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学位論文名	**ヘリコバクター属細菌のホスファチジルエタノールアミンの特性**
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論文内容の要旨

1　研究目的

Helicobacter pylori, a pathogen responsible for gastroduodenal diseases in human, retains myristoyl phosphatidylethanolamine (MPE) in the cell membranes. *H. pylori* MPE is an important glycerophospholipid concerned with the intramembranal absorption of steroids such as cholesterol and pregnenolone with a 3β-hydroxyl. Meanwhile, progesterone with a 3-oxo induces the bacteriolysis to *H. pylori*. An earlier study by our group has demonstrated the possibility that progesterone molecule is a fundamental structure for developing new steroidal anti-*H. pylori* medicines. However, the bactericidal mechanism of progesterone to *H. pylori* has remained unclear. We, therefore, synthesize a novel progesterone derivative to examine whether progesterone targets *H. pylori* MPE due to the induction of the bacteriolysis.

H. pylori PE is considered to bind more selectively to cholesterol than to cholesteryl ester by the myristic acid in its PE molecule. Therefore, the bacterial cell membranes of *H. pylori* selectively absorb cholesterol from a medium supplemented with serum, even though the concentration of cholesterol is conspicuously lower than that of cholesteryl ester in serum. As observed in *H. pylori*, the same bacterial Genus, *H. felis* and *H. cinaedi* likewise selectively absorb cholesterol into the cell membranes, when the bacteria were incubated using serum-supplemented medium. No earlier investigations have, however, elucidated as for whether PE of the two *Helicobacter* bacterial species takes part in the selective absorption of cholesterol into the cell membranes. We, therefore, isolate the PE from the two *Helicobacter* bacteria to examine the hydrophobic interaction between cholesterol and its glycerophospholipid, and to analyze the fatty acid composition of PE of those *Helicobacter* bacteria followed by the decision of PE molecular species.

2　研究方法

Helicobacter bacteria were cultured using a PPLO broth. A novel synthetic progesterone derivative was obtained via the dehydration condensation reaction between the molecules of 17α-hydroxyprogesterone and linoleic acid. Measurement of colony-forming units was performed by the conventional method. Lipids were analyzed by a thin-layer chromatography. Nitric oxide (NO) was measured using a Griess reagent. Cytotoxicity of

progesterone and its derivatives was estimated via the MTT assay. Extraction of lipids was carried out by the organic solvent distribution method. Quantification of cholesterol was carried out by the ferrous chloride-sulfuric acid method. PE was purified by an Iatrobead-column chromatography. Fatty acid composition of PE was analyzed by GC-MS. PE molecular species were analyzed by LC-MS.

3　研究成果

A novel synthetic progesterone derivative 17α-hydroxyprogesterone linoleate (17hPL) conferred the effective bacteriolytic action against *H. pylori*. MPE of this bacterium turned out to exhibit higher binding affinity for 17hPL than palmitoyl PE of *Escherichia coli* used as a prevalent PE species of Gram-negative bacteria. As correlated with the selective binding affinity of 17hPL for *H. pylori* MPE, this progesterone derivative selectively killed *H. pylori* and had no influence on the viability of other commonplace bacteria. One of the hormonal effects of progesterone is the inhibition of nitric oxide (NO) production from macrophages stimulated with lipopolysaccharide (LPS). We, therefore, examined the capability of 17hPL to inhibit the NO production in murine macrophage-like cells activated by LPS. As such, 17hPL turned out to be relatively weaker in its capability to inhibit NO production in LPS-activated cells than progesterone.

PE of both *H. felis* and *H. cinaedi* interacted more selectively with cholesterol than with cholesteryl ester. These results suggested the possibility that the bacterial cells of *H. felis* and *H. cinaedi* may abundantly contain PE with myristic acid. On this basis, we analyzed the PE molecular species of the two *Helicobacter* bacteria and demonstrated that the PE containing myristic acid accounts for more than 35% in the total PE of each *Helicobacter* bacterium.

4　考察

17hPL was considered to bind to MPE in the cell membranes of *H. pylori*, to induce the destabilization of the membrane structures, and to ultimately elicit the bacteriolysis. In addition, the hormonal effect of progesterone on mammalian cells seemed to be attenuated by the modification of it with a linoleic acid.
MPE of *H. felis* and *H. cinaedi* seemed to take part in the selective absorption of cholesterol in the bacterial cell membranes.

5　結論

Based on this study, we demonstrated that progesterone molecule could become to a fundamental structure for developing new steroidal antibacterial medicines for selectively acting on *H. pylori*.
In addition, we revealed that *H. felis* and *H. cinaedi* retain the unique PE molecular species distinguishable from the PE molecular species of *H. pylori*.

論文審査の結果の要旨

ヘリコバクター属細菌の細胞膜にはホスファチジールエタノールアミン(PE)である myristoyl PE が存在する。myristoyl PE がプロゲステロンの誘導体である 17α-hydroxyprogesterone (17hPL)に疎水結合し、ヘリコバクター属細菌を溶菌する。*Helicobacter pylori*（*H. pylori*）に対しても 17hPL は選択的に結合し、溶菌作用により殺菌する。しかし、*H. pylori* 以外のグラム陰性

細菌の主要な PE は palmitoyl PE であり 17-hPL は結合しないので、17hPL の *H. pylori* に対する溶菌作用は特異的であり、*H. pylori* 以外の細菌に対しては影響がないことを明らかにした。学位論文の内容は既に 2 つの論文として publish されている(Journal od steroid Biochemistry and Molecular Biology 2014;140;17-25, Lipids 2015;50;799-804)。提出された論文の内容に関しては問題がないと判断され、特段の修正は必要ないとの結論に達した。以上より申請された論文はそのままの状態で受領され合格とした。

最終試験の結果の要旨

研究の背景の説明などで、*H. pylori* の臨床における重要性および細菌学的な性質のみならず、治療について十分な知識があることが認められた。プロゲステロンの誘導体である 17hPL の in vitro における *H. pylori* に対する溶菌作用のメカニズムの詳細を十分に説明することができた。in vivo でのヒトへの臨床応用が最終目標であり、臨床応用に先立つマウスへの in vivo の実験に関する質問が集中した。しかし、in vivo の実験が進んでいなかった。accept された 2 つの論文は 2014 年と 2015 年であり、その後の実験としての in vivo のデータが実験途中であっても発表されることが望ましいところであったがデータがなくその点が残念であった。しかし、ヘリコバクター属細菌に対する 17hPL の溶菌による殺菌作用の詳細なメカニズムの解析は既に 2 つの論文としてまとめられている。質疑応答においても十分な知識と技能を備えていると認められたため合格とした。