氏　名（生年月日）	劉　姝（りゅう　しゅ）（1983年2月23日）
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学位論文題目	The development of novel microneedle arrays fabricated from hyaluronic acid , and their application in the transdermal delivery of diabetes drugs (新規ヒアルロン酸マイクロニードル製剤の開発及び糖尿病治療薬の経費吸収改善への応用に関する研究)
論文審査委員	(主査)　教授　山本　昌
	(副査)　教授　西口工司
	(副査)　教授　栄田敏之

論文内容の要旨

Traditional non-invasive transdermal patch systems are simple and comfortable to use, but the choice of therapeutics is limited to small molecules due to the existence of stratum corneum. To overcome this skin barrier, microneedle arrays (MNs) have gained increasing attention as a novel minimally invasive method and they are able to deliver a variety of molecules into the skin, including small drugs, macromolecules, nanoparticles and fluid extracts. MNs have been demonstrated to effectively penetrate the stratum corneum into the epidermis and/or superficial dermis to administer compounds into the skin for local or systemic administration. Their sharp tips and target insertion depth reduce the risk of encountering the nerves that perceive pain. Among numerous research works regarding MNs, the ones made of biocompatible and biodegradable polymers and carbohydrates appear to be an attractive drug delivery system. They have the potential for loading drugs into a matrix of needles and efficiently releasing them in the skin by biodegradation or dissolution in the interstitial fluid; a one-step application. If left in the skin, these types of needles safely degrade and eventually disappear, which are free from the risk of complications of the ones fabricated from silicon, metal and glass.

Based on these observations, in this study, novel dissolving MNs fabricated from hyaluronic acid (HA) were developed, evaluated their characteristics and assessed the improvement on transdermal delivery of relatively high molecular weight drugs. Moreover, insulin-loaded MNs with insulin containing in whole needles were fabricated and characterized their ability of transdermal delivery of insulin to type 1diabetic rats in an *in vivo* system. Furthermore, in order to minimize the wastage of drug, accurately control the drug dose and achieve a fast drug delivery, exendin-4 tip-loaded MNs were developed and investigated their acute efficacy in type 2 diabetic GK/Slc rats in an *in vivo* system.

1. The characteristics of novel microneedle arrays fabricated from hyaluronic acid

MNs fabricated from HA have 190 microneedles in a circular array with a diameter of 10 mm. Each needle was approximately 800 μm in height, and had a diameter of 160 μm at the base and 40 μm at the tip. After being inserted into the dermis, they created drug permeation pathways that had a similar shape to the inserted MNs in the histological cross-section of skin. The MNs containing blue dyes uniformly created pathways on surface of rat skin, and the blue dots corresponded to the

injection sites of the MNs. After the application onto rat skin *in vivo*, MNs appeared to slightly dissolve at 5 min and approximately completely dissolve within 1 h. Moreover, the MNs significantly increased transepidermal water loss and reduced transcutaneous electrical resistance, indicating that they could puncture the skin and create drug permeation pathways successfully. Both of the value almost recovered to baseline levels in the MN group, and relatively small pathways created by the microneedles rapidly recovered as compared with those created by a tape stripping treatment. Slight erythema but no edema appeared at the injection sites at 1 h and disappeared within 24 h. The primary irritation index of the MNs was calculated to be 1.7, indicating that irritation and skin damage caused by MNs were slight. Furthermore, in this study, fluorescein isothiocyanate-labeled dextran with an average molecular weight of 4 kDa (FD4) was used as a model drug with a relatively high molecular weight. It was found that the transdermal permeability of FD4 using the MNs was much higher than that of the FD4 solution. Furthermore, the MNs were much more effective for increasing the amount of FD4 accumulated in the skin. These findings indicated that using novel MNs fabricated from HA is a very useful and effective strategy to improve the transdermal delivery of drugs, especially relatively high molecular weight drugs without seriously damaging the skin.

2. Application in the transdermal delivery of diabetes drugs

Insulin-loaded MNs with insulin containing in whole needles were uniform in size with sharp tips. They maintained their skin piercing abilities for at least 1 h, even at a relative humidity of 75 %. After storing insulin-loaded MNs for a month at −40, 4, 20, and 40 °C, more than 90 % of insulin remained in MNs at all temperatures. It was also found that insulin was released from MNs at a relatively constant rate via an *in vitro* release study, and the majority of the insulin was released within 1 h. These findings were consistent with the complete dissolution of MNs within 1 h of application to rat skin *in vivo*. Therefore, the novel HA MNs possess self-dissolving properties after their dermal application, and insulin appears to be rapidly released from these MNs. Furthermore, a dose-dependent hypoglycemic effect and transdermal delivery of insulin were observed after a dermal treatment with insulin-loaded MNs *in vivo*. A continuous hypoglycemic effect was observed after 0.25 IU of insulin was administered to skin via MNs. Additionally, lower peak plasma glucose levels, but higher plasma insulin concentrations after 2 h, were achieved with 0.25 IU of insulin administered via MNs as compared to the subcutaneous administration of insulin of the same dose. Pharmacodynamic and pharmacokinetic parameters indicated that insulin administered via MNs was almost completely absorbed from the skin into the systemic circulation, and that the hypoglycemic effect of insulin-loaded MNs was almost similar to that of the subcutaneous injection of insulin. These findings indicate that the novel insulin-loaded MNs fabricated from HA are a very useful alternative method of delivering insulin via the skin into the systemic circulation without inducing serious skin damage.

In order to further improve the efficacy of soluble MNs, drug tip-loaded MNs were designed and developed. FD4 was selected as a model drug, and content in FD4 tip-loaded MNs was shown with standard error below 10 % in the microneedles with dosage of 1, 2 and 4 μg/patch. *In vitro* release of drug was rapid even at 30 s at the beginning, and the majority of the FD4 was released within 5 min. Furthermore, in the acute efficacy study in type 2 diabetic GK/Slc rats, exendin-4 tip-loaded MNs showed a nearly equivalent efficacy as subcutaneous injection on glucose tolerance and enhancement of insulin secretion. The pharmacokinetic property of exendin-4 tip loaded MNs was observed closely matched the same dosage exendin-4 subcutaneous injection.

In conclusion, these findings indicate that the novel soluble MNs fabricated with HA were very useful alternative method to deliver drug from the skin to the systemic circulation without serious skin damage. Therefore, the HA MNs might be effective and safe dosage form for transdermal delivery of insulin and exendin-4 in clinical applications for the treatment of diabetes.

審査の結果の要旨

一般に、インスリンなどの生理活性を有するペプチド・タンパク性医薬品は、臨床上、注射により投与されている。しかしながら、注射は痛みを伴い、またアレルギーなどの副作用を発現しやすいという欠点を有することから、注射に代わる新しい投与経路が注目されている。これら投与経路のうち、経皮投与は、薬物の全身作用発現を期待する投与経路として注目されているが、皮膚の最外層には角質層が存在し、薬物の透過の最大の透過障壁となるため、水溶性や高分子薬物の透過が制限されている。したがって、薬物の経皮適用を可能にするためには、薬物の皮膚透過性を改善する必要がある。

こうした観点から、現在までに経皮吸収促進剤の利用、プロドラッグ修飾、イオントフォレシスの利用、ソノフォレシスの利用などの様々な経皮吸収改善方法が試みられているが、高分子薬物の経皮吸収性を有効かつ安全に改善した例は少ないのが現状である。そこで本研究では生体内で自己溶解し、安全性が高いヒアルロン酸を素材としたマイクロニードル (MN) を用いて、糖尿病治療薬であるインスリン及び Exendin-4 の経皮吸収性の改善を試みた。

まず、MN の製剤特性や安全性について検討した。その結果、MN を除毛したラット皮膚に投与したところ、MN は皮膚に均一に挿入できることが認められた。また、MN は皮膚適用後体液より 1 時間内で速やかに溶解し、薬物を皮膚内で放出、拡散した。また、MN 適用による皮膚の水分蒸散量や皮膚電気抵抗値がコントロール群と比べ変化したことから、MN は角質層バリアー機能を低下させることが明らかになった。しかしながら、これらの変化は Tape stripping 群と比べ軽微であり可逆的であることから、MN がきわめて安全であることが確認された。

次に MN を用いた糖尿病治療薬インスリンの経皮吸収性の改善について検討した。その結果、MN 中のインスリン含有量は均一であり、長期の高温保存においても安定であることが明らかになった。また、*in vitro* における MN の適用によりインスリンの経皮透過性が顕著に増大し、インスリンの累積透過量曲線のラグタイムを短縮したことが認められた。また、*in vivo* における MN によるインスリンの経皮投与は、糖尿病モデルラットの血糖降下及び血中インスリン増加を顕著に促進し、その変化はインスリン投与量に依存的であることが明らかになった。さらに、MN によりインスリンを投与した際の血糖値および血中インスリン濃度変化は、皮下投与に比べほぼ同等の値になることが確認できた。また、MN の各種投与方法のうち、コーティング MN 及びインスリン含有 MN を適用した際にインスリンの経皮吸収性が顕著に改善することが認められた。

さらに、GLP-1 のアナログである Exendin-4 を先端に搭載した生体分解性 MN を調製し、２型糖尿病のモデル動物である GK/Slc ラットを用いて Exendin-4 含有 MN の皮膚適用後の有効性について検討した。その結果、Exendin-4 含有 MN の皮膚適用後、グルコース耐糖効果ならびにインスリン分泌の増大が観察され、その効果は皮下投与の場合とほぼ同等であることが認められた。したがって、Exendin-4 含有 MN は、２型糖尿病治療の際に Exendin-4 の皮下投与に代わるきわめて有用な投与形態であることが確認できた。

以上のことから、本 MN は、糖尿病治療薬であるインスリン及び Exendin-4 の経皮吸収改善に有効であることが認められた。本 MN は安全性にも優れていることから、今後、有効かつ安全性の高い経皮適用製剤としての臨床応用が期待される。

学位論文とその基礎となる報文の内容を審査した結果、本論文は博士（薬学）の学位論文としての価値を有するものと判断する。