Necrostatin-1 Protects against Ischemia/Reperfusion Injury by Inhibition of RIP-1 (Receptor-interacting protein 1) in a Rat Flap Model

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INTRODUCTION: The failure of reconstructive surgeries remains a challenge for plastic surgeons. Ischemia reperfusion (I/R) injury is considered to be one of the major problems in flap surgery. Necroptosis is a recently discovered, caspase-3 independent programmed necrosis. Necrostatin-1 (Nec-1) is a specific inhibitor of necroptosis. Reports indicate that Nec-1 provides protection in ischemic models, such as brain, kidney and heart. The aim of this research is to investigate whether this effect is also applicable to the field of flap surgery.

MATERIALS AND METHODS: The extended epigastric skin flap (6×9cm) of rats was used. Three hours of complete ischemia was performed using a clamp and then the clamp was removed to reperfusion the flap. Sixteen rats were randomly assigned into 2 groups: (1) Control: Fifteen min before ischemia onset and 15min after the onset of reperfusion, PBS (1.65ml/kg) was administrated intraperitoneal. (2) Nec-1: Fifteen min before ischemia onset and 15min after the onset of reperfusion, Nec-1 (1.65mg/kg) was applied intraperitoneal. Twenty-four hours after the onset of the reperfusion, the rats were assessed for flap survival and perfusion analysis. The flap was divided into 54 segments of equal size (1×1cm) for HE and immuno-staining, electron microscope, and ELISA analysis (Figure1).

RESULTS: The flap survival rates in Nec-1 group were significantly higher than control group (79.86%±5.79 % vs. 66.54%±9.69 %, P=0.016). The mean vascular flows in Nec-1 group and control flap were 55.93±13.02PU and 39.98±9.14 PU, respectively (P=0.034) (Figure2). Electron microscope detected necrotic cells started to present 7cm from the pedicle in Nec-1 group while 5cm in the control group. Immuno-staining showed increased levels of RIP1 at 8 and 9cm from the pedicle in Nec-1 group, while it increased at 7, 8 and 9cm from the pedicle in control group. There was no significant difference in Caspase-3 between 2 groups.

CONCLUSIONS: Nec-1 has a protective effect against I/R injury by the inhibition of RIP1 on the skin island flap model, which made it a promising novel strategy in clinical setting.

REFERENCES:


FIGURE LEGEND:

Figure 1. (A) Flap model. (B) The flap was divided into 54 segments. (C) Schematic of the protocol.

Figure 2. (A) Flaps 24 h after the reperfusion. (B,C) Flap survival rates and mean vascular flows in Nec-1 group were significantly higher than the control group.