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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INTRODUCRION: 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.[1] Reports indicate that Nec-1 provides protection in ischemic models, such as brain, kidney and heart.[2] 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:

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2. Linkermann A, Bräsen JH, Himmerkus N, Liu S, Huber TB, Kunzendorf U, Krautwald S. Rip1 (Receptor-interacting protein kinase 1) mediates necroptosis and contributes to renal ischemia/reperfusion injury. Kindey International. 2012;81:751-761.

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.

Figure1

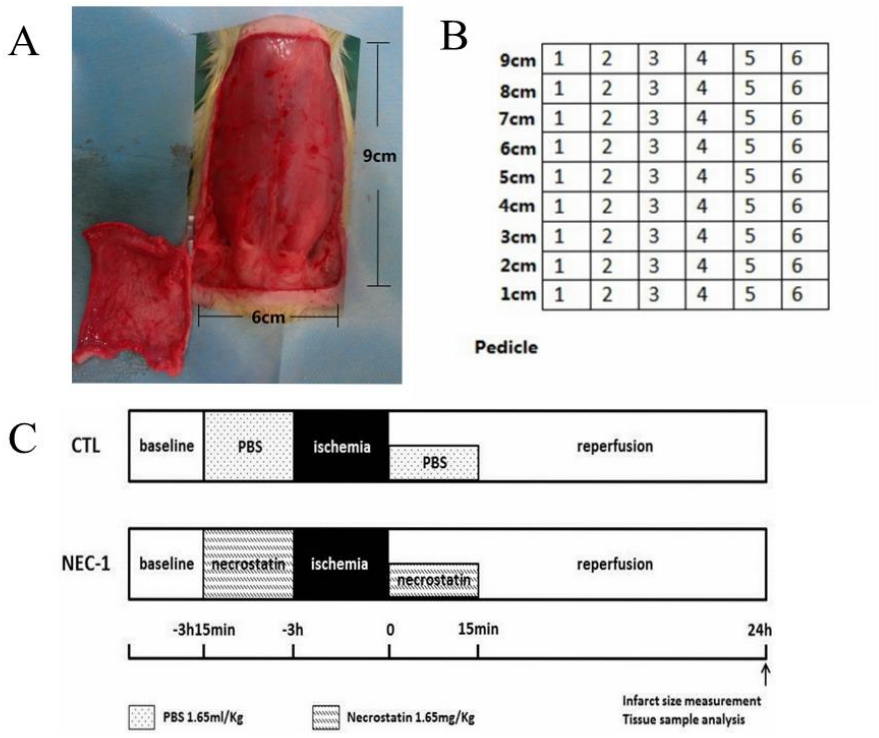
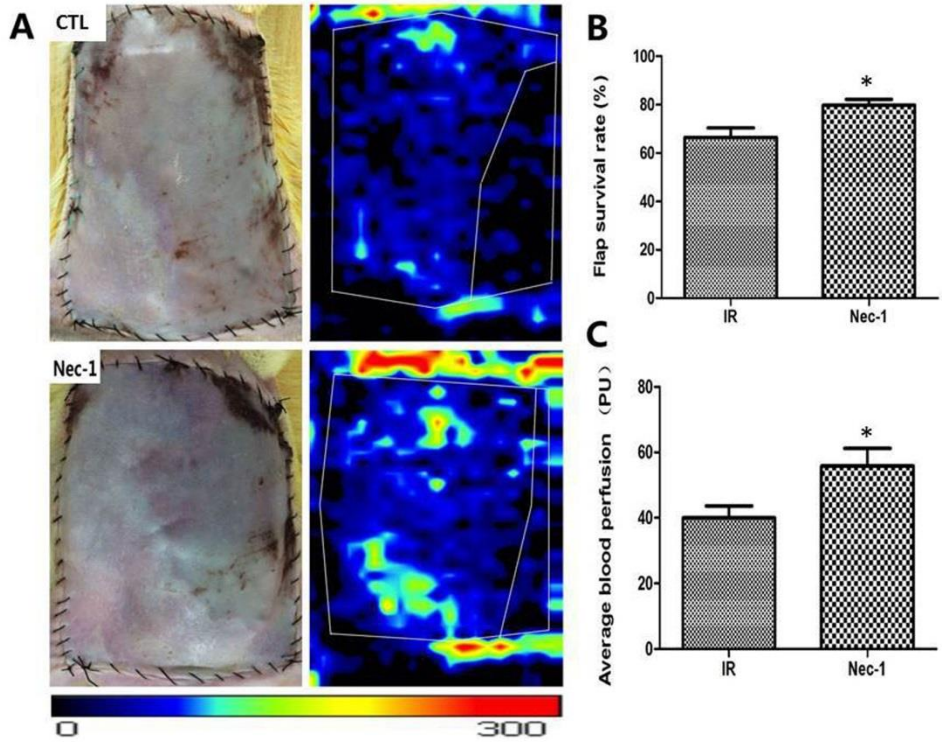

A
9cm
6cm
B
9cm 1 2 3 4 5 6
8cm 1 2 3 4 5 6
7cm 1 2 3 4 5 6
6cm 1 2 3 4 5 6
5cm 1 2 3 4 5 6
4cm 1 2 3 4 5 6
3cm 1 2 3 4 5 6
2cm 1 2 3 4 5 6
1cm 1 2 3 4 5 6
Pedicle
C
CTL
baseline
PBS
ischemia
PBS
reperfusion
NEC-1
baseline
necrostatin
ischemia
necrostatin
reperfusion
-3h15min
-3h
0
15min
24h
PBS 1.65ml/Kg
Necrostatin 1.65mg/Kg
Infarct size measurement
Tissue sample analysis

Figure2


A
CTL
Nec-1
0
300
B
Flap survival rate (%)
100
80
60
40
20
0
IR
Nec-1
*
C
Average blood perfusion （PU）
80
60
40
20
0
IR
Nec-1
*