともに灼熱感も与えるのは、TRPM8 チャネルだけでなく TRPA1 チャネルも活性化するためであることが知られていた。高石氏は、より清涼感を与える物質を、ヒト TRPM8 チャネルを活性化しヒト TRPA1 チャネルを活性化しないことを指標として種々の精油から探索し、eucalyptus oil、なかでもその成分のひとつである 1,8-cineole が優れていることを新規に見出した。

次に、高石氏は、ヒト TRPA1 チャネルを活性化しないという条件で有用な構造類似物質の探索を行い、ヒト TRPA1 阻害物質として borneol、fenchyl alcohol、2-methylisoborneol を新規に同定した。また、これらの物質が高濃度の menthol によるヒトにおける灼熱感を確かに抑制することを確認した。さらに、変異体を用いた解析により、ヒト TRPA1 チャネルにおける作用部位として、menthol と共通する第 5 膜貫通部位の 2 つのアミノ酸残基 Ser873, Thr874 を同定するとともに、borneol の有する 6 員環の-OH 基が高親和性の結合に寄与していることも明らかにした。また、AITC 等のアゴニストにより活性化されたヒト TRPA1 チャネル電流が、borneol 等の阻害剤の追投与により抑制された後、阻害剤のみを洗い流した直後に著しく増強するという興味深い現象も見出した。

Menthol は鎮痛作用も有することが知られてきたが、その分子基盤は十分には明らかにされていなかった。高石氏は、menthol がヒト TRPM8, ヒト TRPA1 に作用するのみならず、ヒト TRPV1 の capsaicin および高温刺激による活性化を阻害することを新たに見出した。さらに、その作用部位の同定を目的として変異体を用いた解析を行い、menthol がヒト TRPV1 の capsaicin の作用部位として知られる Tyr511, Ser512, Thr550 とは異なる部位に作用することを明らかにした。

このように、高石氏は、一貫した主題のもと、密接に関連した三つのテーマについて連綿と優れた研究成果を挙げてきた。さらに、これらの研究成果は、高石氏を筆頭著者とする英文原著論文 3 編として既に発表されている。また、高石氏の知見は、1,8-cineole を成分とする新しい清涼塗布材の開発を導いた。

以上、本研究は、分子生物学的手法を組み合わせた細胞生理学的解析およびヒト感覚生理学的解析の実験結果を統合して、種々の精油成分の TRP チャネルに対する作用の新側面とその構造基盤を明らかにしたもので、その科学的価値はもちろんのこと応用開発における価値

(別紙様式 3)
(Separate Form 3)
も高く評価できる。以上の理由から、審査委員会は全員一致で、本論文が学位論文として相応しいものであると判断した。