Pneumatic Injection Therapy-Induced Transcutaneous Penetration of Hypertonic Glucose Solution: Macro- and Microscopic Analyses of Human and Rat Tissues

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Background and Objectives

Pneumatic injection therapy (PIT) is a needle-free, penetration-enhanced, transdermal method of delivering drugs into the skin. This study evaluated topographically the PIT-induced transcutaneous infiltration of a 20% hypertonic glucose solution in fresh human cadavers and outlined the microscopic patterns and reactions to PIT with a 20% glucose solution in in vivo rat skin.

Materials and Methods

The penetration depths of a 20% glucose solution by PIT were analyzed macroscopically in 10 hemifaces from fresh cadavers. In addition, the infiltration patterns of PIT with a 20% glucose solution performed in vivo in rats were analyzed through a histology evaluation.

Results

Pneumatic injections of a hypertonic glucose solution generated PIT zones in the subcutaneous fat and superficial temporal fascia layers (10 out of 10 hemifaces), superficial layer of the deep temporal fascia (DTF; 8 out of 10 hemifaces), and deep layer of the DTF (7 out of 10 hemifaces). In addition, distinctive PIT zones were generated in rat tissues from the upper papillary deep to the muscle and fascia layers.

Conclusion

The data suggest that PIT-induced, jet infiltration of a 20% hypertonic glucose solution contributes to clinical improvement of skin laxity by stimulating the wound repair processes and increasing collagen production throughout multiple layers of the skin.

Key words

Transcutaneous pneumatic injection; Prolotherapy; Hyperglycemic solution; Cadaver; Rat; Ultrasound

INTRODUCTION

Prolotherapy refers to perilesional injection therapy of hypertonic glucose solution to induce a hyperglycemic state for treating chronically painful musculoskeletal conditions.^{1,2} The hyperglycemic condition induces wound repair processes marked by infiltration of inflammatory cells; moreover, phenotypical activation of fibroblasts is achieved by mechanical loading of infiltrated solution.³⁻⁸ Thus, the suggested action mechanisms of prolotherapy include the induction of fibroblast proliferation, collagen deposition, and vascular proliferation, as well as the thickening of ligamentous structures.^{1,2,9,10}

Pneumatic injection therapy (PIT) is a needle-free, penetration-enhanced, transdermal method of delivering drugs into targeted areas of the skin.^{11,12} A variety of solutions, including normal saline, 5% or 20% glucose solution, botulinum toxins, and soft tissue fillers, can be forcefully, but with minimal invasiveness, injected into the skin and subdermal layers, as well as the superficial musculoaponeurotic system (SMAS) or muscle layers, depending on the pressure settings.¹¹⁻¹⁵ By doing so, PIT characteristically generates semi-circular or cylindrical zones of tissue infiltration and leaves pin-point entry points in the epidermis.^{11,12} Our study group previously compared PIT-induced tissue reactions with 5% isotonic and 20% hypertonic glucose solutions in *in vivo* micropig skin and tissue-mimicking phantom.¹² PIT of 20% hypertonic glucose immediately generated smaller, homogeneous glucose droplets in the dermis, and elicited more remarkable post-procedural inflammatory cell infiltration, compared to PIT of 5% isotonic glucose solution.¹²

In this study, we aimed to topographically evaluate PIT-induced transcutaneous penetration of 20% hypertonic glucose solution in human fresh cadavers. The layer-dependent infiltration patterns were analyzed immediately after PIT by dissecting the temple of the cadavers. The order of dissection thereof was as follows: the skin, subcutaneous fat, superficial temporal fascia (STF), and the superficial and deep layers of the deep temporal fascia (DTF). Immediate *in vivo* human tissue reactions by PIT were also evaluated using two-dimensional ultrasound. Additionally, in the aforementioned *in vivo* experimental study using micropig skin, we sought to compare the infiltration patterns of different injectable solutions; however, the depths of infiltration over the dermosubcutaneous junction were significantly limited by thick dermal collagen bundles.¹² Therefore, we also performed an *in vivo* study in experimental rats to evaluate the patterns of and reactions to infiltration of 20% hypertonic glucose solution

into the subcutaneous fat and fibromuscular layers.

MATERIALS AND METHODS

Pneumatic injection therapy system and injectable solution

A PIT device (Shemax™; Shenb Co., Ltd., Seoul, Korea) that delivers compressed pneumatic pressure to a disposable nozzle filled with injectable solution was utilized in this study. Depending on the therapeutic purposes and the targeted injection sites, the pneumatic pressure can be adjusted as described in a previous report.¹² The filling volume of injectable solution is determined by regulating the opening duration of the nozzle. Additionally, 20% hypertonic glucose solution (Huons, Bundang, Korea) was used as an injectable solution, and was pretreated with 5% (v/v) methylene blue for macroscopic visualization.

Pneumatic injection therapy on human cadaveric tissue

To evaluate the penetration depths of the hypertonic glucose solution, 10 hemifaces from five legally donated, fresh cadavers (4 males and 1 female; median age: 62 years; age range: 59-65 years) were macroscopically analyzed in this study. Each hemiface was treated with a single pass of PIT on the skin of the temple area. Approximately 30 PIT shots at intervals of 1-2 cm were delivered to each hemiface. The trigger point injection settings comprised a pneumatic pressure of 4.13 bars and an injection volume of 0.08 ml, with a nozzle opening duration of 57 msec/shot. Immediately after the jet infiltration of hypertonic glucose solution, the temple areas of the cadaveric skin were dissected gently in the following order: the skin, subcutaneous fat, STF, and superficial and deep layers of the DTF. PIT zones in each anatomic layer were measured using Image J software (Version 1.48; National Institutes of Health, Bethesda, MD, USA), and the relative areas thereof, which were calculated in reference to the area of PIT zones in the subcutaneous fat layer, are presented as means $\pm$ standard deviation.

Ultrasound examination

Immediate *in vivo* human tissue reactions at various pressure settings were evaluated using two-dimensional ultrasound study in a 40-year-old male volunteer. After obtaining written informed consent, a single pass of PIT was made along the non-hair-bearing area of the frontal hairline and abdominal skin after cleansing with 70% ethanol in the supine position without topical or systemic anesthesia. PIT was performed with the pneumatic pres-

sure settings of 2.05, 2.55, 3.15, 3.63, 4.13, and 4.64 bars; an injection volume of 0.12 ml/shot; and a nozzle opening duration of 61 msec/shot. Each shot was spaced at least 2 cm from the others to avoid overlapping effects from other experimental conditions. Immediately after PIT, two-dimensional ultrasound (ECUBE15; Alpinion Medical Systems Co., Ltd., Seoul, Korea) was emitted at the entry points and treatment sites to evaluate the characteristic features of the infiltrated skin layers. Two-dimensional ultrasound images were obtained using a linear high-frequency hockey stick transducer (IO8-17; Alpinion) at a frequency of 8-17 MHz, measuring maximally 3 cm deep for the frontal hairline and 5 cm deep for the abdomen. The heights and widths of PIT zones are presented as means $\pm$ standard deviation. This study was approved by the Institutional Review Board of International St. Mary's Hospital, Catholic Kwandong University College of Medicine.

Pneumatic injection therapy on *in vivo* rat tissue

All experimental protocols were approved by the ethics committee of the Soonchunhyang University Institutional Animal Care and Use Committee. Eight male, Sprague-Dawley rats were purchased (Orient Bio Corp., Seongnam, Korea) at the age of 6 weeks, and the *in vivo* experiments were performed at the age of 14 weeks at weights of 450-500 g. After cleansing with a mild soap and 70% alcohol, each rat was treated with a single pass of PIT

along the back skin, with a total shot count of 30 shots at intervals of 1-2 cm, under ether anesthesia. The trigger point injection settings comprised a pneumatic pressure of 3.63 bars, an injection volume of 0.06 ml of 20% hypertonic glucose solution, and a nozzle opening duration of 55 msec/shot.

Histologic evaluation

The experimental rats were sacrificed for sampling the treated tissue in a humane manner according to standard protocols. Immediately and 1, 2, and 3 weeks after treatment, full thickness tissue specimens, including the epidermis, dermis, subcutaneous fat, muscle, and fascia, were obtained for microscopic evaluation. Each sample was fixed in 10% buffered formalin and embedded in paraffin. Then, serial tissue sections of 4- μ m thickness for each treatment setting were prepared and stained with hematoxylin and eosin and Masson's trichrome.

RESULTS

Pneumatic injection therapy on human cadaveric tissue

Immediately after PIT, bluish, uniform, pin-point entry points were found in the epidermis in all cadaveric samples (Fig. 1A). Approximately 1-cm, skin-colored papules were generated around the entry points. After dissecting the epidermis, dermis, and subcutaneous fat, round to

Fig. 1. Pneumatic injection therapy (PIT) on human cadaveric tissue. (A) PIT-induced bluish pin-point entry points on the epidermis. The PIT zones of 20% hypertonic glucose solution (B) in the subcutaneous fat (SC) layer and superficial temporal fascia (STF), (C) the superficial layer of the deep temporal fascia (sDTF), and (D) the deep layer of the deep temporal fascia (dDTF). SK, skin.

oval zones of bluish 20% hypertonic glucose solution were found in the subcutaneous fat layer in all hemifaces (Fig. 1B). Moreover, the bluish PIT zones were also found in the STF in all hemifaces, and the relative mean area thereof was remarkably larger (2 ± 0.5) than the infiltrated zones in the subcutaneous fat layer (1 ± 0.7) (Fig. 2).

After removing the STF, the DTF was identified and further divided into the superficial and deep layers. In the superficial layer of the DTF, bluish PIT zones were found in eight (80%) of 10 hemifaces, with a relative mean area of 3.3 ± 1.2 (Fig. 1C). Moreover, the zones of glucose infiltration were found in seven (70%) of 10 hemifaces in the deep layer of the DTF, with a relative mean area of 2.2 ± 0.8 (Fig. 1D). Among the seven hemifaces wherein glucose infiltrated the deep layer of the DTF, the greatest number

Fig. 2. Relative areas of PIT zones in human cadaveric tissue. The values are presented as means $\pm$ standard deviation. The relative area of PIT zones in each of the STF, sDTF, and dDTF was calculated in reference to the PIT area in the subcutaneous fat layer.

of infiltrated zones was found in the subcutaneous fat layer, disregarding the epidermis where the solution entered the skin, and the fewest was observed in the deep layer of the DTF. Meanwhile, the largest zones of glucose infiltration were generated in the superficial layer of the DTF, and the smallest zones were found in the epidermis.

Immediate tissue reactions on two-dimensional ultrasound

Immediately after applying PIT with 20% hypertonic glucose solution on *in vivo* human tissue, the pressure- and site-dependent features of tissue infiltration were evaluated by two-dimensional ultrasound examination. To do so, PIT was delivered on the non-hair-bearing area of the frontal hairline and abdominal skin to obtain ultrasound images free from interference by hair shafts. The PIT settings of 2.05 bars and 0.12 ml/shot elicited well-demarcated, heterogeneous infiltration of glucose solution throughout the dermis and in the dermosubcutaneous fat junction (mean height, 3.5 ± 0.5 mm; mean width, 9.2 ± 0.4 mm), with remarkable postacoustic shadowing (Fig. 3A). The settings of 2.55 bars and 0.12 ml/shot also generated well-demarcated, heterogeneous, oval PIT zones throughout the dermis and subcutaneous fat layer (mean height, 5.1 ± 0.4 mm; mean width, 14 ± 1.9 mm) (Fig. 3B). Moreover, the central and superficial parts of PIT zones, which surrounded the entry points, showed hyperechogenic infiltration, compared to the peripheral and deeper parts of PIT zones, in the settings of both 2.05 and 2.55 bars.

At the pressure settings of 3.15, 3.63, and 4.13 bars and an injection volume of 0.12 ml/shot, well-demarcated,

Fig. 3. Ultrasound examination. Two-dimensional ultrasound images exhibiting PIT zones (white circles) immediately after PIT on the non-hair-bearing area of the frontal hairline using 20% hypertonic glucose solution in *in vivo* human. The PIT settings included an injection volume of 0.12 ml/shot and a pressure of (A) 2.05 bars, (B) 2.55 bars, (C) 3.15 bars, and (D) 3.63 bars. A linear high-frequency hockey stick transducer at a frequency of 8-17 MHz. Asterisks indicate postacoustic shadowing. E, epidermis; D, dermis; S, subcutaneous fat layer; FM, fascia and muscle; PO, periosteum.

heterogeneous, round to oval PIT zones were observed at the levels of the dermis, subcutaneous fat, facial muscle and fascia, and periosteum (Fig. 3C, D, and 4A). Meanwhile, however, the PIT zones became wider and deeper with higher pneumatic pressures (3.15 bars, mean height of 7.6 ± 0.2 mm and mean width of 9.6 ± 0.2 mm; 3.63 bars, mean height of 8.6 ± 0.2 mm and mean width of 12.4 ± 0.6 mm; 4.13 bars, mean height of 8.3 ± 0.2 mm and mean width of 11.5 ± 0.5 mm). Additionally, PIT on abdominal skin at the pressure settings of 3.63, 4.13, and 4.64 bars and an injection volume of 0.12 ml/shot exhibited narrower, but deeper infiltration patterns of 20% glucose solution, compared to PIT on the frontal hairline (Fig. 4B-D). The PIT zones showed a mean height of 17.5 ± 0.3 mm and a mean width of 14.5 ± 0.6 mm at the pressure of 3.63 bars, a mean height of 16.8 ± 0.2 mm and a mean width of 15.8 ± 0.5 mm at 4.13 bars, and a mean height of 15.1 ± 0.6 mm and a mean width of 18.1 ± 0.1 mm at 4.63 bars.

Pneumatic injection therapy on *in vivo* rat tissue

Immediately after PIT with 20% hypertonic glucose solution at the injection volume of 0.06 ml/shot and a pneumatic pressure of 3.63 bars, distinctive PIT zones were generated in rat tissues from the upper papillary deep to the muscle and fascia layers (Fig. 5A-C). Each PIT zone was composed of numerous round to oval droplets that surrounded entry points and of sigmoid-shaped central droplets. The surrounding glucose droplets in the mid and lower dermis and subcutaneous fat tissue were larger in size, compared to those in the upper dermis. Furthermore, the glucose droplets infiltrated extensively into the superficial fascia layer, and several droplets were found

in the muscular layer (Fig. 5D-F). In the deep fascia layer, the glucose solution infiltrated by penetrating through the fibrous septum of skeletal muscles.

At one week post-PIT, glucose droplets were not observed in any layers of the rat skin. Collagen fibers in the dermis, the fibrous septum of the subcutaneous fat and skeletal muscle, and the superficial and deep fascia layers were fragmented and irregularly arranged, but were thicker than in controls (Fig. 6A, D). At two weeks post-PIT, we noted marked increases in thickened collagen fibers in all layers of the rat specimens (Fig. 6B, E). At three weeks post-PIT, the amounts of the thicker collagen bundles had remarkably increased in the dermis, subcutaneous fat, and superficial fascia layers, compared to control, 1 week post-PIT, and 2 weeks post-PIT specimens (Fig. 6C, F).

DISCUSSION

In this study, we aimed to topographically evaluate the patterns of PIT-induced, layer-dependent, glucose infiltration immediately after delivering PIT on the temporal scalp. To do so, we obtained horizontal macroscopic views of the layers of fresh cadaveric tissues by gently dissecting each layer of the skin and subcutaneous fat tissue, STF, and superficial and deep layers of the DTF.

In previous cadaveric study, injectable solutions were shown to generate semi-circular PIT zones throughout the epidermis, dermis, subcutaneous fat, and SMAS layers at the pressure setting of 6 bars.¹¹ The size of PIT zones was deemed to be greatest in the SMAS, followed by the subcutaneous fat tissue and upper part of the der-

Fig. 4. Ultrasound examination. Two-dimensional ultrasound images demonstrating PIT zones (white circles) immediately after PIT on the non-hair-bearing area of the (A) frontal hairline and (B-D) abdomen using 20% hypertonic glucose solution in *in vivo* human. The PIT settings included an injection volume of 0.12 ml/shot and a pressure of (A) 4.13 bars, (B) 3.63 bars, (C) 4.13 bars, and (D) 4.64 bars. A linear high-frequency hockey stick transducer at a frequency of 8-17 MHz. Asterisks indicate post-acoustic shadowing. E, epidermis; D, dermis; S, subcutaneous fat layer; FM, fascia and muscle; PO, periosteum.

Fig. 5. Immediate PIT-induced tissue reactions in *in vivo* rat tissue. (A, D) Untreated rat tissue. (B, C, E, F) Immediate PIT-induced tissue reactions in *in vivo* rat tissue after the jet-infiltration of 20% hypertonic glucose solution. (B, E) Insets show magnified areas of (C) the epidermis and dermis and (F) the fibromuscular layers. Masson's trichrome stain, original magnification (A, B, E) $\times 40$ and (C, D, F) $\times 100$, scale bar = 200 μm .

Fig. 6. Delayed PIT-induced tissue reactions in *in vivo* rat tissue. Post-PIT tissue reactions in the epidermis, dermis, subcutaneous fat, and fibromuscular layers at (A, D) one week, (B, E) two weeks, and (C, F) three weeks after treatment. Masson's trichrome stain, original magnification (A-C) $\times 40$ and (D-F) $\times 100$, scale bar = 200 μm .

mis.¹¹ Meanwhile, PIT at an 8.5-bar pressure setting histologically exhibited cylindrical infiltration zones throughout the epidermis, dermis, subcutaneous fat, SMAS, and

masseter muscle layers. Thereby, the size of PIT zones were deemed uniform throughout the infiltrated layers.¹¹

In our cadaveric study, distinctive PIT zones were mac-

roscopically found in the epidermis, dermis, subcutaneous fat, STF, and DTF layers at the pressure setting of 4.13 bars. The layered dissection of post-PIT cadaveric tissues revealed the generation of greater PIT zones in the STF and DTF in most of the cases, compared to the subcutaneous fat layer. We suggest that the relatively lower pressure setting could effectively reach target layers of the STF and DTF during face lifting; however, the degrees and patterns of infiltration differed in individual cadaveric tissues.

In this study, two-dimensional ultrasound examination exhibited well-demarcated PIT zones of tissue infiltration immediately after PIT using 20% glucose solution in *in vivo* human tissue. At lower-pressure settings, such as 2.05 and 2.55 bars, in the frontal hairline, PIT zones were observed throughout the epidermis and dermis with central hyperechogenicity, and the largest area of tissue infiltration was found in the mid portions of the dermis. Meanwhile, at higher pressure settings, such as 3.15, 3.63, and 4.13 bars, in the frontal hairline, PIT zones were generated throughout the skin, subcutaneous fat, muscle and fascia, and periosteum layers with peripheral hyperechogenicity. Moreover, the largest relative area of tissue infiltration was found deeper in the subcutaneous fat or muscle and fascia layers at higher pressure settings.

Our previous *in vivo* micropig study compared the infiltration patterns of 5% isotonic and 20% hypertonic glucose solutions injected with PIT.¹² Therein, PIT zones generated by 20% glucose solution were composed of small, homogeneous glucose droplets in the dermis, whereas 5% glucose solution generated PIT zones of heterogeneous glucose droplets of varying sizes in the dermis.¹² Unfortunately, the thick collagen bundles in the dermis of the micropig skin limited the depth of penetration, and as such, the PIT-induced infiltration patterns in the subcutaneous fat, muscle, and fascia layers could not be evaluated. Therefore, in this study, we performed PIT on rat skin, and full-thickness tissue specimens, which contained skin, subcutaneous fat, superficial fascia, skeletal muscle, and deep fascia layers, were evaluated.

The thickness of the temple area, which consists of six or seven layers, decreases with age.¹⁶⁻¹⁸ The age-related volume loss of the superficial or deep part of fat tissues or temporalis muscle has been suggested to contribute thereto.¹⁷⁻¹⁹ Because decreased volumes in the topographic components in the temple area are associated with loosening among the skin layers, the laxity of the face can eventually be aggravated.¹⁷⁻¹⁹ By inducing the wound repair process and tightening among the topographic layers of the temple area, prolotherapy is theoretically

effective for the restoration of facial skin laxity. Our microscopic *in vivo* experimental rat study revealed that PIT-induced jet-infiltration of 20% glucose solution reached the layers of skeletal muscle and deep fascia. Furthermore, PIT-induced tissue injury and accompanying wound repair resulted in marked increases in collagen production throughout the infiltrated zones over the duration of 3 weeks post-PIT. Accordingly, in light of these results, we propose that PIT-induced wound repair and increased collagen fibers throughout the multilayers of the temporal area elicited by jet-infiltration of 20% hypertonic glucose solution can effectively contribute to clinical improvement in face lifting.

In conclusion, our topographical study using human fresh cadavers demonstrated that PIT with 20% glucose solution penetrates to the deep part of the DTF. Moreover, our *in vivo* human study demonstrated that the injectable solution could be more effectively delivered peripherally and deeply into the target tissue at higher pressure settings. Finally, *in vivo* experimental rat study revealed that the jet-infiltrated glucose solution penetrates to the deeper fascia layers and stimulates wound repair processes and collagen production. Our findings suggest that PIT jet-infiltration of 20% hypertonic glucose solution can contribute to clinical improvements in skin laxity by stimulating wound repair processes and increasing collagen production throughout multiple layers of targeted skin tissue.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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