

EVIDENCE THAT AMPICILLIN AND STREPTOMYCIN CAN SUPPRESS AUTOTOMIC BEHAVIOUR IN RATS

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Autotomy is a phenomenon of deafferentation of the rat limb by transection of the sciatic and saphenous nerves, inducing an automutilation of the limb as a response to painful stimuli generated from the amputation neuroma. Autotomy has been proposed as an experimental model for neuralgia pain. The present study was designed to investigate the influence of streptomycin, ampicillin and chloramphenicol applied either in the vicinity of the nerve stump or intramuscularly. The course of autotomy was followed during 8 weeks using the scoring system of Wall et al. (1979). It was shown that streptomycin and ampicillin can suppress autotomy when applied topically whereas systemic use of the investigated antibiotics was efficient only when streptomycin was injected.

Key words: autotomy phenomenon, ampicillin, streptomycin, chloramphenicol.

INTRODUCTION

Wall et al. (1979) were able to produce automutilation behaviour in rats after the sciatic and saphenous nerves had been transected. This behaviour of attacking denervated limbs was named the autotomy phenomenon and was taken as an experimental model for neuralgia pain. The advantage of this finding is reflected in the possibility of comparison and representation of pain intensity (Sporel et al., 1991). Based on the extent of the damage to the limb, an autotomy degree scale was formed (Wall et al., 1979). There are two components of painful impulses causing the autotomy phenomenon. According to Wall et al (1979), the first one is the ectopic activity from neuroma formation. The second one, being central, is related to CNS activities (Kaupilla et al., 1991).

Published studies have been focused on substances and drugs blocking pain receptors or excitatory neurotransmitter receptors. According to Puke et al. (1993), opioid application and cryotherapy (Wagner et al., 1995) have been known to reduce the autotomy scores. Seltzer et al. (1991) showed that amitriptylene, a tricyclic antidepressant, reduced autotomic behavior. Yamamoto et al. (1992) suppressed autotomy with oral morphine in the sciatic nerve in rats. Stajcic (1992) showed that topically applied streptomycin significantly reduced

the autotomy phenomenon after sciatic nerve section in rats. Also, Stajcic *et al.* (2002) showed that morphological changes may be responsible for the antineuralgic effects of streptomycin. Suaudeau *et al.* (1989) prevented automutilation behavior in rats by subcutaneous application of chloramphenicol and amoxicillin. According to Best *et al.* (1999) aminoglycosides, tetracyclines and lincosamides have neuromuscular blocking side effects.

This study was designed to investigate whether the mode of application of antibiotics can have an influence on the rate of suppression of the autotomic phenomenon in rats.

MATERIALS AND METHODS

Experimental animals

Study approval was obtained from the Ethical committee of the Faculty of Stomatology, Belgrade University. The study was performed on 60 female Albino Wistar rats, weighing 200-300 grams. The experiment was terminated by etherisation of the animals.

Experimental procedure

The animals were anaesthetized with an intraperitoneal injection of Nembutal (35 mg/kg).

The sciatic nerve was identified, isolated and transected posterior to the last side branch. The saphenous nerve was also transected and blood vessels ligated. The proximal end of the sciatic nerve was pulled into a 10mm length of polyethylene (PE) tube which was secured in place with a suture and closed with sterile bone wax on the distal part of the tube (Fig. 1). Two weeks after the surgical



Figure 1. The proximal end of the sciatic nerve is placed into the polyethylene tube

procedure animals usually started biting their claws and toes of the feet. Autotomic behavior was measured by the extent of the limb lesions expressed as the autotomy score according to Wall *et al.* (1979). Scores from one to five (A_1 - A_5) are for bitten claws (Fig. 2). Scores from five to ten (A_5 - A_{10}) represent bitten phalanges (Fig. 3). The maximal autotomy score (A_{13}) represents the whole foot bitten (Fig. 4). All rats were examined daily in the first two weeks.



Figure 2. Four nails bitten



Figure 3. An animal limb without two phalanges



Figure 4. The whole foot bitten off

Experimental groups

Two experimental groups were formed when the animals exhibited autotomy scores between A_1 to A_5 . In *Group 1*, the animals were operated on again according to the previous protocol and tissue fluid was carefully aspirated from the PE tube with a 27-gauge needle, taking care not to touch the nerve. 0.01 ml of 50% solution of streptomycin, ampicillin, chloramphenicol and saline were slowly injected into the PE tube, beside the nerve in the different groups of animals. The animals were examined for autotomy score on a weekly basis during 8 weeks or until the autotomy score reached A_{13} . In *Group 2*, when the animals exhibited autotomy scores between A_1 to A_5 , streptomycin, ampicillin, chloramphenicol and saline as a control were applied intramuscularly in daily doses of 250 mg/kg in a two-week period, and the autotomy score was followed during the experimental period.

Tested drugs

The following drugs were used:

Ampicillin (Pentrexyl-Galenika), Streptomycin (Streptomycin sulfate-Galenika), Chloramphenicol sodium- Mediochemine-LTD, Saline (Zdravlje, Leskovac) and Nembutal (Sodium pentotal-Galenika)

Statistical evaluation

The weekly mean autotomy scores and standard deviations of the mean were calculated for each group. The Mann-Whitney test was used to test differences between the groups.

RESULTS

Streptomycin injected in to the PE tube, in *Group 1*, suppressed the autotomy scores during the entire postinjection period (Table1). At the eighth postinjection week, none of rats from this group reached the maximal autotomy score.

Table 1. Autotomy scores after antibiotics or saline applied postoperatively in the PE tube

Statistical parameters	Antibiotics 0.01ml - 50% solution			
	streptomycin	ampicillin	chlo-ramphenicol	saline
No. of animals	15	15	15	15
Minimal autotomy score	1.00	2.00	5.00	5.00
Maximal autotomy score	7.00	13.00	13.00	13.00
Average value of the autotomy score	4.00***	7.07**	10.13	10.33
Standard error	1.69	2.91	2.97	2.99

MANN-WHITNEY z -TEST-

*** p < 0.001 Statistical significance comparing streptomycin to saline

** p < 0.01 Statistical significance comparing ampicillin to saline

Nine animals (60%) did not change their autotomy score until the end of the experimental period (Table 2).

Table 2. Autotomy score changes after streptomycin was administered postoperatively

Autotomy score before re-operation	Autotomy score at the end of experimental period							No. of animals
	A1	A2	A3	A4	A5	A6	A7	
A1	1	1						2
A2		2	1	1	1			5
A3						1		1
A4				2				2
A5					4		1	5
Number of animals	1	3	1	3	5	1	1	15

 Number of animals without changes of autotomy score

Statistical significance showed in the animals where *ampicillin* was injected in the PE in relation to saline ($p < 0.01$), but not when chloramphenicol was compared with saline (Table 1). When animals were treated with antibiotics and saline systemically in *Group 2*, only streptomycin suppressed the autotomy score when compared with saline (Table 3).

Table 3. The autotomy score after antibiotics were applied systemically

Statistical parameters	Antibiotics 250 mg/kg			
	streptomycin	ampicillin	chloramphenicol	saline
No. of animals	15	15	15	15
Minimal autotomy score	1.00	5.00	2.00	1.00
Maximal autotomy score	13.00	13.00	13.00	13.00
Average value of the autotomy score	4.73*	9.33	10.27	8.20
Standard error	3.79	3.13	3.84	4.57

* MANN-WHITNEY z-TEST

P < 0.05 Statistical significance comparing streptomycin to saline

DISCUSSION

The prevailing opinion in the literature is that the autotomy phenomenon is caused by painful impulses originating from neuroma at the proximal end of the cut nerve (Abbot *et al.*, 1991). Based on the current findings it can be strongly suggested that an artificially created neuroma in the closed PE tube is directly responsible for the autotomy behavior in experimental animals, since the latency period of two weeks corresponds to the time required for a neuroma to be formed. It was established that many antibiotics including aminoglycosides block neuromuscular transmission, decreasing acetylcholine release or postinjectional sensitivity (Best *et al.*, 1999). However, a potential peripheral site for drugs effectively reduces autotomy by suppression of the afferent nociceptive input from neuroma. Stajcic *et al.* (2002) demonstrated that streptomycin could cause disintegration of the nerve fibers when applied topically on the rat infraorbital nerve. Having in mind this finding and the results obtained in this study, it may be suggested that streptomycin applied postoperatively in the PE tube suppresses the autotomy degree. As a consequence, this protective effect against autotomy found with antibiotics may be interpreted as an analgesic effect. On the other hand, antibiotic agents are not known to have analgesic effects. The action of the antibiotics could also have been related to chronic infection of the wounded limb,

but standard surgery in rats generally does not use very stringent asepsis care because these animals are very resistant to infections. Therefore, an infection at the surgical site does not appear to be an explanation for the autotomic behaviour. It has also been reported that streptomycin stabilizes the nerve cell membrane (Sokoll and Gergis, 1981) and inhibits acetylcholine release at nerve endings (Alkadhi and Mclsaac, 1978). The mechanism of the protective effect of ampicillin against autotomy is largely unknown. It could be suggested that ampicillin as well as streptomycin, produces some degenerative changes of the nerve stump, which have been confirmed by Stajcic *et al.* (2002). On the other hand, it is interesting that glycerol (hypertonic solution) had a destructive influence on the nerve fibers and consequently reduced the ectopic discharge from the transected nerve by an osmotic effect (Rappaport *et al.*, 1986). In this study we used 50% ampicillin solution which is hypertonic and would answer for the local influence on the autotomy degree. However, chloramphenicol had the same osmotic concentration but no clear effect on autotomy. Several classes of antibiotics have neuromuscular blocking side effects. These include aminoglycosides, tetracyclines and lincosamides (Pittinger *et al.*, 1970). Significant research has been done to explore the mechanism of antibiotic-induced neuromuscular blockade. The hypothesised mechanisms of neuromuscular blockade for aminoglycosides are based on decreased acetylcholine release or decreased postinjectional sensitivity (Best *et al.*, 1999). This would explain how streptomycin applied systematically reduced the autotomy score. It has been seen that certain antibiotics also suppress autotomy when injected daily, chloramphenicol and amoxicillin being the most effective (Sauadeu *et al.*, 1989). These two substances are known to cross the blood-brain barrier at a 50% rate but in our experiments chloramphenicol and ampicillin did not show any effect on the autotomy degree when applied systemically. The hypothesis could also be advanced that the effect of ampicillin applied postoperatively on the degree of autotomy is the consequence of another side effect, which could be explored, in a further neurophysiological investigation.

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DOKAZ DA AMPICILIN I STREPTOMICIN MOGU DA SPREČE AUTOTOMIJU KOD PACOVA

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SADRŽAJ

Eksperiment je izveden na 60 Albino Wistar pacova kod kojih je izvršeno presecanje n. ischiadicus-a i n. saphenus-a što je za posledicu imalo pojavu fenomena autotomije. Ovaj fenomen predstavlja eksperimentalni model bola, a ispoljava se samoodgrizanjem delova denervisanog stopala eksperimentalne životinje. Većina autora smatra da supstance i lekovi koji sprečavaju autotomiju utiču i na supresiju bola. Imajući ovo u vidu, cilj naših istraživanja bio je da ispitivane antibiotike primenimo na eksperimentalnom modelu posle ispoljavanja fenomena autotomije, kako bi utvrdili njihovo dejstvo na supresiju ovog fenomena. U isto vreme moguće je razmatrati i eventualno korišćenje ovih lekova u terapiji bola. Rezultati naših istraživanja pokazuju da streptomycin primenjen lokalno i intramuskularno sprečava autotomno ponašanje kod pacova, dok ampicilin sprečava razvoj ovog fenomena primenjen lokalno. Mehanizam delovanja ovih antibiotika još uvek nije u potpunosti razjašnjen, ali to ne umanjuje njihovu eventualnu primenu u terapiji hroničnog bola.