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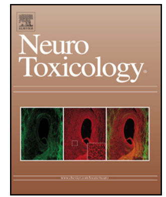

Functional and histopathological changes induced by intraparenchymal injection of kainic acid in the rat cervical spinal cord

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ABSTRACT

Kainic acid (KA) is an analog of the neurotransmitter glutamate and is widely used as an excitotoxic agent to lesion spinal cord networks, thus, providing an interesting model to learn basic mechanisms of spinal cord injury. The present work was aimed to evaluate motor and sensory performance of rats and analyze morphometric parameters of spinal cord neurons after KA injection. Animals were injected either with 0.75, 1 or 1.25 mM of KA at the C5 segment of the cervical spinal cord. Motor and sensory performance of the rats were evaluate at day 0 (before injection) and at days 1, 2, 3 and 7 post-injection (pi) and compared with those of saline-treated and non-operated animals. Animals were sacrificed at each time point for morphometric and histopathological analysis and compared among groups. All KA-treated animals showed a significant impairment at the motor and sensory tests for the ipsilateral forelimb in a concentration-dependent manner in comparison to saline-treated and non-operated animals. Neuronal cell count showed a significant loss of neurons at C4, C5 and C6 cervical segments when compared with those of saline-treated and non-operated animals. The contralateral side of the cervical segments in KA-treated rats remained unchanged. Some improvement at the motor and sensory tests was observed in animals injected with 0.75 and 1 mM KA. Moreover, a mild increase in the neuronal count of the damaged segments was also recorded. The improvement recorded in the motor and sensory tests by day 7 pi may be a consequence of a neuron repairing mechanism triggered soon after the KA excitotoxic effect.

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1. Introduction

Kanic acid (KA) is a cyclic analog of the major stimulatory brain neurotransmitter glutamate. It acts on neuronal receptors inducing an excitotoxic effect, causing neuronal death in different regions of the CNS (Bayrakdar et al., 2013; Ben-Ari and Kainate, 2000; Kuzhandaivel et al., 2010; Pereno et al., 2011). Taccola et al. (2008) showed that 24 h after a time-constrained (1 h) kainate application, KA causes extensive neuronal network damage and early irreversible loss of neuronal activity. It has been shown that high extracellular concentrations of glutamate evoke spinal damage *in vivo* (Liu et al., 1999; Matsui et al., 2005). KA induces an intracellular influx of Ca^{2+} that leads to the production of free radicals, the hyperactivation of the intracellular enzymes poly (ADP) ribose polymerase-1 and ATPase, thus triggering energy failure, DNA damage and neuronal death (Mazzone and Nistri, 2011; Mitra et al., 2013).

Different experimental methods have been used to study spinal cord injury (SCI) and neurodegenerative diseases. Some of the most commonly experimental models used are the weight drop (contusion) (Chvatal et al., 2008; von Euler et al., 1996), the transection of the dorsal section of the spinal cord (Kunkel-Bagden et al., 1993; Schrimsher and Reier, 1993) and the injection of a specific compound into the spinal cord (neurotoxin) (Klein et al., 2009). Thus, a reliable test protocol, suitable for the injury model is

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essential for proper evaluation of motor and sensory dysfunctions, as well as functional recovery after SCI.

At present, KA is used as a useful model to replicate glutamate excitotoxicity in *in vivo* models, in neuron cultures as well as in isolated organotypic slices of spinal cord (Calderó et al., 2010; Kuzhandaivel et al., 2010; Magnuson et al., 1999; Mazzone et al., 2010; Taccola et al., 2008). Mazzone et al. (2010) were able to evoke a reproducible pattern of spinal network lesions, and to assess the functional outcomes in terms of motor neuronal network activity *in vitro*.

Although KA is known as a neurotoxic drug, to our knowledge there are no reports of its effects after intraparenchymal injection in the rat cervical spinal cord. There are few reports using KA with the intrathecal via (Mitra et al., 2013), and others using intraparenchymal injection of KA analogs, such as AMPA, quisqualic acid and NBQX (Corona and Tapia, 2004; Hirata et al., 1997). The aim of the present research was to perform a systematic study of motor and sensory functional changes and histopathological lesions that different concentrations of KA may cause at the C5 cervical segment of inoculated rats. The data are expected to be relevant for the design of future trials for the prevention and treatment of other neurodegenerative diseases at the cervical spinal cord.

2. Materials and methods

2.1. Animals

Young (3–4 mo. 200–300 g) (n = 75) male Sprague-Dawley rats, raised in our rat colony, were used. Animals were housed in a temperature-controlled room (22 ± 2 °C) on a 14:10 h light/dark cycle. Food and water were available *ad libitum*. All experiments with animals were performed according to the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of INIBIOLP's Animal Welfare Assurance No. A5647-01.

2.2. Toxin administration

On experimental day 0, rats were anesthetized with ketamine hydrochloride (40 mg/kg; ip) plus xylazine (8 mg/kg; im) and placed in prone position. Kainic acid injection was performed as previously described (Nishida et al., 2014). Briefly, to gain access to the C5 segment a trepanation at the C4–C5 fibrous joint 1 mm lateral from the midline (dorsal spinal process) of the vertebral column was performed. For injecting the solution a 10 μl Hamilton® syringe fitted with a 26G needle was hand-held. At certain distance from the tip, the needle had a mark to indicate the limit of penetration into the spinal cord. The needle was vertically introduced 1.5 mm down on the right side of the spinal cord in order to reach the Lamina-VI of that side (ipsilateral). Once introduced into the spinal cord, the needle was held in place for 2 min. The discharge of the solution lasted for 5 min. Before removing the needle it was held in place for 2 more min to avoid leaking of the solution. Five microliters either of the KA solution or saline were discharged at that point at a rate of 1 μl/min. In all cases, the histological feature of the needle trace was checked to determine the accuracy of the technique. After surgery animals were returned to their cages and checked periodically until they woke up. In no case animals required manual emptying of the bladder.

2.3. Drug injection

Kainic acid (Sigma–Aldrich, Inc., St. Louis, MO. USA) was dissolved in 0.9% saline and kept at 4 °C until use. Animals were injected either with 0.75 mM KA (KA0.75); 1 mM KA (KA1), 1.25 mM KA (KA1.25) or saline (saline-injected group). The selection of the kainate concentrations was based on the results reported by Taccola et al. (2008), who found the appropriate concentration to induce an irreversible loss of motor activity. Five rats of each KA group were sacrificed either at 1, 2, 3 or 7 post-injection (pi) days. Three rats of the saline-injected group were killed at the same time points. Three non-operated animals were used as a control group in all experiments.

2.4. Clinical assessment

2.4.1. Weight measurement

All control and experimental animals were weighed before surgery (day 0) and at every pi time point before sacrifice.

2.4.2. Behavioral tests

All rats were submitted to a set of motor and sensory tests adapted from Biesiadecki et al. (1999), Sedý et al. (2008) and Wallace et al. (1980) before and after treatment at each sacrifice time point.

2.4.2.1. Withdrawal reflex. Withdrawal reflexes are a group of stimulus-based reflex response reactions. To evaluate these reflexes the speed and the force of limb withdrawal after extension, pain, or pressure were assessed. To this end we pricked the foot pad with a needle (pain). The reflex response was considered as 0 = no withdrawal; 1 = normal withdrawal; −1 = delayed withdrawal (Gale et al., 1985; Von Euler et al., 1996).

2.4.2.2. Heat sensitivity test. The hot-plate test was carried out according to the method previously described (Milano et al., 2008). In these experiments, the hot-plate apparatus was set at 55 ± 1 °C. Animals were placed on a 15 cm diameter heated surface surrounded by four acrylic walls, and the time (measured in s) between placement (time zero) and licking of their forepaws or jumping (whichever occurred first), was recorded and considered as the response latency. A 20 s cut-off was used to prevent tissue damage. Three measures at 2-min-intervals were taken before (baseline) and after drug treatment and their means were considered as basal or experimental latencies, respectively.

2.4.2.3. Suspension from a horizontal wire mesh pole. The time during which rats could sustain their own weight was determined by placing the animals on a horizontal wire mesh pole. An 8 cm diameter pole was covered by a nylon mesh (pore size: 0.5 cm). The pole was immediately rotated so as the animals were left suspended from the wire mesh 50 cm over a water tank (Nishida et al., 2011). The latency taken by the animals to fall was recorded as the average of three consecutive sessions.

2.4.2.4. Ladder rung walking test. This test was carried out to examine forelimb coordination during skilled walking in rats. The horizontal ladder rung walking test device has side walls made of clear acrylic and metal rungs (3 mm diameter) which could be inserted to create a floor with a minimum distance of 1 cm between rungs. The side walls were 1 m long and 19 cm high measured from the height of the rungs. The ladder was placed 30 cm above the ground. The width of the alley was 1 cm wider than the rat to prevent the animal from turning around. Rats were trained to walk across the ladder rung walking device three times per session for a week before experimental day 0.

Limb coordination and skilled walking patterns for the forepaw were analyzed according to a scoring system published by Metz and Whishaw (2002) ranging from 0 (abnormal) to 6 (perfect). All scores between 0 and 2 were considered to be an error (total miss,

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