Paravertebral fascial massage promotes brain development of neonatal rats via the insulin-like growth factor 1 pathway**☆

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Abstract
Massage in traditional Chinese medicine can promote body and brain development of premature and normal newborn infants. In the present study, neonatal rats (1 day old) underwent paravertebral fascial massage (15 consecutive days), followed by subcutaneous injection of insulin-like growth factor 1 receptor antagonist, JB1 (9 consecutive days). Paravertebral fascial massage significantly increased insulin-like growth factor 1 expression and cell proliferation in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus. However, JB1 inhibited this increase. Results suggest that paravertebral fascial massage can promote brain development of neonatal rats via the insulin-like growth factor 1 pathway.

Key Words
paravertebral fascial massage; insulin-like growth factor 1; neurogenesis; immunohistochemistry; neural regeneration

Abbreviations
IGF-1, insulin-like growth factor 1; BrdU, 5-bromodeoxyuridine

INTRODUCTION
Massage is an established treatment in traditional Chinese medicine, which over the past few decades has grown in popularity on account of unraveling its scientific methodology[1-2]. Understanding the biological mechanisms promoted by massage, allows targeted patient intervention. In recent years, controversy has surrounded research into the biological mechanisms of massage in determining whether regulation occurs through myofascial or neurophysiologic pathways[3-5]. It has been previously proposed that the fascia is the provides the morphological basis of channels (meridians) and collaterals[6-7] which permit the therapeutic benefits of traditional massage, which might be key to understanding its biological mechanism. Massage up-regulates expression of insulin-like growth factor 1 (IGF-1) in premature and normal newborn infants, promoting their growth and development. Furthermore, IGF-1 can pass through the blood-brain-barrier and promote brain development[8-10]. Growth hormone/IGF-1 deficiency is prevalent in children with cerebral palsy[11-12]. Physical therapy and hormone replacement therapy, with exogenous recombinant growth hormone/IGF-1, have been found to be beneficial to recovery of motor function in children with cerebral palsy, suggesting IGF-1 in the blood circulation can promote recovery of brain function[13]. Serous IGF-1 is an important medium in brain activity and


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plays a leading role in brain function\cite{14}. In the present study, changes in IGF-1 expression and neural stem cell proliferation were observed following paravertebral fascial massage. Using IGF-1 receptor antagonist, we aimed to determine whether IGF-1 play a major role in brain development of neonatal rats.

RESULTS

Quantitative analysis of animals
A total of 60 rats (1 day old) were randomly assigned to five groups: massage (paravertebral fascial massage for 15 days), maternal separation (no massage), control (living with mother with no massage), massage (9 days) plus antagonist JB1 and massage (9 days) plus subcutaneous injection (days 1–9) of saline. Twelve rats were used for each group. All 60 rats were included in the final analysis.

IGF-1 expression in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus
Immunohistochemical staining revealed that after day 15 of paravertebral fascial massage, the number of IGF-1- and 5-bromodeoxuryridine (BrdU)-positive cells was significantly increased in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus in the massage group compared with the maternal separation and control groups ($P < 0.05$; Figures 1, 2).

Figure 1  Representative immunohistochemistry images of insulin-like growth factor 1 (IGF-1)-positive cells (brown stain) and graphs showing the number of IGF-1-positive cells in the subventricular zone (SVZ) of the lateral ventricle and dentate gyrus (DG) of the hippocampus 15 days after massage (immunohistochemical staining). IGF-1-positive cells were observed in the SVZ of the lateral ventricle (A–C) and DG of the hippocampus (D–F) in the massage, maternal separation and control groups. Original magnification: $\times$ 400. Scale bars: 100 µm.

The number of IGF-1-positive cells in the SVZ of the lateral ventricle (G) and DG of the hippocampus (H) are shown for the massage, maternal separation and maternal non separation groups. Data are expressed as mean ± SD ($n = 12$). $aP < 0.05$, vs. the other two groups.
JB1 suppressed IGF-1 expression in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus

Immunohistochemical staining revealed that after day 9 of paravertebral fascial massage, increased IGF-1 expression in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus was blocked by JB1. In contrast, IGF-1 expression levels were normal in the massage plus saline group. The number of BrdU-positive cells in the massage plus JB1 group significantly decreased in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus compared with the massage plus saline group ($P < 0.05$; Figures 3, 4).
**DISCUSSION**

Massage is beneficial to the growth and development of the neonatus. A possible underlying mechanism includes induction of vagal activity, which causes gastric motility and increased insulin and IGF-1 levels\[^{9,15-17}\]. However, this mechanism fails to explain how massage improves motor function in cerebral palsy\[^{18}\] and brain function in autistic children\[^{19-20}\]. This study aimed to determine how...
massage promotes brain development and whether it is regulated by the myofascial pathophysiologic or neurophysiologic function, or a combination of both. This study also addressed whether the fascia regulates a specific region of the brain. In this study, high IGF-1 expression and newly generated cells were observed in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus, after manipulating the paravertebral fascia of neonatal rats. IGF-1 is an important neuroprotective peptide, which can promote brain development in normal physiological conditions. Massage can increase serous IGF-1, likely produced by the fascia tissue of liver, adipose, muscle, kidney and spleen, whereby it is secreted into the blood circulation where it elicits endocrine effects on target tissues\textsuperscript{[21-23]}. Alternatively, IGF-1 can act directly on microglia, astrocytes and neurons of the neurogenesis region and elicit paracrine effects on target tissues\textsuperscript{[8]}. IGF-1 can pass the blood-brain-barrier and migrate to neurogenic regions, like the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus, activating the mitogen-activated protein kinase (MAPK) signaling pathway in neural stem cells and promoting their proliferation\textsuperscript{[24]}. In the present study, subcutaneous injection of IGF-1 receptor antagonist, JB1, resulted in an absence of IGF-1 expression in neurogenic regions, including the subventricular zone of the lateral ventricle and the dentate gyrus of the hippocampus. Furthermore, the neurogenic capacity was obviously decreased. A possible mechanism for this involves the competition of JB1 with the IGF-1 receptor, which inhibits serous IGF-1 from crossing the blood-brain-barrier\textsuperscript{[25]}, causing IGF-1 to lose its biological function by blocking the mitogen-activated protein kinase signaling in neural stem cells\textsuperscript{[8, 24]}. Alternatively, the biological mechanism could involve inhibition of IGF-1 by JB1 to enhance other growth factors, which in turn act on the biological function of neural stem cells\textsuperscript{[26-27]}, significantly decreasing their proliferation. In conclusion, the present study shows all body organs together via the fascia framework, promoting adipose, bone, muscle, kidney and spleen tissues to produce autocrine IGF-1\textsuperscript{[28]}, which is secreted into the blood circulation to cross the blood-brain-barrier, affecting a specific region of brain tissue; (3) massage acts on the nerve endings in the fascia, which feed back to a specific region of brain tissue via neurophysiologic regulation. For example, microglia, astroglia or neurons produce paracrine IGF-1\textsuperscript{[8]}, which directly affects neurogenic areas, promoting proliferation of adult neural stem cells. Despite these proposed mechanisms, limitations in this study exist. The impact of massage on the level of IGF-1 in subcutaneous fascia tissue and the blood circulation was not determined. Therefore, the results cannot completely explain the relationship between the fascia and the brain.

**MATERIALS AND METHODS**

**Design**
A randomized, controlled, animal experiment.

**Time and setting**
The experiment was performed at the Laboratory of Anatomy, Southern Medical University, Guangzhou, China, from March 2010 to July 2011.

**Materials**
Ten litters of Sprague-Dawley rats were housed at 21°C in a 12-hour light/dark cycle with free access to food and water. Parturition was checked for on a daily basis and the day of birth was considered postnatal day 0 (P0). The mother and its litter were housed in $26 \times 42 \times 18 \text{cm}^3$ Plexiglas cages. All rats were provided by the Laboratory Animal Center of the Southern Medical University (certificate No. SCXK (Yue) 2006-0015). All experiments were performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China\textsuperscript{[29]}.

**Methods**

**Massage and JB1 injection**
In the massage group, neonatal rats were separated from their mother twice daily (8:00 a.m. and 4:00 p.m.) from P1 to P15 and received massage. Massage therapy was carried out as follows; the neonatal rat was stationed from their mother twice daily (8:00 a.m. and 4:00 p.m.) from P1 to P15 and received massage. Massage therapy was carried out as follows; the neonatal rat was stationed to a specific region of brain tissue; (3) massage acts on the nerve endings in the fascia, which feed back to a specific region of brain tissue via neurophysiologic regulation. For example, microglia, astroglia or neurons produce paracrine IGF-1\textsuperscript{[8]}, which directly affects neurogenic areas, promoting proliferation of adult neural stem cells. Despite these proposed mechanisms, limitations in this study exist. The impact of massage on the level of IGF-1 in subcutaneous fascia tissue and the blood circulation was not determined. Therefore, the results cannot completely explain the relationship between the fascia and the brain.

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subcutaneous fascia was manipulated from tail to neck, nine times for one treatment. This technique is called Knead Ridge Therapy in traditional Chinese medicine[30]. In the maternal separation group, neonatal rats were separated from their mother at the same period of day and for the same amount of time, but did not receive massage. Control group neonatal rats were not massaged and remained with their mother. In the massage plus JB1 group, JB1 (Bachem, batch: 1035419, Bubendorf, Switzerland) was subcutaneously injected into the rats from P1 to P9. For each rat, a single injection per day was performed following massage (10 a.m.) over 9 consecutive days. JB1 concentration was 50 ng/µL and the volume of injection was weight adjusted for a dose of 18 ng/g[8, 31]. The control massage plus saline group underwent treatment with subcutaneous injection of identical volume with normal saline for 9 consecutive days. BrdU (sigma; 50 mg/kg) was injected intraperitoneally at 2, 4 and 6 hours pre-cardiac perfusion to detect cell proliferation.

**Tissue preparation**
Rats were anesthetized with diethyl ether and perfused transcardially with phosphate buffer (100 mM, pH 7.4) followed by ice-cold 4% paraformaldehyde. The rats were decapitated. The brain was removed and postfixed 1.5 mm bregma to 2.52 mm interaural using a cryostat (Leica, Nussloch, Germany) and stored at 4°C.

**Immunohistochemistry for IGF-1 and BrdU**
Immunohistochemical staining for IGF-1 or BrdU was conducted using rabbit anti-IGF-1 (1:200) polyclonal antibody (Santa Cruz, SC9013, USA), or mouse anti-BrdU (1:1 000) monoclonal antibody (Millipore, MAB3424, Massachusetts, USA) respectively at 4°C overnight. Secondary antibody (SP-9000 Histostain TM-Plus Kits, source: Goats) was applied at room temperature for 2 hours, using the free-floating avidin biotin complex method as previously described[32]. To detect BrdU-labeled cells, free-floating sections were rinsed extensively with phosphate buffered saline (0.05 M, pH 7.4) and incubated in HCl (2N) at 37°C for 30 minutes to denature the DNA. Slides were then developed with nickel-intensified diaminobenzidine.

**Cell quantification**
The number of immune-positive cells in the dentate gyrus was quantified bilaterally in each animal from six dorsal hippocampal sections, separated by six section intervals. The results were expressed as a mean value. The dentate gyrus, including the hilus, subventricular zone of lateral ventricle and the inner third of the granular-cell layer were observed for BrdU positive cells. The number of immunopositive cells in the dorsolateral angle of the subventricular zone of the lateral ventricle was quantified from one of five random visions at high magnification (× 400) in each animal from six dorsal hippocampal sections, separated by six section intervals.

**Statistical analysis**
All data were statistically processed using SPSS 13.0 software (SPSS, Chicago, IL, USA) and were expressed as mean ± SD. All intergroup group differences were compared using one-way analysis of variance. Comparisons between groups was performed using the t-test. A value of $P < 0.05$ was considered statistically significant.

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**Ethical approval:** This experiment received permission from the Animal Care and Research Committee of Southern Medical University, China.

**REFERENCES**


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