

Preservation of Amputated Limbs by Arterial Perfusion with HTK Solution in Experimental Rabbits

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Abstract

Objective: To evaluate the effect of HTK infusion on the protection and replantation of amputated limb. **Methods:** In experiment 1, the amputated limbs of male New Zealand white rabbits were preserved by different preservation methods, including low-temperature HTK solution perfusion group, low-temperature normal saline perfusion group, low-temperature preservation group and non-perfusion group at normal temperature. The homogenates of muscle tissue at different time points were collected for biochemical detection. H&E staining was performed on muscle tissue. Bax, Bcl-2 protein immunohistochemical staining and Tunel method were used to detect apoptosis in muscle, nerve and vascular tissues. The ultrastructure of cells was observed by transmission electron microscope. In experiment 2, the amputated limbs of rabbits were treated with HTK perfusion and cryopreservation without perfusion, respectively. The survival of rabbits and their limbs were observed, and detected by H&E, TUNEL and scanning electron microscopy. **Results:** Experiment 1: With the extension of disconnection time, there were statistical differences between the biochemical indexes of the HTK liquid perfusion group and those of the non-low temperature non-perfusion group, while some indexes of the other groups were different. Immunohistochemical staining of tissues (muscle, nerve and blood vessel) in each group showed that the number of cells stained by anti-apoptotic protein BCL-2 in each group significantly increased with the extension of time, while the number of cells stained by pro-apoptotic protein BAX2 in each group significantly decreased. Tunel test showed that compared with other treatment groups, the apoptosis rate of the HTK liquid group was significantly decreased. Experiment 2: The survival rate of the experimental group was 60%, and that of the control group was only 30%. H&E staining, the TUNEL method and scanning electron microscopy suggested that HTK infusion could reduce ischemia-reperfusion injury in muscle tissue. **Conclusions:** 1. Perfusion preservation of severed limbs at 4 °C can effectively reduce tissue damage caused by ischemia and hypoxia, and the effect is stronger than direct preservation. 2. HTK solution and normal saline were used for one-time perfusion of the severed limbs of rabbits, and the preservation effect of HTK solution was the best. 3. HTK solution was used for one-time perfusion of severed hind limbs of rabbits, which was refrigerated at 4 °C and then replanted (ischemia time was 3-5 h). Compared with the replanted limbs after cold storage alone, the survival rate was higher, and the postoperative ischemia and hypoxia injury of surviving limbs were less.

Keywords

Amputated Limb, Organ Preservation Solution, Perfusion, HTK Infusion, Back Planting

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