

SATURATED PICRIC ACID PREVENTS AUTOPHAGIA AND SELF-MUTILATION IN LABORATORY RATS

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Abstract- The dysesthesia and paresthesia that occurs in laboratory rats after spinal cord injury (SCI) results in autophagia. This self-destructive behavior interferes with functional assessments in designed studies and jeopardizes the health of the injured rat. In this study, we evaluated role of saturated picric acid in the prevention of autophagia and self-mutilation. All rats were anesthetized with an intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) for the SCI procedures. In the first 39 rats, no solution applied to the hind limbs, but in the next 26 cases, we smeared the saturated picric acid on the tail, lower extremities, pelvic, and abdomen of the rats immediately after SCI. In the rats without picric acid, 23 rats died following autophagia, but in the 26 rats with picric acid, there was no autophagia ($P < 0.001$). Picric acid side effects in skin and gastrointestinal signs such as irritation, redness and diarrhea were not seen in any rat. Saturated picric acid is a topical solution that if used appropriately and carefully, might be safe and effectively prevents autophagia and self-mutilation. When the solution is applied to the lower abdomen and limbs, we presume that its bitterness effectively prevents the rat from licking and biting the limb.

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Key words: Autophagia, self-mutilation behavior, rat, spinal cord injury

INTRODUCTION

Laboratory rats are the most commonly used animals for experimental spinal cord injuries. After this type of injury, rats may develop sensory dysfunction. Dysesthesia and paresthesia lead rats to lick and then to chew their nails, toes, and foot (1-3). Repeated chewing of the wound could cause severe infection of the affected limb. This self-destructive behavior interferes with functional assessments in designed studies and jeopardizes the health of the injured rat.

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. This strategy is useful. However, the solutions lose the effectiveness quickly because the thin coating wears off as the gas evaporates and the sprayed area touches the rough surface of the bedding materials.

Animals avoid anything that has a bitter taste. Thus, Zhang *et al.* added 500 mg metronidazole (2-methyl-5-nitro-medazole-1-ethanol) powder to 1 ml of New Skin and it was effective for a short time (3). The main question is how can we prevent or overcome self-mutilation for a long time?

We performed this study to evaluate the safety and efficacy of saturated picric acid (2, 4, 6 - trinitrophenol) in the prevention of autophagia and self-mutilation.

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MATERIALS AND METHODS

We evaluated saturated picric acid solution on adult (weight, 200 to 300 g) female Ratus Norvegicus Wistar with thoracic SCI, which was produced by applying aneurysm clip [Aesculap mini-clip, Phynox, permanent Yasargil aneurysm clips FE716K; closing force: 119g (1.17N), Range: 1.15-1.41N; 5.0 mm L, 3.5 mm D (max. opening), angled blade] at the ninth thoracic level (4, 5). This severe injury causes paresthesia and paraplegia in all rats. All surgical interventions and pre- and post-surgical care were provided in accordance with the protocol approved by Zahedan University Medical Sciences Animal Care and by National Iranian Ministry of Health.

All rats were anesthetized with an intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) for the SCI procedures. After SCI, the rats were housed in individual cages under standard conditions. They were given normal saline (10 cc, twice daily, subcutaneous for 3-7 days), intraperitoneal injection of buprenorphine (0.03 mg/kg for 10-14 days), prophylactic antibiotics (gentamycin sulfate 1 mg/kg and cephazolin 75 mg/kg for 10 weeks). When we studied the effect of decompression on complete spinal cord injury in rats (4, 5), there was an important complication of autophagia and self-mutilation in a few rats. In the first 39 rats, no solution applied to the hind limbs, but in the next 26 cases, we smeared the saturated picric acid on the tail, lower extremities, pelvic, and abdomen of the rats immediately after SCI. It has a yellow color and covered above-mentioned areas. It was repeated whenever the color (coverage) was going to be removed. Repetition was twice weekly for 10 weeks as an average, but could be from every other day to the once weekly.

Pearson Chi-square test was evaluated for statistical analysis. To perform data analysis, the Stata 8 software applications were used. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Sixty-five anesthetized rats underwent clip compression SCI. In the first 39 rats, twenty-three rats (59%) died following autophagia. It was started suddenly with no apparent reason in animals, without any wound or injury on the skin. Self-mutilated behavior was started from 3 to 21 days following SCI (mean \pm standard deviation was 9.35 ± 5.8). Once the animals had started autophagia they continued with it, without any indications of stopping it. There was no recovery from autophagia and no survival for long time. It varied from one leg to bilateral complete lower limbs amputation including chewing the bone and muscles of lower abdomen and pour out of the small and large intestine. It seems that lateral leg and thigh were the most common sites of initiation of autophagia.

In the second group (26 rats), autophagia was prevented completely by applying saturated picric acid solution to the lower half of the body including abdomen, pelvic, lower limbs and tail postoperative (0%). As far as our observation, no biting and licking was observed in the picric acid applied group of rats ($P < 0.001$) (Table 1).

Picric acid side effects in skin and gastrointestinal signs such as irritation, redness and diarrhea were not seen in any rat.

Table 1. Autophagia in rats with and without application of picric acid solution*

| | Positive | | Negative | | Total | Pearson Chi square |
|-----------------------------|----------|----------|----------|----------|-------|-----------------------|
| | Observed | Expected | Observed | Expected | | |
| Picric acid applied | 0 | 9.2 | 26 | 16.8 | 26 | 23.73 ($P < 0.001$) |
| Picric acid did not applied | 23 | 13.8 | 16 | 25.2 | 39 | |

* Data are given as number.

DISCUSSION

Autophagia is an important problem in the spinal cord injured rats. When self mutilation starts in a spinal rat, his/her life is in serious danger.

There is no common identified prevention from autophagia or solution for this important complication. Solutions such as Chew Guard and New Skin were sprayed on or applied to the limb to prevent the rat from chewing the limb. These solutions lose the effectiveness quickly when the gas evaporates or the sprayed area touches the surfaces of their bed. When Zhang *et al.* added bitter metronidazole powder to the New Skin, it had transient effect (3). Considering our observation and follow-up, it seems not only picric acid has no hazard to the personnel and rats, but also it can be safe and effective in the prevention of autophagia and self-mutilation. Twice weekly application of picric acid is enough. Thus, two advantages of picric acid are long lasting coverage and effective prevention in all animals.

There were 59% autophagia in our severe SCI lesion group without application of picric acid. Other investigators have also noted autophagia in spinal animals (2, 4-13). It is not known why some and not all animals indulge in autophagia.

Picric acid could be an occupational health hazard and has a number of undesirable side effects. Topical exposure of picric acid on skin or mucus membranes causes irritation, redness and pain. Inhalation of the agent initiates cough and sore throat, and ingestion causes headache, dizziness, nausea and diarrhea. Effects like this may have animal welfare aspects, but considering our observation, neither side effect nor negative influences on the research model was seen in our 26 rats during husbandry and studies. Furthermore, using mask on the nose and mouth and wearing glove prevented the same risks for the personnel handling the agent.

Saturated picric acid solution, which was applied to the lower extremities and abdomen of the rats, prevented autophagia. Zhang *et al.* reported that the toes of the hind limbs were the affected area in all of their rats (3). In our series of rats, toes were not involved in all of the rats.

Das *et al.* reported that the incidence of autophagia is higher in the animals with 55% lesions than in those with 100% lesion. There were 6.5% autophagia in 100% lesion group, but 29% in 55% lesion group. They concluded that sparing of some spinal cord from lesion seemed to be related to a higher percentage of cases indulging in autophagia (14).

Saade *et al.* showed that autotomy was associated only with spinal cuts that involved the anterolateral column. Their findings strongly suggest that pain resulting from previous exposure to the injury produces a memory trace in the central nervous system, which can account for the phantom pains encountered in various clinical conditions (2). They showed in other study that dorsolateral funiculus lesion, followed immediately by leg denervation, resulted in accelerated regular autotomy (15).

Zhang *et al.* showed that by using bupivacaine powder, a sodium channel blocker, at the locus of transection immediately after nerve injury, the chronic pain behaviors were prevented; the hyperexcitability of wide dynamic range neurons was also substantially reduced (9). Fairbanks *et al.* reported that agmatine, an endogenous neuromodulator, exogenously administered to rodents, decreased hyperalgesia-accompanying inflammation, and reduced autotomy-like behavior and lesion size after excitotoxic spinal cord injury (10). Autophagia often can not be relieved by opiates. It seems the loss of sensitivity to opiates may be associated with the up-regulation of endogenous antioioid substances (13).

We recommend applying saturated picric acid to the abdomen and hind limb of all SCI rats to prevent autophagia. It is an easy, topical way to effectively prevent autophagia in rats with SCI. We feel that these results strongly support the use of preventive treatment in reducing the self-mutilation behavior by anticipating the occurrence of autophagia.

In conclusion, we have found picric acid an effective method to prevent autophagia and though it is known as toxic, we didn't face any unwanted side effects, so it might be a safe method as well.

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Conflict of interests

The authors declare that they have no competing interests.

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