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Original Research

Effects of manual acupuncture with the Japanese traditional needle manipulation technique on skeletal muscle blood flow and arterial blood pressure in rats - a comparison of the techniques -

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Abstract

[Objective] The purpose of this study was to investigate effects of manual acupuncture (MA) with the Japanese traditional needle manipulation technique on skeletal muscle blood flow (MBF) and arterial blood pressure in rats and to compare the effects of these techniques.

[Materials and methods] The experimental animals were male Sprague-Dawley rats (270 - 350 g, N = 77). MA with one of the techniques was applied to both right tibial anterior (TA) and extensor digitorum longus (EDL) simultaneously. The techniques were single inserting technique (SI), twisting technique (Tw), rotating technique (Ro), sparrow pecking technique (SP) and retaining technique (Re). SI, Tw, Ro or SP was applied for 1 minute and Re was done for 8 minutes. In addition, the combination of SP for 1 minute and Re for 7 minutes was examined (SP&Re). MBFs of TA, EDL, soleus (Sol), plantaris (Pla) and gastrocnemius (Gas) were measured by radiolabeled microspheres. The arterial blood pressure was recorded via a catheter inserted into the right common carotid artery before, during and after MA.

[Results] Both SP and SP&Re significantly increased MBF compared to non-stimulated control group (Cnt) in both TA ($P < 0.001$ and 0.05, respectively) and EDL ($P < 0.05$ and 0.01, respectively). However, there were no significant differences of MBF between SP and SP&Re in each muscle. On the other hand, there was no significant difference of MBF between techniques in Sol, Pla and Gas that were not punctured directly. Although the mean arterial blood pressure (MAP) showed a tendency to decrease during stimulation in each group, there was no significant difference between before and after stimulation.

[Conclusion] These results suggest that the effect of MA on muscle circulation would depend on tissue injury caused by the needle manipulation technique. On the other hand, there were few effects of the single or the combined Re on it.

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Key words: *manual acupuncture, needle manipulation techniques, muscle blood flow, radiolabeled microspheres*

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Ⅰ. Introduction

Acupuncture has been applied to various skeletal disorders, eg shoulder pain[1], low back pain[2] and chronic knee pain[3,4]. In clinical practice, acupuncturists select manual acupuncture (MA) and/or electroacupuncture (EA) in consideration of physical and pathological conditions of patients.

EA has been used in many clinical and experimental studies. The reasons includes that control of the stimulus condition is easy. EA is used at low frequency and high intensity, high frequency and low intensity or a mixture of these in clinical practice. On the other hand, MA has many needle manipulation techniques that are used singly or in combination. In Japan, the 17 techniques have been educated traditionally[5]. In these techniques, single inserting technique (SI), twisting technique (Tw), rotating technique (Ro), sparrow pecking technique (SP) and retaining technique (Re) are basic ones. However, there are few studies that compared these effects. Langevin et al presented that unidirectional rotation (corresponds to Ro) or bidirectional rotation (Tw) was greater than no rotation (SI or Re) in the force necessary to pull an acupuncture needle out of the skin, and that the former (Ro) was greater than the later (Tw)[6]. Kim et al reported that both twirling manipulation (Ro) and lifting-thrusting manipulation (SP) significantly enhanced analgesia compared to acupuncture without a manipulation (SI or Re) in rats, and that the former (Ro) was more effective than the later (SP)[7]. Takakura et al showed that SP suppressed vibration-induced finger flexion reflex than in-site technique (Re) in human[8]. These findings suggested that a more noxious technique might be more effective.

In our previous study, we examined effects of MA with SP on muscle blood flow (MBF) using radio-labelled microspheres in rats and showed that MA could increase MBF locally and stimulus-dependently[9]. The radiolabeled microspheres technique is a useful and powerful method to measure blood flow of various tissues and organs, eg skeletal muscle [9,10], heart[11], gastro-intestinal tract [12], uterus[13] and brain[14]. This also has advantages to measure blood flow of multiple muscles simultaneously and quantitatively without damaging the target ones and being influenced by motion. These are suitable for studies of MA and EA that are accompanied with the small tissue injury and motion.

Thus, in the present study, we investigated effects of MA with one of the Japanese traditional needle manipulation techniques (SI, Tw, Ro, SP and Re) on MBF and arterial blood pressure in rats and compared the effects of these techniques. Additionally, we examine the effect of the combination of SP and Re on them. For MBF measurement, the radiolabeled microsphere technique was used, as well as our previous study[9].

II. Materials and methods

1. Experimental Animal

Male Sprague-Dawley rats (270 - 350 g, N = 77, Japan SLC, Inc, Shizuoka, Japan) were used under anaesthesia with urethane (1.2 g/kg ip). They were housed in an air-conditioned room, under 12:12 light-and-dark cycle and had free access to water and standard rodent diet. This study was approved by the ethical committee of Meiji University of Integrative Medicine (No. 17-44).

2. Acupuncture stimulation and experimental groups

For acupuncture stimulation stainless-steel acupuncture needles (0.20 × 30 mm, Seirin Inc, Shizuoka, Japan) were used. The stimulus point was on the midline of tibial anterior muscle (TA) at a 7 - 8 mm point below the knee joint. The needle was inserted toward the ankle joint. The needle was penetrated both right TA and extensor digitorum longus (EDL) locating on the dorsum of TA. As a result, the needle was inserted in a 15 - 18 mm.

In SI, a needle was inserted and removed at once. In Tw, it was inserted and twisted 30 times. It took 1 second for a half-turn. In Ro, it was inserted and rotated 60 times clockwise. It took 1 second for a turn. In SP, it was inserted and moved 30 times up and down at amplitude of 8 - 10 mm. It took 1 second for a movement (up or down). In Re, it was inserted and retained for 8 minutes. In a combination of SP and Re (SP&Re) is Re for 7 minutes after SP for 1 minute.

Thus, we set following 7 experimental groups; SI, Tw, Ro, SP, Re, SP&Re and Cnt which has no stimulation. Each group was 11 rats (N =11).

3. Muscle blood flow measurement

The radiolabeled microsphere technique was used for muscle blood flow measurement. The method followed Laughlin's report[10]. We used the ^{51}Cr-labeled microspheres (15 micrometer in diameter) dissolved in saline with 10% Tween 80 (PerkinElmer Japan Co Ltd, Japan), as well as our previous study[9]. The measurement was

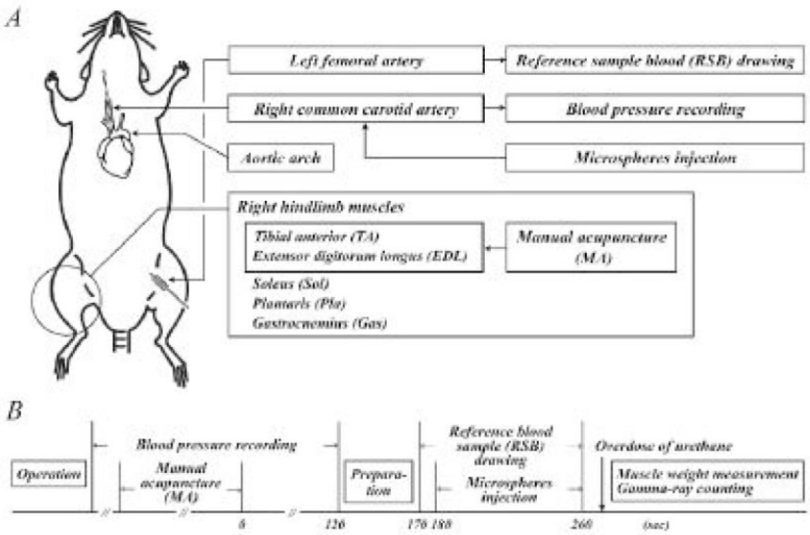

Figure 1. **MBF measurement with the radiolabeled microspheres**
Figure 1A shows the operation of MBF measurement with the radiolabeled microspheres and the target muscles. The blood pressure was recorded from a polyethylene tube inserted into the right common carotid artery until injection of the radiolabeled microspheres. The microspheres were injected to the aortic arch through the tube. RSB was drawn from a tube inserted into the left femoral artery during the microspheres injection. The target muscles were the right hindlimb muscles (TA, EDL, Sol, Pla and Gas). Both TA and EDL were stimulated by acupuncture needle simultaneously. *Figure 1B* shows the protocol of the MBF measurement. Refer to the text for the details.

done in the following procedures (*Figure 1*): 1) the reference sample blood (RSB) was withdrawn from the left femoral artery by pull pump (0.66 ml/min); 2) 10 seconds later, the microsphere solution was injected into the aortic arch by push pump (0.66 ml/ min) through a catheter inserted into the right common carotid artery, and the remaining microspheres in the catheter were continuously flushed with saline for 120 seconds after the start of microsphere injection; 3) the rat was killed with an overdose of urethane; 4) the right hindlimb muscles, which were TA, EDL, soleus (Sol), plantaris (Pla) and gastrocnemius (Gas), were excised and weighed; 5) the gamma radiation of RSB and each muscle was counted by gamma counter (Auto Well Gamma System ARS-600, ALOKA Co Ltd, Japan); and 6) MBF was calculated using the following equation:

$$MBF = 100*Rm*V/Rb*W \text{ (ml/min/100 g)}$$

where Rm and Rb are the amounts of gamma radiation (cpm) of muscle and RSB, respectively; V is pull pump speed for drawing blood; and W is muscle weight (g). The microspheres was injected at 3 minutes after removing a needle.

4. Blood pressure recording

The arterial blood pressure was recorded via a catheter inserted into the right common carotid artery before, during and after MA (*Figure 1*). This catheter was also used to inject the microspheres. Thus, the recording was stopped 1 minute before the start of microspheres injection.

5. Statistical analysis

The GraphPad Prism 5 for Windows (GraphPad Software Inc., USA) was used for statistical analysis. The one-way ANOVA was applied to compare MBF data between experimental groups. When there was a significant difference between groups, the post hoc Tukey's test was subsequently done. The repeated one-way ANOVA was applied to compare MAP data between groups. The post hoc Dunnett's test were subsequently used to compare MAP data between before and after stimulation.

The significance level (*P*) was set at < 0.05. All data were expressed as mean ± standard error (SE).

III. Results

Figure 2 shows MBF after MA with a different needle manipulation technique. In TA, MBFs of stimulated groups were higher than Cnt (15 ± 3 ml/min/100 g) in the following order: SP (50 ± 6 ml/min/100 g), SP&Re (42 ± 7 ml/min/100 g), Ro (38 ± 7 ml/min/100 g), Tw (31 ± 4 ml/min/100 g), Re (24 ± 5 ml/min/100 g) and SI (21 ± 4 ml/min/100 g). There were significant differences between SP and Cnt, SI or Re ($P < 0.001$, 0.01 and 0.05, respectively) and between SP&Re and Cnt ($P < 0.05$). In EDL, MBFs showed a similar tendency to those of TA, but not so clear. There were significant differences between SP&Re and Cnt, SI or Re ($P < 0.01$, 0.05 and 0.05, respectively) and between SP and Cnt ($P < 0.05$). On the other hand, there were no significant differences in Sol, Pla and Gas which were not punctured directly by needle ($P = 0.33$, 0.38 and 0.82, respectively). In addition, there were no a significant differences between Cnt and SI, Re or Tw in all muscles.

Table 1 presents MAP before, during and after MA. The values of each time point were calculated by averaging the temporal MAP data for every 1 minute. Note that there was no MAP data during stimulation in SI, because the stimulus time was very short. In Cnt, SI, Tw, SP, Re and SP&Re, there was no difference in the repeated one-

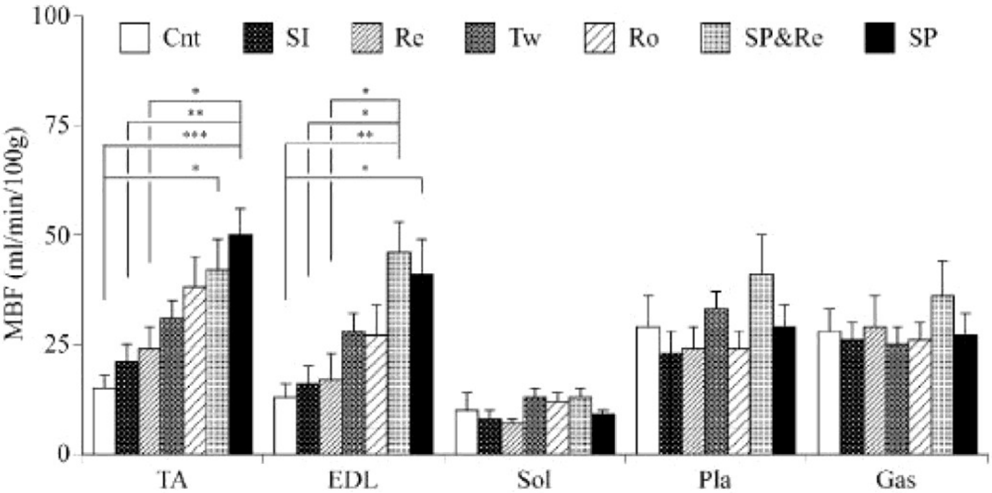

***Figure 2.* MBF after MA with the Japanese traditional needle manipulation technique in rat hindlimb**
In TA, there were significant differences between SP and Cnt, SI and Re ($P < 0.001$, 0.01 and 0.05, respectively) and between SP&Re and Cnt ($P < 0.05$). In EDL, there were significant differences between SP and Cnt ($P < 0.05$) and between SP&Re and Cnt, SI and Re ($P < 0.001$, 0.05 and 0.05, respectively). There was no significant difference in Sol, Pla and Gas that were not punctured by needle directly. The number of animal was 11 each. Data were expressed as Mean ± SE. ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$.

Table 1. MAP before, during and after MA with a different needle manipulation technique

Experimental Group	N	MAP [mmHg] Before [min] -1 - 0	During [min] 0 - 1	During 1 - 2	During 2 - 3	During 3 - 4	During 4 - 5	During 5 - 6	During 6 - 7	During 7 - 8	After [min] 0 - 1	After 1 - 2
Cnt	11	86 ± 3	86 ± 3	-	-	-	-	-	-	-	86 ± 3	87 ± 3
SI	11	77 ± 4	-	-	-	-	-	-	-	-	79 ± 5	81 ± 5
Tw	11	83 ± 3	80 ± 3	-	-	-	-	-	-	-	82 ± 3	83 ± 3
Ro	11	89 ± 5	85 ± 4*	-	-	-	-	-	-	-	89 ± 4	90 ± 4
SP	11	81 ± 4	79 ± 3	-	-	-	-	-	-	-	81 ± 4	82 ± 3
Re	11	84 ± 2	85 ± 2	86 ± 3	85 ± 3	85 ± 2	84 ± 2	83 ± 2	83 ± 2	82 ± 2	85 ± 3	85 ± 4
SP&Re	11	88 ± 2	83 ± 3*	83 ± 3**	87 ± 2	91 ± 2	93 ± 2	93 ± 2	93 ± 2	92 ± 2	91 ± 2	90 ± 3

*$P < 0.05$ and **$P < 0.01$ vs Before MA in the post hoc Dunnett's test. Data were expressed as Mean ± SE.

way ANOVA (P = 0.36, 0.08, 0.12, 0.54 and 0.71, respectively). On the other hand, there was a significant difference between before and during acupuncture stimulation (P < 0.05) in Ro. In addition, there was a significant difference between before and 1 or 2 min after stimulation start in SP&Re (P < 0.05 and 0.01, respectively). However, there was no significant difference between MAPs before and after stimulation in all groups and each MAP recovered to a value before MA at 1 min before microspheres injection.

IV. Discussion

At first, note that the stimulus time for each manipulation technique was different in this study. We spent 1 second for one needle action to stimulate it precisely. Ro (clockwise) was one action and both SP (up and down) and Tw (clockwise and anticlockwise) were two actions. On the other hand, the stimulus time for each technique was 60 seconds. Thus, the stimulus times for Ro, SP and Tw were 60, 30 and 30 times, respectively.

SP is easy to get acupuncture needling-specific sensation, so called 'De-qi' and, thus, this is often used in clinical and experimental studies. In this study, SP increased MBF most in all techniques. This suggests that SP is the strongest stimulation in the manipulation techniques, because this increased MBF depending on stimulus intensity (1, 10 and 30 pecking times) in our previous study[9]. The increase in MBF indicates muscle tissue injury. It is difficult to move a needle up and down precisely, because muscle tissue is tender. So, the needle slightly moved radially. As a result, we speculate that SP would stimulate the largest tissue volume in the other techniques.

Ro is easy to get 'De-qi' and is able to stimulate it quantitatively compared to SP, because the depth of an inserted needle is fixed. However, it is easy to be a strong stimulation and to get cause 'a difficulty to withdrawal a needle' which would be induced by tissue winding and/or muscle contraction[6].Ro increased MBF more than two times in comparison with Cnt in both TA and EDL, however, there was no significant difference. This may be due to a statistical analysis for multiple comparisons, because it is difficult to detect a small significant difference between two groups when there is a big significant one between the other groups. In fact, there was a significant difference of MBF between Ro and Cnt in TA (the Dunnett's test; P < 0.05). The Dunnett's test is able to compare between a standard group (control) and one of the other groups, but not to compare between two of all groups. Thus, we assume that Ro could obviously increase MBF, but is inferior to SP. However, the increase in MBF of EDL by Ro was at the same level as that by Tw. This reason is unclear. EDL is a small muscle located behind TA. Thus, we may not insert a needle in it correctly.

The study of Kim et al reported that the analgesic effect of Ro was larger than that of SP[7]. However, the increased MBF of Ro was smaller than that of SP. This discrepancy might be responsible for a difference of stimulated volumes of these techniques. A needle would penetrate the same point of muscle precisely in SP in Kim's study. On the other hand, as mentioned above, a needle radially stimulated muscles (TA and EDL) in SP in this study. Thus, the stimulated volume was larger than that of Ro. If we stimulated muscle with a needle precisely, the increased MBF of Ro may be larger than that of SP.

Tw is able to stimulate quantitatively as well as Ro, but is relatively hard to cause 'a difficulty to withdrawal a needle'. Thus, it is used in many experimental studies investigating effects of MA on visceral functions via somato-autonomic nerve reflex, eg gastric motility[15], arterial pressure[16] catecholamine secretion and adrenal sympathetic nerve activity[17]. Tw increased MBF, but not significantly, in this study. The increase was limited to the half of SP in TA and EDL. This indicates that Tw would be weaker noxious stimulation than SP and Ro. This did not contradict the Langevin's report[6] and that the 'De-qi' induced by Tw is said to be milder than that by these techniques generally[5]. The stimulus volume and the winding tissue on a needle in Tw would be less than that in those. Thus, we think that the increase in MBF by Tw was mild.

Re is often used in combination with SP, Ro or Tw in hope of the continuous stimulation. In this study, Re slightly increased MBF but not significantly, and there is no significant difference of MBF between SP and SP&Re. Goto et al reported that C fiber mechano heat nociceptor units of human skin were not excited during Re in microneurogram study[18]. The increase in MBF by SP (30 times pecking) was maintained for at least 60 minutes after withdrawing a needle in our previous study[9]. These findings suggest that Re would have a little effect on muscle circulation and would not enhance the effect of the other technique. Although Re is often used in clinical practice with the other techniques, the effects on biological functions are not clear. The further study is needed.

SI is said to be the weakest stimulation in needle manipulation techniques and, thus, is applied to weak and sensitive patients[5]. In agreement with these, SI increased MBF only slightly and hardly influenced muscle circulation in this study.

The increase in MBF was observed only in directly punctured muscles (TA and EDL). On the other hand, there were no significant differences between techniques in Sol, Pla and Gas which were not stimulated directly. These results correspond to ones of our previous study[9]. The increase almost coincided with the stimulus intensity that has been said so far. These results suggest that the increase might depend on tissue injury caused by needle manipulation.

In Tw, Ro, SP and SP&Re, MAP decreased during stimulation and recovered after it. The significant decrease was observed in both Ro and SP&Re. This decrease has been considered to be caused by somato-autonomic reflex[19,20]. Although both SP and SP&Re were given the same manipulation (stimulation), the significant decrease was observed only in SP&Re. This reason is unclear. But, the MAP of SP was lower than that of SP&Re before MA. This difference might be the cause. In this way, there were some changes of MAP in each groups, however, there was not a significant difference between MAPs before and after MA. Thus, MAP seems to have nothing to do with the increased MBF by MA.

In both the present and our previous studies, the increased MBF with MA was only observed in muscles punctured by needle without MAP change[9]. These results suggest that the increase might be caused by local vasodilators. In addition, the increase was not influenced by cutting the sciatic nerve in our previous one[9]. Axon reflex is one of the local circulation control and is assumed to be responsible for the increase in MBF by MA[21]. Our results are not contradictory to the assumption. However, the mechanism of increase in MBF by MA is still unclear, because various factors participate in the local circulation control complicatedly. Thus, the further study is needed to clarify it.

V. Conclusion

We investigated effects of MA with the Japanese traditional needle manipulation technique on MBF and arterial blood pressure in rats. MBF was roughly increased by MA in order of SP, Ro, Tw, Re and SI, regardless of arterial blood pressure. Thus, the increase in MBF coincided with the stimulus intensity generally said. It is suggested that the increase might be caused by local vasodilators. On the other hand, there were few effects of the single and the combined Re on muscle circulation.

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