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Short communication

Dermal administration of manganese porphyrin by iontophoresis

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ABSTRACT

The present study describes a technique for dermal administration of cationic manganese porphyrin (Mn-porphyrin), an antioxidant with superoxide dismutase (SOD) activity, in hairless mouse. In general, the stratum corneum on the surface of the skin represents a barrier to passive diffusion of therapeutic agents by standard dermal administration. The present study investigated whether, dermal administration of Mn-porphyrin solution using iontophoresis, the electrical dermal administration technique, could overcome this barrier. We visually confirmed that Mn-porphyrin had penetrated to the reverse side of the hairless mouse skin after iontophoresis for a short period. With prolonged iontophoresis, the ratio of detectable Mn-porphyrin solution on the reverse side of the hairless mouse skin increased.
In the future, this technique could provide an innovative approach for delivery of this antioxidant in intractable disease.

1. Introduction

Porphyrin has been used effectively in laser photo-dynamic therapy (PDT) for cancer. However, PDT must have been living in a dark room after treatment by side effect on the photosensitive epilepsy, which are needed to improve the quality of life (QOL) for patient. In addition, PDT has only been applied to cancer therapy to date and may not be suitable for other intractable diseases.

Manganese porphyrins (Mn-porphyrin) are metal complexes produced by introducing manganese into porphyrins, and provide promising superoxide dismutase (SOD) mimics with chemical versatility [1]. They have been widely and successfully used in different models of oxidative stress and are either progressing towards, or have entered, phase 1 clinical trials. SOD is an essential enzyme that eliminates superoxide radical anion ($O_2^{\bullet -}$). In this way, it contributes to protecting cells from damage induced by reactive oxygen species (ROS) in diseases, such as cancer, Parkinson's disease, and Alzheimer's disease [2,3]. The potential applications of porphyrin–metal complexes with SOD activity research have been actively investigated over the past decade [2–5]. To expand the range of potential of biomedical applications, novel delivery routes should be explored.

Iontophoresis is a ground-breaking technique facilitating the migration of ions in solution into the body under the influence of an electrical current [6]. As shown in Fig. 1(a), the therapeutic agent may fail to penetrate the stratum corneum by passive diffusion, but when an electrical field is applied, the therapeutic agent moves away from the electrode of the same charge and enters the stratum corneum, mainly via hair pores and sweat ducts (Fig. 1(b)). This potentially enables drugs to enter blood vessels close to the skin surface rapidly, without the invasiveness of an injection. The speed of this process and concentration of drug delivered by iontophoresis depends on its molecular weight, whereby larger drugs show slower passage through the skin [6]. The ease of application, the minimization of systemic side effects, and the increased drug penetration directly into the target region have resulted in extensive clinical use of iontophoresis, mainly in the field of transdermal drug delivery. Transdermal delivery of Mn-porphyrin by iontophoresis has advantages over the use of porphyrin only, such as improved QOL, expanding its application to other intractable diseases and improving treatment speed. The present study explored the development of a successful technique using iontophoresis for efficient transdermal delivery of an aqueous solution of Mn-porphyrin.

2. Materials and Methods

2.1. Materials

The following compounds were purchased from the companies indicated in parentheses: Propionic acid (Kanto Chemical Co., Inc.), pyrrole (Kanto Chemical Co., Inc.), 4-pyridinecarboxaldehyde (Tokyo Kasei Co., Ltd.), tetrahydrofuran of extra pure grade (THF,

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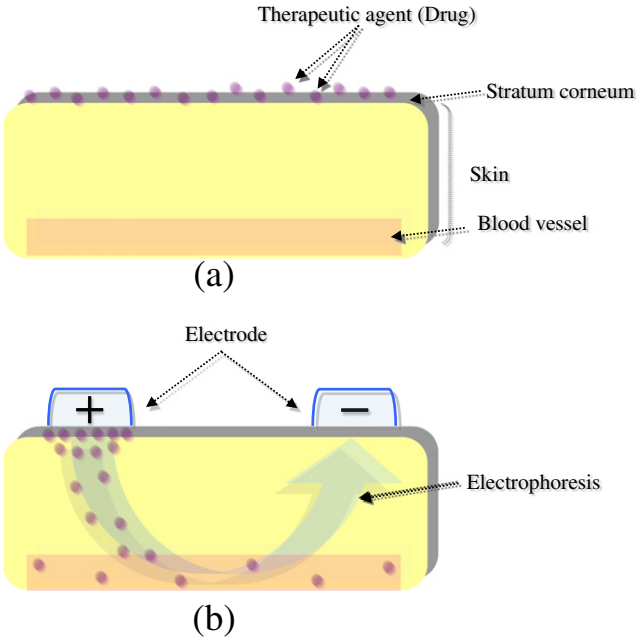

Fig. 1. A schematic of dermal administration (a) without iontophoresis (passive diffusion) and (b) with iontophoresis.

Kanto Chemical Co., Inc.), *N,N*-dimethylformamide (DMF, Kanto Chemical Co., Inc.), methyl *p*-toluenesulfonate (Kanto Chemical Co., Inc.), ammonium hexafluorophosphate (NH_4PF_6, Aldrich), tetraethylammonium chloride (TEAC, Tokyo Kasei Co., Inc.), and manganese (II) acetate tetrahydrate (Nacalai Tesque, Inc.).

2.2. Synthesis of Mn-porphyrin

Cationic manganese porphyrin (Mn-porphyrin, MnTM4PyP) was synthesized, as reported previously [7,8]. Briefly, 250 mL of propionic acid was stirred at 100 °C under nitrogen. After adding 4-pyridinecarboxaldehyde to the propionic acid under these conditions, pyrrole was continuously added. After reaction termination, the solution was cooled to room temperature and then dried. The sample was neutralized by adding ammonia water; tetrakis-(4-pyridyl)-porphyrin (T4PyP) was produced with an aspirator after dissolved impurity by THF.

This produced 0.2 g of T4PyP, which was dissolved with 80 mL of DMF. Methyl *p*-toluenesulfonate (10 mL) was added and it was then heated to 100 °C. After 20 h, the sample was cooled to room temperature and then dried. After removing methyl *p*-toluenesulfonate from the sample, ammonium hexafluorophosphate was continuously added and the sample was cooled. The collected sample was dissolved with acetone and stirred for 1 h after adding a small amount of tetraethylammonium chloride (TEAC). Then, the sample was cooled for 2 h and the resulting porphyrin was dissolved with methanol. Tetrakis-(4-*N*-methylpyridyniumyl)-porphyrin (TM4PyP) was produced by evaporating this solution.

The TM4PyP produced (0.2 g) was dissolved with methanol and the solution was heated to 80 °C. Manganese (II) acetate tetrahydrate (0.24 g) was added to the heated solution. After reaction termination, the solution was cooled to room temperature and the sample was evaporated. After dissolving with water, a little NH_4PF_6 was added and stirred for 1 h. Then, the sample was cooled and collected. The collected samples were dissolved with acetone and a small amount of TEAC. The reacted samples were collected and dissolved in methanol. MnTM4PyP (Mn-porphyrin) was produced by evaporating this solution.

Confirmation of TM4PyP and MnTM4PyP synthesis was carried out as reported in the literature [1,8]. TM4PyP was characterized by nuclear magnetic resonance (^{1}H-NMR), as shown in Fig. 2. The peak indicated provided exact confirmation of TM4PyP. Porphyrin origin: −3.1 ppm (1H of inner of porpyrin); 4.3 ppm (methyl group of methyl pyridinium); 9.0 and 9.5 ppm (1H of methyl pyridinium); and 9.2 ppm (singlet peak of β pyrrole). Conter-ion origin: 2.3 ppm (methyl group of methyl p-toluenesulfonate) and 7.1 and 7.5 ppm (benzene ring origin of methyl p-toluenesulfonate). Solvent: 2.5 ppm (dimethyl sulfoxide) and 3.4 ppm (water).

MnTM4PyP was characterized by spectrometry at 463 nm. The chemical formula of the Mn-porphyrin synthesized is presented in Fig. 3.

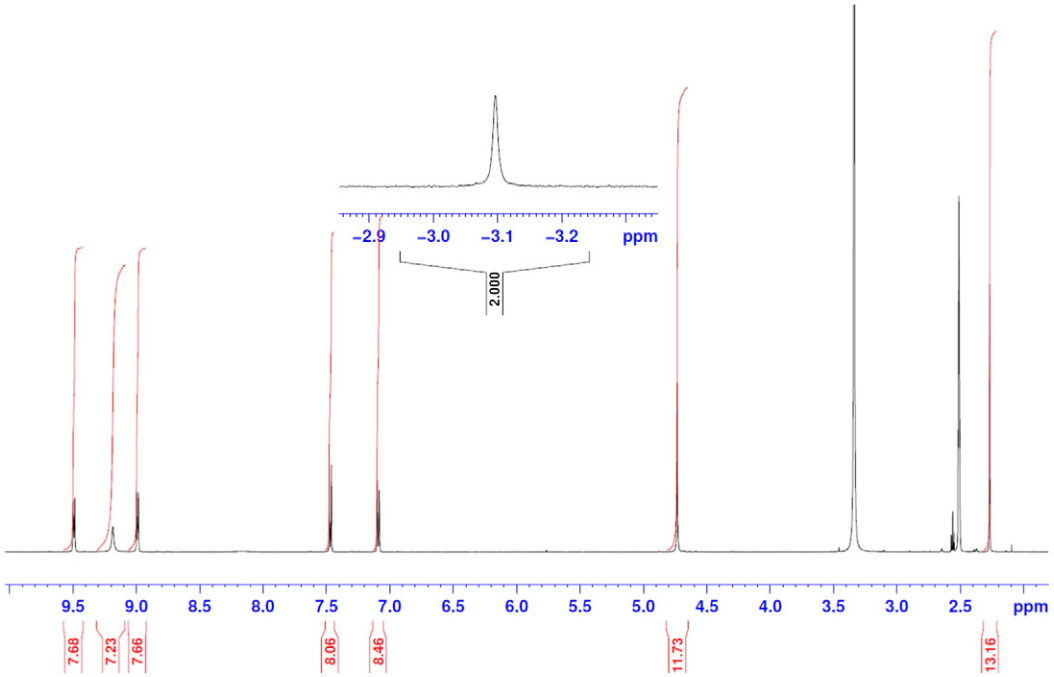

Fig. 2. The chart of nuclear magnetic resonance (^{1}H-NMR) of TM4PyP.