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STRATEGY FOR PREVENTION OF MYONEPHROPATHIC METABOLIC SYNDROME (MNMS): COMPARISON OF COOLING AND PERFUSION

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INDEXING WORDS

acute arterial occlusion; myonephropathic metabolic syndrome; cooling; perfusion

SYNOPSIS

To evaluate the usefulness of cooling and perfusion methods for acute ischemic legs, we performed the experimental study using adult mongrel dogs. As creation of acute ischemic legs, the abdominal aorta was cross-clamped below the renal arteries, where all branches were ligated, for 6 hours. After release of clamp, the various parameters were examined for 18 hours. All dogs were divided into four groups according to the suppressive methods. Control group: cross-clamping for 6 hours with no suppressive method, Group 1: cooling legs using crushed ice during 6 hour-clamping, Group 2: perfusion by heparinized saline of 2.5-10.0 ml/kg before declamping, Group 3: cooling during latter half of 6-hour clamping and perfusion same as Group 2.

The serum levels of metabolites and the tissue pressure of the thigh showed significantly lower in the suppression groups than Control group after declamping. In addition, GOT, CPK and aldolase revealed significantly lower values in Group 3 than Group 2. The tissue blood flow of the thigh recovered to the same extent as before ischemia in Groups 2 and 3, while it did not so in Control group and Group 1. Microscopic findings in hematoxylin and eosin (H-E) staining indicated a marked destruction of muscle fiber and interstitial edema in the striated muscle in Control group. These changes were not found so much in Group 2, and almost no changes in Groups 1 and 3. The immunostaining for Mb in the striated muscle showed negative in Control group and Group 2, while it showed positive staining in Groups 1 and 3. The immunostaining for Mb in the kidnies showed the most dense deposits of Mb in the renal tubules in Control group.

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These changes were less in Group 2, little in Group 3, and no change in Group 1.

In conclusions, cooling method revealed the minimal metabolic change, and combined method with perfusion was still more effective for prevention of MNMS.

INTRODUCTION

Acute arterial occlusion of the legs often causes serious sequelae such as myonephropathic metabolic syndrome (MNMS) or sudden death after revascularization. As you know at Hanshin-Awaji earthquake in Japan 1995, lots of people were overlaid by houses and died of crush syndrome.[16] Acute arterial occlusion is similar to crush syndrome in pathogenicity and treatment.[14,23] Since the report of Haimovici,[8-10] lots of suppressive methods for MNMS have been introduced, such as pharmacological infusions,[3,5,6,13,15,17,18,20,22] perfusion method[1,2,6,7,12,13,15,19,22] or hemofiltration.[4,18,21]

In this paper, we performed the experimental study in dogs to evaluate the usefulness of cooling, perfusion method or both for prevention of MNMS.

MATERIALS AND METHODS

Acute arterial occlusion model

Twenty adult mongrel dogs weighing from 10 to 15 kg were subjected to this study. All animal experiments were conducted according to the "Guidelines for Animal Experimentation at Kobe University School of Medicine". As creation of ischemic legs, laparotomy was made under general anesthesia by sodium pentobarbital (20 mg/kg), and the abdominal aorta was exposed and all branches were ligated below the renal arteries to the femoral arteries. Then, the abdominal aorta was cross-clamped below the renal arteries for 6 hours. After release of clamp, they were observed for 18 hours. All dogs were divided into four groups according to the suppressive methods. In Control group, the aorta was cross-clamped for 6 hours and declamped with no suppressive method (n=5). In Group 1, legs were cooled by crushed ice during 6 hour-clamping (n=5). In Group 2, vascular bed of the ischemic legs were perfused using heparinized saline prior to declamping (n=5). In Group 3, legs were cooled during latter half of 6 hour-clamping and perfused same as Group 2 (n=5).

Cooling method

Ischemic legs were directly cooled using crushed ice in the box. The temperature of legs went down to 9.8 °C in Group 1 and 14.4 °C in Group 3 at the lowest point. Both of them recovered to the normal temperature about 6 hours after declamping.

Perfusion method

The cannulas (16 Fr.) were inserted into the femoral arteries and veins, and heparinized saline was infused into the femoral arteries and same dose was drained from the femoral veins prior to declamping. The perfused volume was divided into 4 steps of 2.5, 5, 7.5 and 10 ml/kg. Elimination ratio was defined as the ratio of material in discarded perfusate to the whole blood. Effectiveness of elimination was defined as the elimination ratio to the perfusion volume.

Various parameters

Circumferencial length and tissue pressure of the thigh were measured for evaluation of the degree of edema. The tissue pressure was defined as air pressure to overcome the tissue pressure within closed compartment. This method was according to Whitesides' report in 1975.[24] The tissue blood flow of the thigh was measured using Laser Doppler Perfusion Monitor (PeriFlux PF3, Perimed, Sweden) attached onto the muscle bundle. Serum glutamic oxaloacetic transaminase (GOT), creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aldolase, blood urea nitrogen (BUN), creatinine (Cr), potassium (K) and myoglobin (serum-Mb) were also obtained in systemic venous blood as well as urinary myoglobin (urine-Mb). All parameters were measured at 1, 3, 6, 12 and 18 hours after declamping.

Histological evaluation

All of the dogs were sacrificed at 18 hours after declamping. The striated muscles of the thigh were inspected in hematoxylin and eosin (H-E) staining, and were also examined in immunostaining for Mb microscopically. In addition, the kidnies were also inspected in immunostaining for Mb.

Statistical analysis

Results were expressed as mean ± standard error. Statistical comparisons between groups were made using ANOVA. P value of 0.05 or less was considered to be statistically significant.

RESULTS

Circumferencial length and tissue pressure

The circumferencial length of the thigh did not change in Group 1 throughout the observation period, but gradually increased in the other 3 groups after declamping. The maximum increase ratios were 1.15 ± 0.06, 1.15 ± 0.07 and 1.07 ± 0.04 at 18 hours in Control group, Group 2 and 3 respectively. Significant differences were seen between Control group and Group 1 at 12 and 18 hours ($p<0.05$). There were no significant differences between the other groups at any time (Fig. 1).

The tissue pressure of the thigh also showed significantly higher value in Control group than the other 3 groups. The maximum level was 15.7 ± 2.9 mmHg at 6 hours in Control group. Significant differences were seen between Control group and the other 3 groups at 6 and 12 hours

($p<0.01$). In contrast, there were no significant differences among the other groups at any time (Fig. 1).

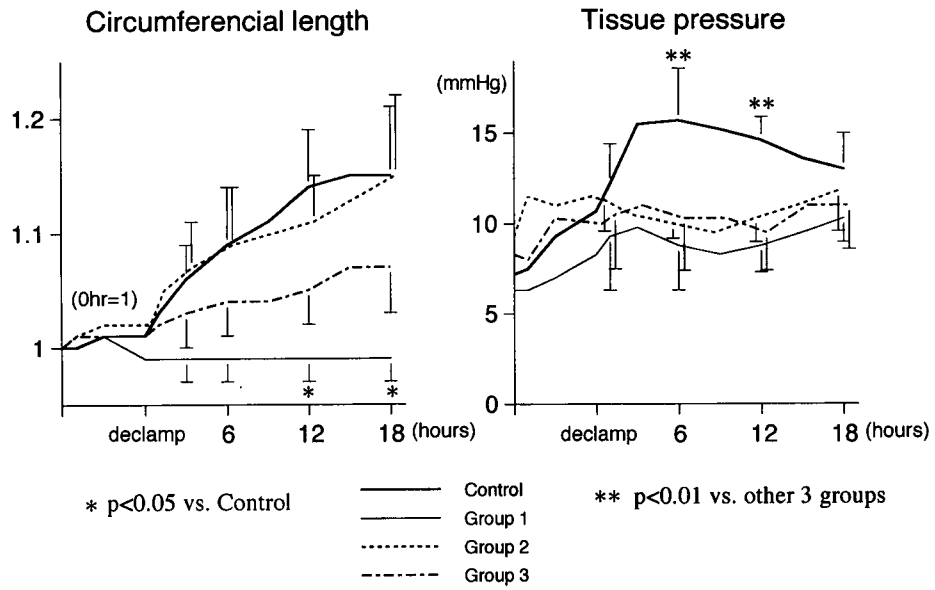

Fig. 1. Circumferencial length & tissue pressure of the thigh in ischemic leg.
The circumferencial length of the thigh did not change in Group 1 throughout the observation period, but gradually increased in the other 3 groups after declamping. Significant differences were seen between Control group and Group 1 at 12 and 18 hours ($p<0.05$). The tissue pressure of the thigh also showed significantly higher value in Control group than the other 3 groups. Significant differences were seen between Control group and the other 3 groups at 6 and 12 hours ($p<0.01$).

Serum metabolites parameters

Serum GOT, CPK, LDH and aldolase showed significantly higher values in Control group than the other 3 groups. Significant differences were seen between Control group and the other 3 groups in GOT, CPK and LDH at 3, 6, 12 and 18 hours, and in aldolase at 6, 12 and 18 hours ($p<0.01$). In Control group, the maximum levels of GOT was 1,839 ± 395 IU/l at 6 hours, CPK was 88,986 ± 16,432 IU/l at 12 hours, LDH was 1,960 ± 258 IU/l at 6 hours and aldolase was 955 ± 205 IU/l at 12 hours. All of these four parameters showed the lowest values in Group 1. In addition, GOT, CPK and aldolase showed significantly lower values in Group 3 than Group 2. Significant differences were observed between these groups in GOT at 6 ($p<0.05$), 12 ($p<0.01$) and 18 ($p<0.05$) hours, and in CPK and aldolase at 6, 12 and 18 hours ($p<0.01$) (Fig. 2).

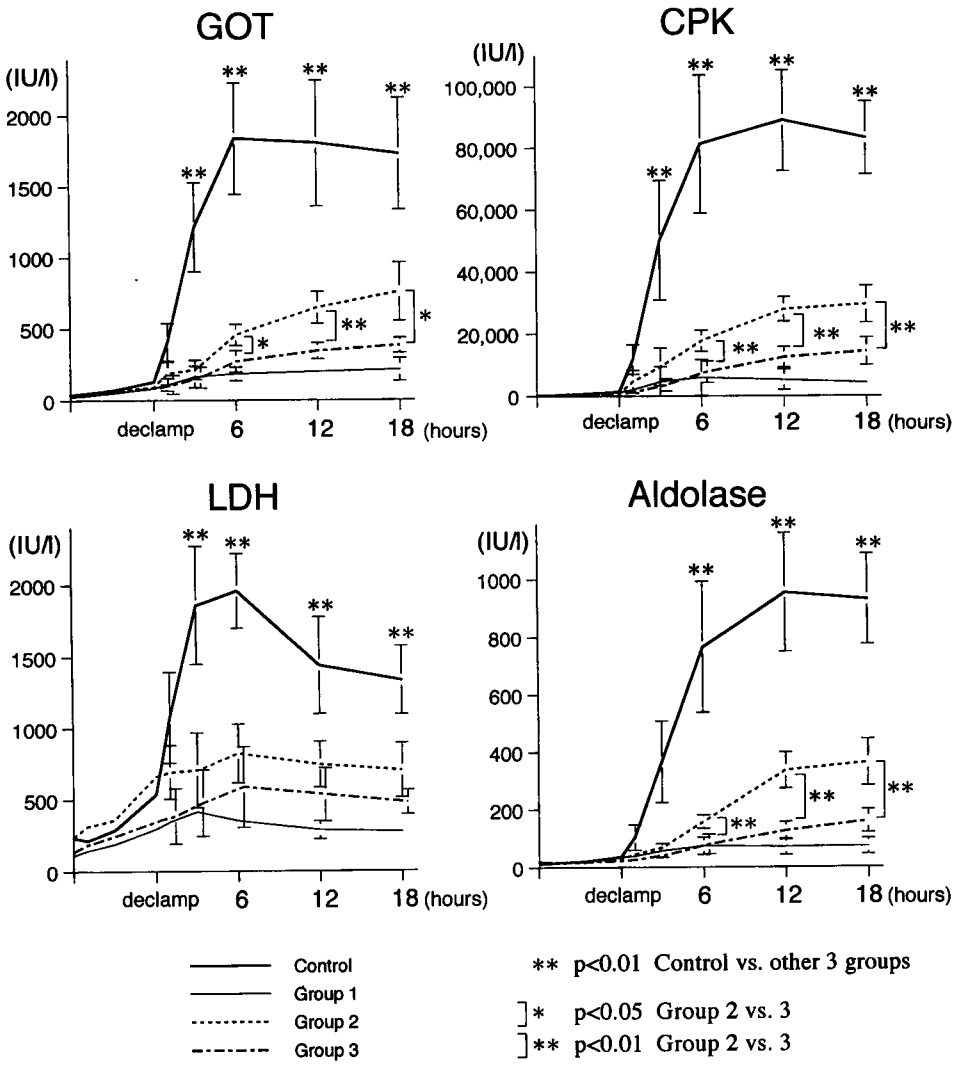

Fig. 2. Serum metabolites parameters.

Serum GOT, CPK, LDH and aldolase showed significantly higher values in Control group than the other 3 groups. All of these four parameters showed the lowest values in Group 1. In addition, GOT, CPK and aldolase showed significantly lower values in Group 3 than Group 2.

Renal function

Serum BUN, creatinine and potassium showed significant increases after declamping in Control group. In contrast, in the other 3 groups, these parameters showed no significant increases. Significant differences were found between Control group and the other 3 groups in creatinine at 3, 6 ($p<0.01$), 12 and 18 ($p<0.05$) hours, but no significant differences were seen in BUN and potassium at any time. In Control group, the maximum level of BUN was 32.3 ± 16.1 mg/dl, creatinine was 2.1 ± 0.7 mg/dl at 18 hours and potassium was 5.2 ± 1.5 mEq/l at 6 hours (Fig. 3).

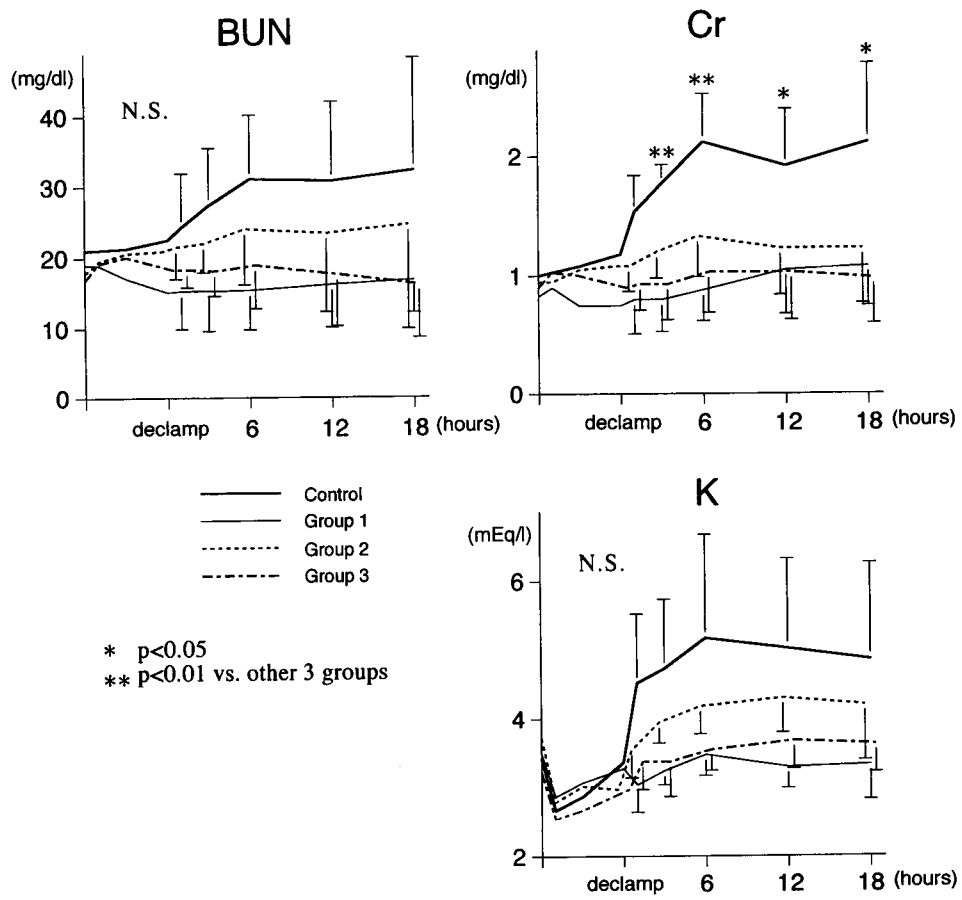

Fig. 3. Renal function.
Serum BUN, creatinine and potassium showed significant increases after declamping in Control group. In contrast, in the other 3 groups, these parameters showed no significant increases. Significant differences were found between Control group and the other 3 groups in creatinine at 3, 6 ($p<0.01$), 12 and 18 ($p<0.05$) hours.

Myoglobin

The serum-Mb and urine-Mb showed remarkably increase after declamping in Control group and moderately increase in Group 2, but little increase in Groups 1 and 3. No significant differences were seen among 4 groups at any time. The maximum level of serum-Mb was 115,300 ng/ml at 12 hours in Control group, 1,251 ng/ml at 12 hours in Group 1, 66,200 ng/ml at 1 hours in Group 2 and 5,420 ng/ml at 12 hours in Group 3. The maximum level of urine-Mb was 3,250,000 ng/ml at 6 hours, 260,000 ng/ml at 12 hours, 1,820,000 ng/ml at 6 hours and 500,000 ng/ml at 12 hours in each group, respectively (Fig. 4).

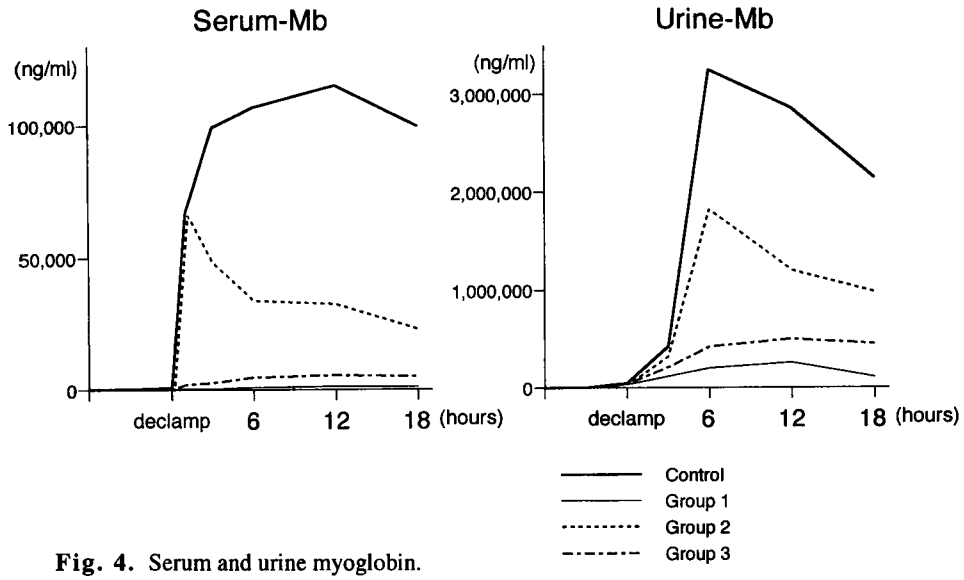

Fig. 4. Serum and urine myoglobin.
The serum-Mb and urine-Mb showed remarkably increase after declamping in Control group and moderately increase in Group 2, but little increase in Groups 1 and 3. No significant differences were seen among 4 groups at any time.

Perfusion effect

The perfusion volume increased more, the elimination ratio also did more. It attained the maximum level of 10.4 ± 9.5 % at 10 ml/kg. Effectiveness of elimination attained the maximum level of 1.37 ± 0.39 %/ml/kg at 5 ml/kg. However, in these parameters, there were no significant differences among 4 steps (Fig. 5).

The tissue blood flow of the thigh recovered to the same extent as before ischemia in Groups 2 and 3. In contrast, it did not recover only to the maximum level of 0.72 ± 0.13 (under definition of "1.00" before ischemia) in Control group and Group 1 (Fig. 5).

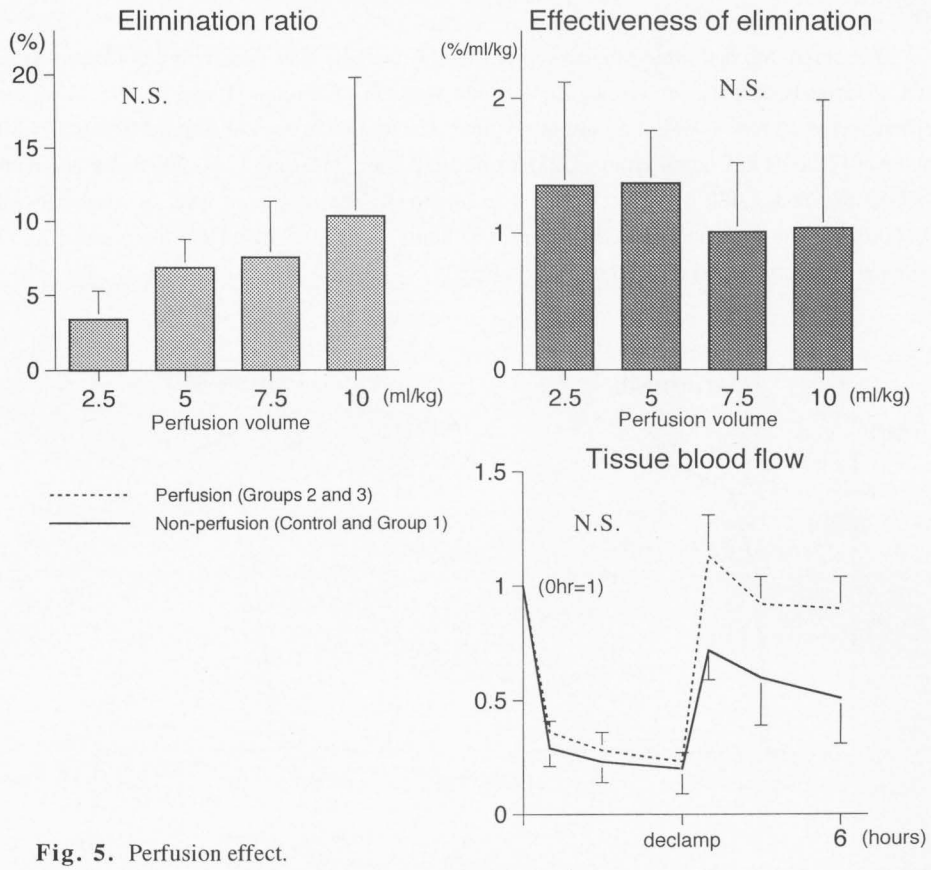

Fig. 5. Perfusion effect.

The perfusion volume increased more, the elimination ratio also did more. Effectiveness of elimination attained the maximum level of 1.37 ± 0.39 %/ml/kg at 5 ml/kg. The tissue blood flow of the thigh recovered to the same extent as before ischemia in Groups 2 and 3. In contrast, it did not recover only to the maximum level of 0.72 ± 0.13 (under definition of "1.00" before ischemia) in Control group and Group 1.

Histological evaluation

Microscopic findings in hematoxylin and eosin (H-E) staining showed a marked destruction of muscle fiber and interstitial edema in the striated muscle of Control group. These changes were not found so much in Group 2, and almost no changes in Groups 1 and 3 (Fig. 6). The immunostaining for Mb in the striated muscle showed negative in Control group and Group 2, because a large amount of Mb was dissolved and carried away. In contrast, it showed positive staining in Groups 1 and 3 (Fig. 7). The immunostaining for Mb in the kidnies revealed the most dense deposits of Mb in the renal tubules in Control group. These changes were less in Group 2, little in Group 3, and no change in Group 1 (Fig. 8).

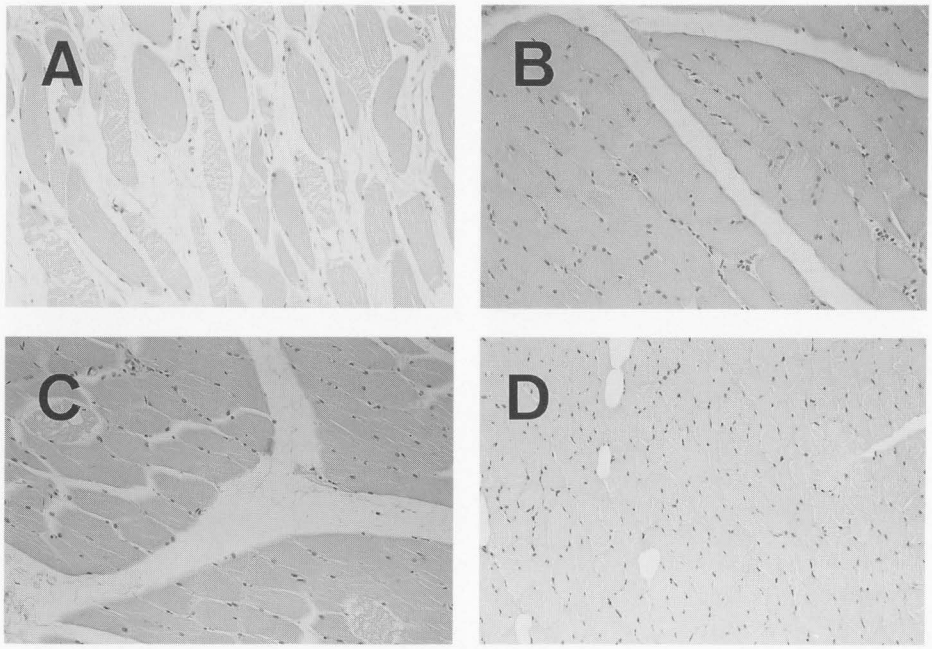

Fig. 6. Striated muscle (HE x 100).
(A) Control (B) Group 1 (C) Group 2 (D) Group 3
Microscopic findings in hematoxylin and eosin (H-E) staining showed a marked destruction of muscle fiber and interstitial edema in the striated muscle of Control group. These changes were not found so much in Group 2, and almost no changes in Groups 1 and 3.

DISCUSSION

Basic pathological status of MNMS is characterized by myoglobinuria, hyperkalemia, elevation of muscle-derived enzyme, metabolic acidosis and renal failure. To prevent such a serious sequel due to acute arterial occlusion, lots of suppressive methods have been introduced including primitive amputation of legs, THAM infusion for correction of serum pH,[6,13,15,22] perfusion for collecting ischemic metabolites[1,2,7,12,13,15,19,22] and hemofiltration for elimination of them.[4,21] In addition, MNMS is possible to be related to free radical, therefore infusion of scavenger is considered to be effective.[18,20] Regarding simple cooling method, Kugimiya et al. reported its efficacy for preventing MNMS under cardiopulmonary bypass,[11] but other investigators have not proved it. In this paper, we performed the experimental study to inspect the usefulness of cooling, perfusion method or both for prevention of MNMS.

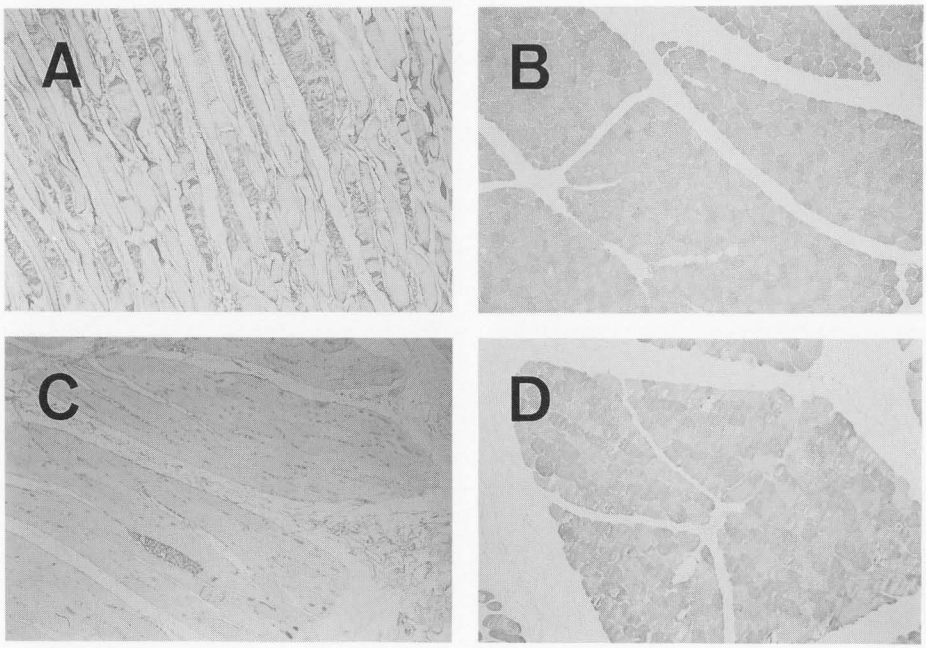

Fig. 7. Striated muscle (immunostaining for Mb x 100).
(A) Control (B) Group 1 (C) Group 2 (D) Group 3
The immunostaining for Mb in the striated muscle showed negative in Control group and Group 2, because a large amount of Mb was dissolved and carried away. In contrast, it showed positive staining in Groups 1 and 3.

As an acute arterial occlusion model, occlusion time was defined to be 6 hours that some previous papers[12] regarded as "golden time". Cooling is a simple and useful method for preservation of organs, and is considered to be applicable to the ischemic legs. In this study, almost all parameters were maintained at the levels of preischemia in 6-hour cooling method. In addition, the microscopic findings showed no destruction of muscle fiber nor interstitial edema in H-E staining, and positive staining in immunostaining for Mb in the striated muscle and no deposits of Mb in immunostaining of the kidney. Therefore, immediate cooling minimizes the metabolic changes due to acute arterial occlusion, and is considered to be most effective for prevention of MNMS. In Group 3, all legs were cooled for latter 3 hours to examine whether a short time cooling prior to revascularization is effective, because there is often time lag from diagnosis to initiation of treatment in actual clinical cases. In Group 3, GOT, CPK and aldolase showed significantly lower values than in Group 2, and the microscopic findings revealed almost no change in Group 3. Therefore, a short time cooling also proved to be effective for prevention of MNMS.

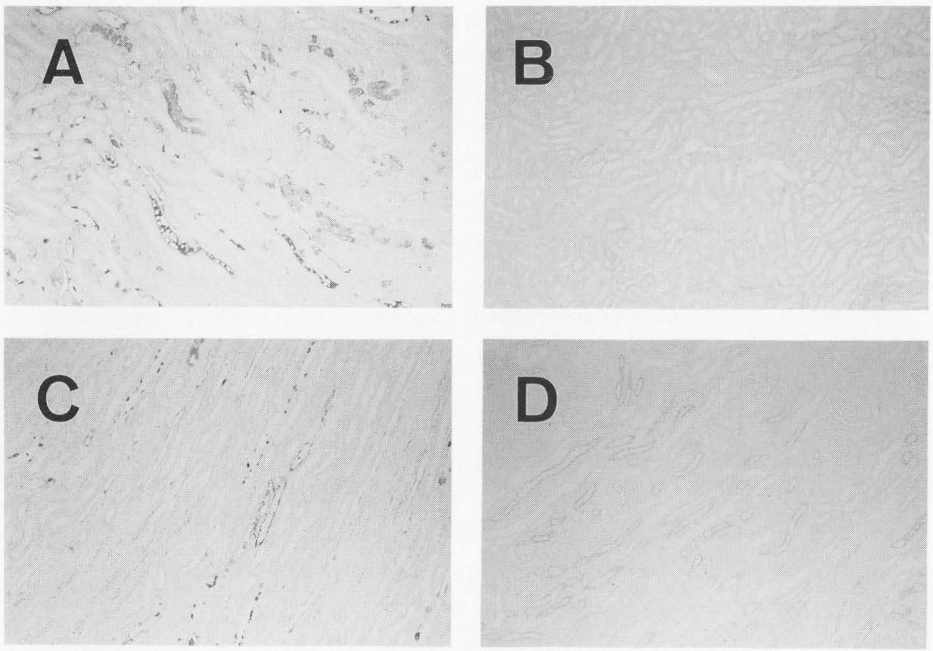

Fig. 8. Kidney (immunostaining for Mb x 100).
(A) Control (B) Group 1 (C) Group 2 (D) Group 3
The immunostaining for Mb in the kidnies revealed the most dense deposits of Mb in the renal tubules in Control group. These changes were less in Group 2, little in Group 3, and no change in Group 1.

Regarding perfusion method, almost all parameters were lower in Group 2 than in Control group, and significant differences were observed in tissue pressure, GOT, CPK, aldolase and creatinine between these groups. In addition, the microscopic findings showed that the destruction of muscle fiber and interstitial edema in H-E staining of the striated muscle were obviously less in Group 2 than in Control group, so were also deposits of Mb in immunostaining of the kidney. Perfusion effect may be due to protecting from the injury to mitochondrial enzyme. Some previous papers insisted on the differences of its efficacy according to content and volume of the perfusate.[1,2,7,12,13,15,19,22] Esato[6] and Nakano[15] reported 30 ml/kg of lactated Ringel's solution was most effective. In this study, simple heparinized saline was also effective,[7,12,13] because it possessed an elimination effect of ischemic metabolites in addition to a wash-out effect. When perfusion volume was divided from 2.5 to 10 ml/kg, the perfusion volume of 5 ml/kg showed the best effectiveness of elimination in this study. Regarding wash-out effect, we observed a complete recovery of the tissue blood flow after declamping and the embolus was not seen microscopically in

the striated muscle in Groups 2 and 3.

From these findings in physical, biochemical and histological examinations in acute arterial occlusion model, cooling method indicated minimal metabolic changes and combined method with perfusion was still more effective for prevention of MNMS.

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