論　文　内　容　の　要　旨
Synopsis of Thesis

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論文題名 Title	Development of LC-MS/MS Quantification of the Novel Antimicrobial Peptide, SR-0379, and its Pharmacokinetics in Rats（抗菌ペプチド SR-0379のLC-MS/MS法を用いた測定法の確立及びラットでの体内動態）

論文内容の要旨

〔目　的(Purpose)〕

While screening of the functional genes for angiogenic molecules via *in silico* analysis, we have developed a novel small, angiogenic peptide with thirty amino acids, named as AG30. From AG30, we further developed a potent angiogenic peptide with anti-microbial activity, named as SR-0379, which has twenty amino acids (under submission). Because both of anti-microbial and angiogenic actions are required in the wound healing process, SR-0379 might be an effective therapeutic agent to treat the wounds such as decubitus ulcer, diabetic ulcer, and burn ulcers.

Recently, functional peptides and proteins have been considered as potential drugs. Bioassays, immunoassays and isotope tracer techniques have been used to evaluate the pharmacokinetics of peptides and proteins. Immunoassays such as ELISA are unable to distinguish such compounds from their metabolites, which is the primary disadvantage of these methods. The total radioactivity detected using an isotope tracer technique is not indicative of the parent compound concentration in biological samples. Mass spectrometry-based technologies can provide many benefits over these methods. The quantitative analysis of peptides and proteins via liquid chromatography/mass spectrometry (LC/MS) might be difficult; however, LC/MS methods that utilize a triple quadrupole, ion trap, and quadrupole time-of-flight (Q-Tof) were recently used to quantify peptides. Based on these techniques, we have newly developed a sensitive method for determining bioactive peptides in rat plasma and tissues.

〔方法ならびに成績(Methods/Results)〕

A 5-μL aliquot of a SR-0379 working solution in 50% methanol/H_2O at various concentrations (5 – 1000 ng/ml) was mixed with 1500 μl phosphoric acid (4%). Next, 495 μl of rat plasma and 10 μl of the internal standard solution (5000 ng/ml) were added. The solution was vortexed for 10 s and centrifuged at 10,000 × g for 5 min. The supernatant was transferred to an Oasis HLB μElution plate that had been previously activated by successive applications of 0.3 ml methanol and 0.3 ml Milli-Q water. This plate was then washed with 0.3 ml of Milli-Q water. The peptide adsorbed onto the surface of the plate was eluted with 150 μl of acetonitrile-water-trifluoroacetic acid (75:25:1). A 10 μl aliquot was transferred to the LC-MS/MS system.

Although SR-0379 was unstable in the rat plasma and subcutaneous tissue samples, pretreatment with EDTA and phosphoric acid (4 %) inhibited its degradation. The lower limits of quantification (LLOQ) for SR-0379 were fully validated as 5 ng/ml in plasma and 5 ng/g in tissue with acceptable linearity, intra- and inter-assay precisions, and accuracy. Measurement of SR-0379 concentration in plasma after intravenous injection via LC-MS/MS yields plasma concentration-time curves ($AUC_{0-\infty}$) with areas of 667 ng·min/ml and an elimination half-life ($t_{1/2}$) of 4.8 min. The concentration of SR-0379 in the subcutaneous tissue samples was 13.1 μg/g tissue at 30 minutes after a single dermal application (1 mg/ml, 50 μl) to a full-thickness excisional wound. Here, a highly sensitive and specific LC-MS/MS assay with a lower limit of quantification of 5 ng/ml was developed and validated to quantify SR-0379 in rat plasma. This method is useful for pharmacokinetic studies of the peptide drugs in rats.

〔総　括(Conclusion)〕

A highly sensitive and specific LC-MS/MS assay with a lower limit of quantification of 5 ng/ml was developed and validated to quantify SR-0379 in rat plasma. This method was fully validated and possessed acceptable linearity, accuracy, and both intra- and inter-assay precisions. Furthermore, this method is useful for performing pharmacokinetic studies in rats.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

血管新生因子の探索的な研究として、遺伝子機能スクリーニングを行い、30残基のペプチド（AG-30/5C）を創製された。AG-30/5Cをリード化合物とし、創薬を目標として、活性の向上、製造コストの低下を目指した新規ペプチドを創製することを目的とした。AG30/5Cの代謝物を検索することから、活性が向上した20残基の新規ペプチドの創製に成功した。さらに、創製した新規ペプチドの血漿中濃度・皮下組織中濃度の測定法を確立し、体内動態を検討した。新規ペプチドは、抗菌活性を有しながら、創傷治癒促進作用を示すユニークな化合物である。ラットの全層欠損に新規ペプチドを滴下した際、皮下組織中濃度は、抗菌活性・創傷治癒促進作用を示す濃度と同程度の濃度を示していたことから、ラットで創傷治癒促進作用を示したことは妥当であると考えられた。

よって、本研究は、新規ペプチドのトランスレーショナルリサーチを行うための用量の妥当性を示した点で有益な研究であり、学位の授与に値すると考えられる。