A Topical Mixture for Preventing, Abolishing, and Treating Autophagia and Self-Mutilation in Laboratory Rats

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The dysesthesia and paresthesia that occurs in laboratory rats after spinal cord injury and peripheral nerve injury results in autophagia and self-mutilation. This self-destructive behavior interferes with functional assessments in designed studies and jeopardizes the health of the injured rat. We developed a topical mixture that prevents, abolishes, and treats autophagia and self-mutilation. When the mixture is applied to the limb, its bitterness effectively prevents the rat from licking and biting the limb. In addition, the mixture has antiseptic properties.

Laboratory rats are the most commonly used animals for experimental spinal cord and peripheral nerve injuries. After these types of injuries, rats may develop sensory dysfunction. Dysesthesia, and especially paresthesia, leads to the rats licking then chewing their nails and the tips of their toes. In severe cases, the whole toe and even the foot may be chewed (1, 2).

This self-destructive behavior of the injured rat should be stopped as soon as possible. Commonly, bleeding and the open wound will cause anemia and body weight loss. Repeated chewing of the wound can cause severe infection of the affected limb. Furthermore, the poor health of these rats combined with swelling of, trauma to, or loss of the limb make neurologic assessment of the limb inaccurate. For humane reasons, these rats are euthanized and excluded from the designed experiment.

There are several ways to disrupt this self-destructive behavior. First, an Elizabethan collar can be placed around the neck to prevent the animal's mouth from reaching the affected limbs. Second, solutions such as Chew Guard (Summit Hill Lab, Navesink, N.J.) and New Skin (Medtech, Jackson, Wyo.) can be sprayed on or applied to the affected limb to prevent the rat from chewing the limb. Both strategies are useful. However, the collar is awkward for injured rats and affects their ability to drink and eat. The solutions lose their effectiveness quickly because the thin coating wears off as the gas evaporates and the sprayed area touches the rough surface of the bedding materials. Moreover, these treatments do not abolish the behavior nor do they treat the wounded limb.

We have developed a mixture to prevent and abolish this self-destructive behavior and to treat the wounded limb of spinal cord- and nerve-injured rats. We were inspired by an old Oriental way of stopping young children from habitually sucking their finger by smearing the bitter juice of a plant (*Coptis chenensis*) on the digit. Animals also will avoid chewing anything that has a bitter taste. Metronidazol (2-methyl-5-nitromedazole-1-ethanol, ICN Biomedical Research Products, Irvine, Calif.) is odorless and has a strong bitter taste. It is a commonly used antitrichomonal and antibiotic to treat anaerobes. New Skin is an antiseptic liquid bandage. It has a mild solvent odor but is tasteless. We added 500 mg Metronidazol powder to 1 ml of New Skin to make our mixture. After smearing this mixture on the rat's limb, the solvents evaporated, and a dried coating of this mixture was left on the target area. Therefore, in contrast to New Skin alone, the combination of Metronidazol with New Skin provides both an aversive bitter-tasting substance to block the self-destructive behavior and a protective covering to allow healing.

We evaluated this mixture on adult (weight, 160 to 200 g) female Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, Ind.) with lumbar spinal cord injury (SCI), which was produced by dropping a 10-g weight from a height of 25 mm onto the L2 spinal cord segment or by injection of kainic acid (RBI, Natick, Mass.), a neurotoxin, into the same segment (3, 4). Both of these injuries cause paresthesia and paraplegia. All surgical interventions and pre- and post-surgical care were provided in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, *Guide for the Care and Use of Laboratory Animals*, and the guidelines of the Animal Care and Use Committee of the University of Louisville. All rats were anesthetized by using pentobarbital (50 mg/kg intraperitoneally) for the SCI procedures. After SCI, the rats were housed individually under standard conditions and did not receive post-surgery analgesics.

For the purpose of studying abolishment and treatment of autophagia and self-mutilation, 24 anesthetized rats underwent contusion SCI. By 10 days post-contusion SCI, autophagia occurred in nine rats. The affected area in all of these rats were the toes of the hindlimbs. Two rats were euthanized because of severe self-mutilation. The other seven rats were treated with the mixture. All of these rats stopped chewing their toes after one treatment. One treated rat required an additional treatment at day 16 post-SCI because autophagia returned.

For the purpose of studying autophagia prevention, another 24 anesthetized rats underwent kainic acid-induced SCI. We smeared the mixture on the toes of 13 of these rats immediately after SCI. At 2 to 3 weeks post-SCI, only one of these rats had chewed its toes. This chewing stopped after one additional treatment. Of the 11 rats that did not receive treatment immediately after SCI, 4 developed autophagia within 10 days post-SCI.

Thus, we have found that the combination of Metronidazol with New Skin is an easy, topical way to effectively prevent, abolish, and treat autophagia in rats with SCI. We feel that these results strongly support the use of preventative treatment in reducing the self-mutilation behavior by anticipating the occurrence of autophagia.

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