EFFECTS OF KINESIOTHERAPY IN ISCHEMIC LESION AND REPERFUSION IN RATS

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ABSTRACT

Objective: To investigate the effect of kinesiotherapy on the functionality of the pelvic limb of rats after ischemic and reperfusion injury. Methods: 10 rats were divided into two groups, GI (control) and GII (kinesiotherapy). All the animals underwent ischemia for a period of three hours, followed by tissue reperfusion. In Group GII, non-resistive systemic kinesiotherapy was performed (swimming) in three weekly sessions of 50 minutes, over a period of four weeks, while the GI animals remained at rest. Functional analysis of motor behavior was evaluated weekly. The animals were then sacrificed, and the soleus and gastrocnemius muscles and the sciatic nerve removed for histopathological analysis. Results: There was a significant recovery of motor behavior with kinesiotherapeutic treatment during the four weeks of treatment. However, the histological examination of the tissues showed no morphological changes of cell injury and repair. Conclusion: It was not possible to affirm that the exercise was effective in cell repair, because neither of the groups (control and experimental) showed any histological difference. On the other hand, systemic kinesiotherapy showed a beneficial effect on functional rehabilitation after ischemia and reperfusion. ***Level of evidence III, Case-Control Study***.

Keywords: Ischemia. Reperfusion. kinesiotherapy. Functional analysis.

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INTRODUCTION

Lower limb ischemia produces signs and symptoms resulting from arterial insufficiency in the tissues of a particular human body segment. If the treatment is not effective, there may be loss of functional conditions.[1] The main consequences of ischemia occur in arteriole-capillary territory and if the ischemia persists, the first areas of capillary and tissue necrosis appear.[2] During ischemia, tissue tolerance is variable and multifactorial, depending on the time of ischemia, the metabolic needs of the tissues, the contribution of collateral circulation and of humoral factors. Accordingly, it is not possible to define an exact period in which each tissue would have its integrity irreversibly impaired vis-à-vis an ischemic period.[3]

If the ischemia persists, necrotic areas appear, and at a given time, there will be areas of normal tissue interposed by areas of functionally altered, yet viable, tissues, together with areas of necrotic tissue. When the reperfusion process is started and circulation is restored, viable tissues recover and resume their function, and the necrotic tissues undergo resorption, organization and fibrosis processes. There will then be biochemical, hemodynamic and anatomopathological consequences in variable space. This will always happen when large areas of muscle are affected and the ischemia lasts for a long time.[2] The inflammatory response, with the release of large quantities of mediators, with chemotactic effect, causes intense leukocyte migration to the blood vessels of the ischemic tissues, and a consequent endothelial adhesion and activation, which could lead to the obstruction of microcirculation, exacerbating the ischemia.[4]

As it is a frequent clinical event, ischemia appears to the vascular surgeon with a strong indication for restorative surgery. Restoration of the blood flow is generally necessary to recover normal cell function. On the other hand, reperfusion of the blood oxygenated in the ischemic tissues may lead to tissue lesions that are even more severe than those provoked by the ischemia, due to the increase in free radicals, a phenomenon called oxidative stress.[5-7] Free radicals are unstable molecules or fragments of molecules with an odd electron in their external orbit. Such molecules act on the lipids, carbohydrates, proteins and nucleic acids and lead to modifications of function and of cell structure, occasioning cell death.[8-10]

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Kinesiotherapy is defined etymologically as the art of curing, using all the techniques of movement of the body or of the body parts to relieve symptoms or to improve function. Kinesiotherapy occupies a prominent position as a physiotherapeutic method for rehabilitation, and is able to determine a set of functional and structural adaptations with the objective of preventing muscle atrophy and of recovering the motricity and sensibility of the areas involved.[11-13]

In this context, there is a shortage of clinical and experimental studies with the purpose of stimulating the repair of ischemic tissues through kinesiotherapy. In an experimental study using kinesiotherapy to promote nerve regeneration and the functional recovery of locomotion in rats with traumatic sciatic nerve injury there was a beneficial effect in the animals' functional recovery.[14]

Thus, the aim of this study is to analyze the effects of systemic kinesiotherapy in tissue repair and functional rehabilitation after ischemia and reperfusion of the pelvic limb of rats.

MATERIAL AND METHODS

The study was approved by the Institutional Research Board of Universidade de Franca under no. 015/09-A.

The survey was conducted at the Experimental Physiotherapy Laboratory of the above university. The sample was composed of 10 male albino Wistar rats, with weight oscillating between 300 and 350 grams, from the vivarium of Universidade de Franca. The animals were distributed in an equal number into two groups: GI (control) and GII (kinesiotherapy), and kept in plastic cages, receiving water and feed ad libitum.

The anesthetic procedure was performed with 0.6 ketamine base solution (50 mg/Kg) (ketamina® - Pfizer do Brasil Ltda, São Paulo, Brazil) and 0.1 xylazine (10 mg/Kg) (rompum® - Bayer do Brasil Ltda, São Paulo, Brazil) injected in the abdominal region. After this ischemia was performed over a three-hour period with the use of a tourniquet at the proximal end of the pelvic limb of the animals, followed by tissue reperfusion.

The non-resistive systemic kinesiotherapy sessions (swimming) started two days after the ischemic procedure in the animals from group GII, in three weekly 50-minute sessions for four weeks in a tank measuring 100cm x 50cm with a depth of 40cm, containing water at 30°C in a sufficient quantity to prevent the animals from resting the end of their tails on the bottom of the tank. The animals from group GI (control) did not undergo any type of activity, remaining at rest throughout the experiment.

The motor behavior evaluation was carried out in a circular arena (open field), made of transparent acrylic, measuring 60cm in diameter by 50cm in height. The floor, also in transparent acrylic, features the drawing of a circle divided into 12 equal parts, used to quantify the frequency of the behavioral responses. The animals were introduced one by one inside the arena, where they could move freely. The ambulation rate (called line crossing) walking and/or running, was measured for 10 minutes by counting the number of sections of the environment crossed by the animal, since it puts its four paws in the other quadrant.[15,16]

The exploratory response of standing on the hind legs which is the response of rearing up on the two hind legs, keeping the front legs elevated, with the animal completely erect in the middle of the arena or semi-leaning against the acrylic wall.[17,18] The functional analysis was performed weekly in the two groups (GI and GII).

At the end of the experiment (after 30 days) the animals were sacrificed in a CO2 chamber and the musculoskeletal tissue (soleus and gastrocnemius) and nerve tissue (sciatic nerve) were collected, immersed in 10% buffered formaldehyde for 24 hours and afterwards dehydrated in an increasing concentration of ethanol, diaphanized in xylol and embedded in paraffin.

The paraffin blocks were sectioned in a rotary microtome, with 4μm-thick histological sections. The sections were gathered on glass slides and stained with Hematoxylin and Eosin (HE) and Gomori Trichrome. The histological analysis was performed using a conventional microscope.

For the statistical analysis we used the Prism 4.0 software for Student's t-test, considered significant when the p-value was below 0.05.

RESULTS

During the ischemic procedure we were able to observe that the animals presented cyanosis, and a decrease in the limb temperature. After removal of the tourniquet and post-anesthetic reestablishment of the animals' functions, it was noted that the animals presented important claudication, which improved over a few weeks and resumed four weeks after the experiment.

We present below the experimental results obtained. The Figures show the temporal evolution over the four weeks of evaluation (frequency of rearing and of crossing).

Figure 1 presents the results of the exploratory behavior assessment. The mean number of rearings of the animals was used as a measurement of the degree of recovery from the injury. A higher number of rearings indicates faster recovery of the animal's muscle movements. In figure 1 we can see the animals' recovery over the weeks of evaluation. In the first two weeks, the two groups evaluated presented similar mean rearings. We can also observe the increase in the number of rearings of the two groups from the first to the second week, which may correspond to the start of the animal's muscle recovery. From the third week on, it is possible to clearly

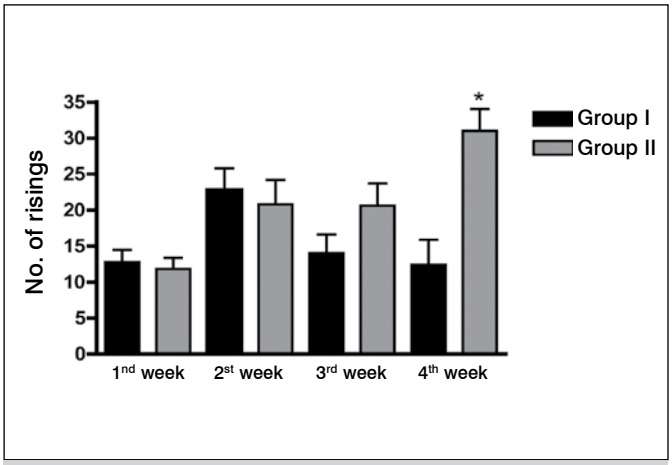

Figure 1. *Mean rearings presented by group I (control) and group II (experimental) during the 4 weeks of evaluation.* Significantly different.*

observe the effect of the kinesiotherapy treatment, since Group II (experimental) presented an increase in the number of rearings, while the control group obtained a much lower mean number of rearings. The results show that, for the experimental scenario used, the kinesiotherapy treatment brought about a significant improvement at the end of the four-week period in the recovery of the muscle movements of the experimental group in relation to the control group of 150%, with significant statistical difference (p=0.0331).

Figure 2 presents the number of crossings of the circular arena. The mean values of the five animals from each group evaluated during the four weeks of evaluation are presented here. The results of Figure 2 present a panorama similar to that of Figure 1, as the two evaluated groups had a similar behavior during the first two weeks and Group II (experimental) presented better recovery from the third week. At the end of the evaluation period, we noted significant improvement (60.4%) in the recovery of the motor activity of the experimental group in relation to the control group (p= 0.0136).

Table 1 summarizes the data presented in the previous two figures. In general, it was observed that from the third week of evaluation the effects of the kinesiotherapy treatment in recovery from the ischemic injury/reperfusion became more evident. It was also noted that in all the measurements evaluated, there was a more accentuated improvement of recovery of Group II (experimental) in relation to Group I (control). Therefore, such an improvement can be attributed to the kinesiotherapy treatment.

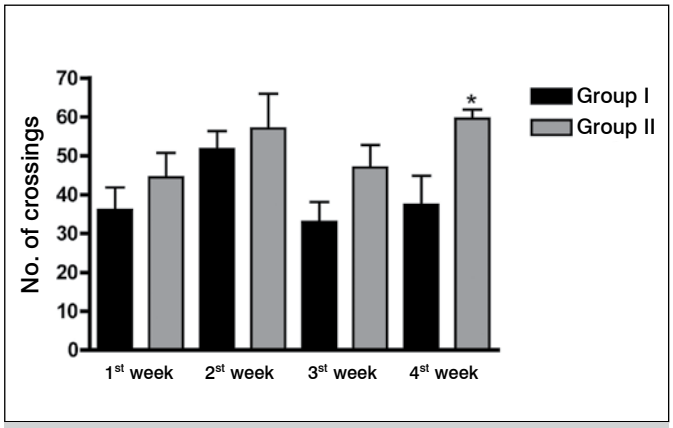

Figure 2. *Mean crossings presented by group I (control) and group II (experimental) during the 4 weeks of evaluation. * Significantly different.*

Table 1. *Data on weekly functional evolution.*

Weeks	Mean Risings		Mean Crossings	
	Group I	Group II	Group I	Group II
1st week	12.8	12	36	44
2nd week	22.8	21	51.8	57
3rd week	14	21	33	47
4th week	12.4	31	37.4	60

There was no inflammatory process, tissue injury and repair process observed in the histopathological analysis, which indicates that the ischemia time was too short to produce morphological lesions in the musculoskeletal and peripheral nerve tissue.

DISCUSSION

In biomedical literature there are other models used to produce tissue ischemia, such as clamping of the unilateral common iliac artery. This model only involves arterial irrigation, leaving venous drainage intact, and is considered more appropriate to simulate an arterial embolism. However, the collateral circulation will become available to irrigate the ischemic territory, which also occurs in the case of arterial embolism.[19]

There are models of skeletal muscle ischemia that use the skin circumference section, subcutaneous tissues and thigh musculature, around the hip joint, only preserving the bone, nerve, femoral artery and vein. Ischemia is produced by the occlusion, under a magnifying glass, of the femoral artery. This method is more traumatic and requires the use of a surgical microscope for its execution, yet it is the ideal model for the total elimination of collateral circulation.[20,21]

The most suitable model for simulating compression trauma or the surgical procedure of tourniquet application used in orthopedic operations is the method that advocates the use of the experimental tourniquet, which involves arterial irrigation and venous drainage simultaneously. In addition, it is argued that this model also favors the vascular lesion, due to the trauma of tourniquet application, and prevents vasomotor and venous response during reperfusion.[22,23] We opted for the experimental tourniquet model as it satisfies the objectives of attempting to simulate the occurrence of ischemia resulting from tourniquet application on limbs in more prolonged surgical procedures and is also applicable in compression traumas, such as victims trapped in crashed cars or under rubble, earth etc.

The functional alterations due to ischemia and to reperfusion of the muscle tissue are associated with fatigue, according to Murthy,[24] caused by the decrease in intracellular pH and imbalance of the Na+/K+ pump, which can lead to a deterioration of muscle contraction capacity or even to the death of this cell.[25]

Kinesiotherapy plays an important role in rehabilitation, and has the ability to promote functional and structural adaptations with the objective of preventing muscle atrophy and recovering the motricity and sensitivity of the areas involved.[11-13] The goals of functional rehabilitation are to promote adequate healing, fast functional return of the limb and to prevent complications caused by disuse such as muscular atrophy and articular contracture.[26,27] Physical exercise has been employed in experimental clinical studies with the purpose of stimulating peripheral nerve regeneration; however, these surveys do not examine the effects of specific types of exercise on regeneration of the peripheral nerve fibers. In the study by Ilha,[14] the possible effects of a program of aerobic training, of muscular resistance and the combination of both programs (for five weeks) in sciatic nerve regeneration in rats after nerve compression injury were tested using functional and morphometric analysis. The results obtained in the study

by Ilha[14] indicate that aerobic training improves functional recovery from the first week of training, and morphological differentiation of the sciatic nerve in regeneration after five weeks of training when compared with the sedentary injured animals. On the other hand, the results of our study indicate that kinesiotherapy had a beneficial effect in functional behavior from the third week, but did not show morphological improvement in the tissues.

In the present study we also observed an increase in the frequency of crossings and rearings in the acrylic arena in the animals submitted to kinesiotherapy when compared with the animals of the control group. The increase in the number of rearings evidences the improvement of these animals, as they managed to bear their weight on their hind legs. This improvement that occurred from the third week may correspond to the start of the animals' muscle recovery and is consistent with a similar study conducted with dogs, where kinesiotherapy positively influenced the functional improvement of the dogs' limb.[28]

A lot of the studies that involve functionality of the pelvic limb of rats are related to sciatic nerve injuries[29-31] and their evaluation is obtained using the SFI (Sciatic Functional Index). However, little is known about the functional evaluation or the recovery of the muscle tissue in injuries caused by ischemia and reperfusion.

The evaluation in the arena is determined by the measurement of the attractive behaviors by placing the animal in a new open space from which escape is prevented by a circular wall. The triggering of these behaviors is dependent on the animal's interaction with a range of factors such as: removal of the animal from a familiar environment, exposure to a new environment, injection of drugs, neural lesions, changes related to the emotional state, besides habituation and learning.[32] Thus, the quantity of movements becomes an indicator of exploratory behavior and indicates an improvement in the animal's tissue and functional repair process.[33] Although Blakley[34] cites that rats with greater stress and anxiety have less exploratory behavior, which can explain the decrease in the number of crossings and rearings in the third week, which was resumed in the fourth week.

It can be observed in the evaluations that the control and experiment groups became accustomed to the environment (arena) from the third week of evaluation. However, comparing groups GI and GII, group GI (control) evidenced greater habituation in the exploratory activity when compared with group GII (experimental). Habituation principles can be understood by mechanisms of behavioral plasticity, characteristic of learning and memory processes[35]. Some studies seek to show habituation in uninjured rats submitted to some kind of stimulation. Varty[36] demonstrated, through analysis of locomotor activity, that both stimulated and control rats exhibit this phenomenon.

Anyhow, the values found in this study suggest that, even with the two groups being evaluated over the four weeks, in the same environment (arena), and, thus being prone to the physiological mechanisms of environment habituation, group GII managed to explore the environment better than group GI, which reinforces the effects of kinesiotherapy in the functional improvement of these animals.

As regards the effects of exercise in tissue repair, we cannot affirm that kinesiotherapy was essential in this study, since it failed to present any morphological difference in the histological analysis both in the control group and in the experimental group. We believe that the microscopy method used to analyze this experiment was not totally adequate and that the biochemical analysis might be able to evidence alterations in the tissues analyzed histologically, since the biochemical alterations of the basic cell functions are related to the decrease in oxygen and energy failure of the cell.[37,38]

In addition, of the articles found on this topic, what stands out is the absence of a pattern in relation to the method used to perform ischemia, besides which there are various ischemia and reperfusion times, and methods to be used as a form of treatment, as well as different methods of evaluation of the results obtained, which makes it very hard to compare the studies.

Although the results do not evidence cell injury, the study demonstrates that the muscle fiber and the peripheral nerve tissue are resistant to morphological alterations arising from ischemia and reperfusion in this experimental model.

CONCLUSION

The muscle fiber and the peripheral nerve proved resistant to the morphological alterations resulting from ischemia and reperfusion; it was not possible to affirm that kinesiotherapy was crucial in tissue repair. On the other hand, exercise presented a beneficial effect in the functional rehabilitation of the pelvic limb of these animals.

REFERENCES

1. Silva JCCB, Burihan E. Diagnóstico clínico da isquemia crítica dos membros. Rev Bras Clin Ter. 1999; 25(2):71-9.
2. Ramaciotti O. Síndrome isquêmica aguda. In: Cordeiro GCL. Manual de angiologia para o clínico. São Paulo: Organon; 1974. p. 89-100.
3. Yoshida WB. Radicais livres na síndrome da isquemia e reperfusão. Cir Vasc Angiol. 1996;12:82-95.
4. Silveira M, Yoshida WB. Isquemia e reperfusão em músculo esquelético. J Vasc Bras. 2004;3(4):367-78.
5. Hearse DJ, Humprey WG, Bullock GR. The oxygen paradox and the calcium paradox: Two facets of the same problem? J Moll Cell Cardiol. 1978;10:641-68.
6. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985;312(3):159-63.
7. Freisleben HJ. Lipoate ameliorates ischemia-reperfusion in animal models. Clin Hemorheol Microcirc. 2000;23(2-4):219-24.
8. Córdova A, Navas JF. Os radicais livres e o dano muscular produzido pelo exercício: papel dos antioxidantes. Rev Bras Med Esporte. 2000;6(5):204-8.
9. Percário S. Alterações oxidativas e da defesa antioxidante no broncoespasmo agudo induzido em cobaias [tese]. São Paulo: Universidade Federal de São Paulo/Escola Paulista de Medicina; 2000.
10. Simonini G, Pignone A, Generini S, Falcini F, Cerinic MM. Emerging potentials for an antioxidant therapy as a new approach to the treatment of systemic sclerosis. Toxicology. 2000;155(1-3):1-15.
11. Herbison GJ, Jaweed MM, Ditunno JF. Histochemical fiber type alterations secondary to exercise training of reinnervating adult rat muscle. Arch Phys Med Rehabil. 1980;61(6):255-7.
12. Sakakima H, Yoshida Y, Sakae K, Morimoto N. Different frequency treadmill running in immobilization-induced muscle atrophy and ankle joint contracture of rats. Scand J Med Sci Sports. 2004;14(3):186-92.
13. Seo TB, Han IS, Yoon JH, Hong KE, Yoon SJ, Namgung U. Involvement of Cdc2 in axonal regeneration enhanced by exercise training in rats. Med Sci

Sports Exerc. 2006;38(7):1267-76.
14. Ilha J. Efeitos do exercício físico na recuperação nervosa periférica após lesão traumática experimental do nervo ciático em ratos adultos [dissertação]. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2007.
15. Stein C, Bueno OF, Xavier GF. Rats do react to stimulus omission. Braz J Med Biol Res. 1994;27(10):2423-30.
16. Denenberg VH. Open-field bheavior in the rat: what does it mean? Ann NY Acad Sci. 1969;159(3):852-9.
17. Birke LIA, Archer J. Some issues and problems in the study of animal exploration. In: Birke LIA, Archer J. editors. Exploration in animals and humans. Cambridge:University Press; 1983. p.279.
18. Oliveira APR. Avaliação clínico-comportamental e histopatológica do encéfalo de Gerbils submetidos à isquemia cerebral experimental induzida por oclusão permanente da artéria carótida [dissertação]. Franca: Universidade de Franca; 2005.
19. Silva MG, Castro AA, Ramos EAG, Peixoto E, Miranda Jr F, Pitta GBB et al. Estudo histológico e bioquímico sérico do alfa-tocoferol na lesão de isquemia e reperfusão em membros pélvicos de ratos. Acta Cir Bras. 2005;20(5):375-81.
20. Kerrigan CL, Stotland MA. Ischemia reperfusion injury: a review. Microsurgery.1993;14(3):165-75.
21. Webster RS, Montero EFS, Fagundes DJ, Zettler CG, Coiro J. O papel do precondicionamento isquêmico na lesão de isquemia e reperfusão do músculo grácil de ratos. Acta Cir Bras. 2006;21(2):80-6.
22. Blaisdell FW. The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: a review. Cardiovasc Surg. 2002;10(6):620-30.
23. Badhwar A, Dungey AA, Harris KA, Scott JA, McCarter SD, Scott JR, et al. Limitations of ischemic tolerance in oxidative skeletal muscle: perfusion vs tissue protection. J Surg Res. 2003;109(1):62-7.
24. Murthy G, Kahan NJ, Hargens AR, Rempel DM. Forearm muscle oxygenation decreases with low levels of voluntary contraction. J Orthop Res. 1997;15(4):507-11.
25. Gazzoni M, Camelia F, Farina D. Conduction velocity of quiescent muscle fibers decreases during sustained contraction. J Neurophysiol. 2005;94(1):387-94.
26. Clark B, McLaughlin RM. Physical rehabilitation in small-animal orthopedic patients. Vet Med. 2001;96(3):234-46.
27. Marsolais GS, Dvorak G, Conzemius MG. Effects of postoperative rehabilitation on limb function after cranial cruciate ligament repair in dogs. J Am Vet Med Assoc. 2002;220(9):1325-30.
28. Souza SF, Mazzanti A, Raiser AG, Salbego FZ, Fonseca ET, Festugatto R, et al. Reabilitação em cães submetidos a artroplastia do joelho. Ciência Rural. 2006;36(5);1456-61.
29. Monte-Raso VV, Barbieri CH, Mazzer N. Índice funcional do ciático nas lesões por esmagamento do nervo ciático de ratos. Avaliação da reprodutibilidade do método entre examinadores. Acta Ortop Bras. 2006;14(3);133-6.
30. Gaparini ALP, Barbieri CH, Mazzer N. Correlação entre diferentes métodos de avaliação funcional da marcha de ratos com lesão por esmagamento do nervo isquiático. Acta Ortop Bras.2007; 15(5):285-9.
31. Costa J, Camargo VM, André ES. Desenvolvimento de um método de baixo custo para avaliação da marcha em ratos. Fisioter Mov. 2008;21(2):115-23.
32. Sachs BD. The development of grooming and its expression in adult animals. Ann N Y Acad Sci. 1988;525:1-17.
33. Berntson GG, Jang JF, Ronca AE. Brainstem systems and grooming behaviors. Ann N Y Acad Sci. 1988;525:350-62.
34. Blakley G, Pohorecky LA. Psychosocial stress alters ethanol's effect on open field behaviors. Pharmacol Biochem Behav. 2006;84(1):51-61.
35. Eichenbaum H. The hippocampus and mechanisms of declarative memory. Behav Brain Res. 1999;103(2):123-33.
36. Varty GB, Paulus MP, Braff DL, Geyer MA. Environmental enrichment and isolation rearing in the rat: effects on locomotor behavior and startle response plasticity. Biol Psychiatry. 2000;47(10):864-73.
37. Perry MO. Compartment syndromes and reperfusion injury. Surg Clin North Am.1988;68(4):853-64.
38. Quinones BWJ, Saleh S. Acute arterial occlusion. J Vasc Surg. 1991;33:578-93.