Journal of Plastic, Reconstructive & Aesthetic Surgery (2015) 68, 104–112

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

Quantification of the effect of Lipo-PGE1 on angiogenesis

SuRak Eo [a,*], Chan Kwon [a], HyunJic Lee [a], SangHun Cho [a], JinYoung Kim [b], GooHyun Baek [c], JeongSeok Yeo [d], ChiYeon Lim [e]

[a] *Department of Plastic and Reconstructive Surgery, DongGuk University Graduate School of Medicine, Seoul, South Korea*
[b] *Department of Orthopaedic Surgery, DongGuk University Graduate School of Medicine, Seoul, South Korea*
[c] *Department of Orthopaedic Surgery, Seoul National University Hospital, Seoul, South Korea*
[d] *Department of Nuclear Medicine, DongGuk University Graduate School of Medicine, Seoul, South Korea*
[e] *Department of Clinical Trial Test, DongGuk University Graduate School of Medicine, Seoul, South Korea*

Received 28 March 2014; accepted 15 September 2014

KEYWORDS
Lipo-PGE1;
Prostaglandin E1;
Angiogenesis-inducing agents;
Vasodilators

Summary Fifteen rabbits were used to assess the effect of Lipo-PGE1 on neovascularization. Merocel® and Alloderm® of the same size were implanted separately under the back skin to act as matrices for vessel growth. Lipo-PGE1 was injected intravenously for 2 weeks in an experimental group of eight rabbits, and they were compared with a control group of seven untreated animals. Blood flow was measured using the $^{99m}TcO_4^-$ clearance technique. The mean blood clearance halftime ($T_{1/2}$) and washout radioactivity were measured. Newly formed vessels were counted by CD31. The mean clearance halftime was 4005 ± 2161.3 and 13840 ± 4644.6 s in the experimental and control group, respectively, in the 1 × 2 × 1.5-cm-sized implants ($p = 0.0125$), and 1560 ± 1174.7 and 3405 ± 807.03 s, respectively, in the 2 × 2 × 1.5-cm-sized implants ($p = 0.0413$). Histological examinations revealed that the mean numbers of newly formed vessels in the experimental and control groups were 11 ± 1.58 and 7.8 ± 1.71, respectively, in the 1 × 2 × 1.5-cm-sized implants ($p = 0.0501$), and 20.19 ± 12.47 and 12.33 ± 3.25, respectively, in the 2 × 2 × 1.5-cm-sized implants ($p = 0.02679$). Lipo-PGE1 was found to be effective in promoting angiogenesis in a rabbit matrix model.

* Corresponding author. Department of Plastic and Reconstructive Surgery, DongGuk University Medical Center, 814 SikSa-dong, IlSandong-gu, GoYang-si, Gyeonggi-do 410-773, South Korea. Tel.: +82 31 961 7330; fax: +82 31 961 7347.
E-mail address: surakeo@yahoo.com (S. Eo).

http://dx.doi.org/10.1016/j.bjps.2014.09.035

Introduction

Prostaglandin E1 (PGE1) is widely used in many clinical fields because of its beneficial effects on vasodilation, platelet disaggregation, blood viscosity, and fibrinolysis.[1,2] It is known to be an effective treatment in various peripheral vascular diseases, such as ischemic limbs,[3,4] diabetic skin ulcers,[5] infarcted myocardium,[6,7] pulmonary hypertension,[8] Raynaud's phenomenon,[9] neurogenic intermittent claudication,[10] spinal canal stenosis,[11] and ischemic gastric tube.[12]

In addition to its well-accepted effects of vasodilation and platelet disaggregation, the angiogenic potential of PGE1 has received considerable attention in the literature.[13] Weiss[3] and Carlson[14] introduced PGE1 as an angiogenic stimulant in clinical practice in 1973, when it was used as a therapeutic strategy for stimulating postnatal neovascularization.

"Therapeutic angiogenesis" exploits the natural process that enhances neovascularization in tissue ischemia, and it is used to stimulate the formation of new blood vessels in ischemic tissues or organs. Angiogenesis is essential for wound healing, tissue growth, and for enhancing the survival of flaps and composite grafts.[1,2] Many trials have been conducted on the pharmaceutical enhancement of therapeutic angiogenesis to recover ischemic tissues, and in a previous study, we showed that Lipo-PGE1 enhances composite graft survival after fingertip amputations.[15] Lipo-PGE1 is a new PGE1 preparation and its lipid component retards metabolic decay in the lung and diminishes adverse reactions.[5,14]

The quantification of neoangiogenesis in ischemic tissues is important and various angiogenesis assays that use different image processing procedures have been devised. Although the merits and demerits of these assays remain topics of study, the monitoring of blood flow using radioisotope tracer-based clearance methods have been shown to be reproducible and accurate.[16] Using our clinical data, we undertook to evaluate and quantify the angiogenic potential of lipo-PGE1 using an in vivo rabbit matrix implantation model and nuclear scanning techniques.

Materials and methods

All animal experiments were performed after obtaining approval from the Institutional Animal Care and Use Committee of the Clinical Research Institute at the DongGuk University Medical Center. National Research Council guidelines for the care and use of laboratory animals (revised in 1996) were observed throughout.

Merocel® and Alloderm®

Merocel® (Medtronic, Jacksonville, FL, USA) is a tampon nasal standard replacement for cellulose- and cotton-based products (Figure 1). It is composed of a biocompatible synthetic material produced by the cross-linking of polyvinyl alcohol and has an open cell structure; that is, all pores are open and it contains no "dead-end pockets" that could contain residues. Merocel® is exceptionally strong and durable, yet soft and comfortable when hydrated. For the above mentioned reasons, we chose it as a matrix for vessel growth. Merocel® was applied in two different sizes, that is, at 1 × 2 × 1.5 cm (four in the experimental group and three in the control group) and 2 × 2 × 1.5 cm (four in each group).

Alloderm® is an acellular tissue matrix that promotes tissue regeneration by supporting rapid revascularization, white cell migration, and cell repopulation, and when transposed into host tissue provides a strong, natural repair.

Merocel® or Alloderm® of the same size was implanted separately under skin flaps on the left and right dorsal skin.

Animal model

All animal experiments were performed in accordance with the guidelines issued by our institution's animal care committee. The study protocol was approved by the Institutional Ethics Committee at the DongGuk University Medical Center. The subjects were 15 male and female New Zealand white rabbits of an average weight of 3.2 kg (range, 3.0—3.5 kg). The animals were randomly allocated to the experimental ($n = 8$) and control ($n = 7$) groups.

For anesthesia, Rompun® (xylazine hydrochloride 23.32 mg/ml, Bayer Korea, Seoul, South Korea) and Zoletil 50® (tiletaminehypochloride 250 mg/5 ml, Virbac, Milperra, Australia) were injected intramuscularly at a dose of 1 ml/kg. Implantation of Merocel® and Alloderm® was carried out using aseptic techniques. Briefly, the dorsal hair was shaved, the skin was wiped with 70% ethanol, and 1-cm-long incisions were made on the left and right sides to create two 2 × 2-cm-sized subcutaneous air pockets. The centers of the Merocel® implants were stitch-marked with 4/0 silk sutures, which were let out through the skin to guide later radioisotope injection. The animals were housed individually in cages and provided with a normal diet and water.

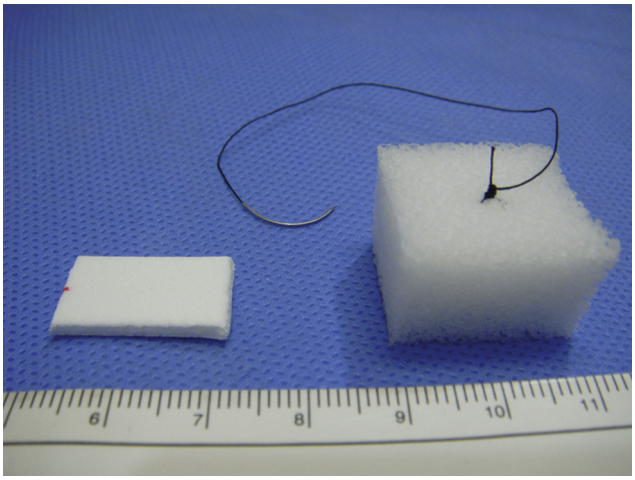

Figure 1 Merocel® is a porous sponge-like material made of cross-linked polyvinyl alcohol, which expands when contacted by water. Two differently sized Merocel® implants (1 × 2 × 1.5 cm and 2 × 2 × 1.5 cm) were inserted subcutaneously under rabbit dorsal skin.