임상의과학석사 학위논문

경막외 주사 후 조직 내 스테로이드 잔량의 정량적 평가: 새로운 토끼 모델

Quantitative assessment of steroid retention in the tissue after epidural steroid injection: a new rabbit model

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Abstract

Quantitative assessment of steroid retention in the tissue after epidural steroid injection: a new rabbit model

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Objectives: To develop a rabbit epidural steroid injection (ESI) model for analyzing steroid retention in the tissue, and to assess the difference in steroid retention in the model according to the location and time elapsed after ESI.

Materials and Methods: Fluoroscopy-guided ESI was performed using the interlaminar approach between the lowest two lumbar segments in 13 female New Zealand white rabbits. Four rabbits were allocated to each of three different groups according to the time of sacrifice: 3, 7, and 15 days post-ESI; the remaining rabbit was sacrificed immediately post-ESI to obtain baseline data. After sacrifice, two segments were harvested: the lowest two lumbar vertebrae and another two lumbar vertebrae immediately above

these. The residual steroid amount (RSA) and residual steroid concentration (RSC) in the collected spinal columns were analyzed. A linear mixed model was used to compare RSAs and RSCs between the injected and adjacent segments, and among the number of days until sacrifice; $p < 0.05$ was considered statistically significant.

Results: Both RSA and RSC of the injected segment were significantly higher than those of the adjacent segment ($p < 0.001$, both). The RSA and RSC significantly decreased over time ($p = 0.009$ and $p = 0.016$, respectively).

Conclusion: The developed rabbit ESI model verified that significantly more steroid was retained at the injected segment than at the adjacent segment and the residual steroid decreased over time. This model could be useful not only for comparing current steroid medications, but also for developing new, better steroid formulations.

Key words: Spine, Epidural injection, Animal study, Fluoroscopy

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Introduction

Epidural steroid injection (ESI) is an important treatment for patients with radicular lower back or neck pain. Due to the increased life expectancy and high prevalence of chronic back pain in older people (1), nonsurgical interventional procedures for spinal pain have significantly increased. In the United States, ESI usage increased 130% from 2000 to 2011 in Medicare beneficiaries; ESI was performed for 4,879 per 100,000 patients in 2011 (2).

Various chronic back pain conditions, which include herniated disc disease, spinal stenosis, and post-lumbar surgery syndrome, are conventional candidates for ESI. Multiple studies have shown robust evidence of the short-term (< 6 months) effectiveness of ESI in patients with disc herniation (3, 4). For treatment, drugs are directly delivered into the epidural space under fluoroscopic or computed tomographic guidance. Steroids are generally used for anti-inflammatory effects, but exert variable adverse systemic effects, including suppression of the hypothalamus-pituitary-adrenal axis, Cushing syndrome, osteoporosis (5), fluid retention, and hyperglycemia. Therefore, current guidelines suggest limiting

the frequency of interventional procedures. The American Society of Interventional Pain Physicians recommends that the interval between epidural injections be 2 months or longer, assuming that more than 50% relief is obtained for 2 months. However, a previous randomized, double-blind trial (6) showed widespread use of more frequent epidural injections, even of particulate steroids.

Accordingly, there is a need for steroid formulations with longer local retention and better symptom relief after ESI. The duration and effectiveness of ESI have usually been assessed by questionnaires of patients' symptoms, but the residual amount of steroid in the tissue has not actually been measured. For quantitative comparison of steroid retention and development of more effective formulations, an adequate animal model is thus essential. However, to the best of our knowledge, there exists no such animal model.

In our laboratory, we designed a rabbit model for applying ESI under fluoroscopic guidance, simulating actual practice. Using this model, we can measure the locally retained steroid amount, which could help determine the duration for which the steroid remained in the injected area, as well as its spread through the epidural space.

Therefore, the purpose of this study was to develop a rabbit ESI model for analyzing steroid retention in the tissue, and to assess the difference in steroid retention in the model according to the location and time elapsed after ESI.

Materials and Methods

Ethical approval

Our study was performed from March 2017 to December 2017. All procedures were approved by the animal care and use committees of our institute (approval number: BA1608-206/050-01).

Animals

Thirteen female New Zealand white rabbits (weight range, 3.1–3.5 kg at the beginning of the study) were obtained from DooYeol Biotech (Seoul, South Korea) and Orient Bio (Seongnam, South Korea). Rabbits were housed in individual cages with controlled light cycle and temperature (21 ± 2 ℃). Standard laboratory chow and tap water were available *ad libitum*.

Experimental design

Twelve of the rabbits were allocated to three different groups according to time of sacrifice: 3, 7, and 15 days after ESI (Fig. 1). The remaining rabbit was sacrificed immediately after ESI to obtain baseline data. At each time-point, the rabbits were sacrificed, and

two lumbar spinal segments were surgically harvested: the lowest two lumbar vertebrae as well as the two lumbar vertebrae immediately above these.

Epidural steroid injection

A radiologist performed ESI under fluoroscopic guidance, in the manner used in actual clinical practice. Rabbits were anesthetized with intramuscular injection of alfaxalone (Alfaxan, 10 mg/mL; Jurox Pty Ltd., Rutherford, NSW, Australia; 5 mg/kg body weight) and xylazine (Rompun, 23.32 mg/mL; Bayer Korea, Ansan, South Korea; 5 mg/kg body weight). Then, the rabbit was placed in the prone position on the fluoroscopy procedure table (Fig. 2A). We selected the interlaminar trajectory between the lowest two lumbar spine segments for epidural injection. A 25-gauge spinal Quincke needle was used, and appropriate needle tip placement was confirmed by fluoroscopy (Fig. 2B) with the injection of approximately 0.5 ml of contrast material (iohexol, Omnipaque 300, 300 mg iodine per ml; GE Healthcare Co. Ltd., Shanghai, China) (Fig. 2C). The injectate was 40 mg (1 mL) triamcinolone acetonide suspension (TA; Triam, 40 mg/ml; Shinpoong Pharmaceuticals, Seoul, South Korea). A fluoroscopic image was obtained following

the injection, to demonstrate washout of the contrast agent.

Tissue harvest

Rabbits were sacrificed after induction of anesthesia, using potassium chloride (150 mg/mL; JW Pharmaceutical, Seoul, South Korea; 150 mg/kg body weight). A longitudinal incision was made along the midline of the prone-positioned rabbit. The level of the tissue harvested was confirmed by palpation of the lumbosacral junction and spinous process of the lumbar vertebra. The paraspinal muscle was incised transversely using a sharp scalpel, and a rongeur was used for dividing the lumbar bony segments. The dorsal section of the rabbit containing the lumbar spine was excised *en bloc* (Fig. 3).

Analysis of the residual steroid amount

Collected spinal columns were sent to an analytical chemistry laboratory (Biological Mass Spectrometry Group at the Dankook University, Cheonan, South Korea) for measuring the residual steroid amount (RSA) and residual steroid concentration (RSC) in the tissue, using a validated high-performance liquid chromatography-mass spectrometry/mass spectrometry (HPLC-

MS/MS) assay. To extract TA from the tissue, the excised whole spine tissue was soaked in 50 mL of methanol and agitated in a shaking incubator for 12 h. The extracted solution (1 mL) was diluted 50 times with methanol and then centrifuged for 10 min at 14,000 rpm. The supernatant (10 μL) was injected into an HPLC–MS/MS system (LC–20 Prominence HPLC system, Shimadzu, Tokyo, Japan; and an API 2000 triple quadrupole mass spectrometer, AB/SCIEX, Poster City, CA, USA). The chromatographic separation was performed using a Phenomenex Luna C18 column (2.0 mm × 150 mm, 5–μm particle size). The mobile phase consisted of 0.1% formic acid in water and acetonitrile at a volume ratio of 50:50, with a flow rate of 0.25 mL/min. The steroidal compound eluted from the column was transferred into the MS/MS instrument with an electrospray ionization source in positive ion mode. The gas temperature was 400 ℃, the ion spray voltage was 5,500 V, and the pressures of curtain and collision gases were 16 and 6 psi, respectively. The TA was monitored using multiple reaction monitoring, with the m/z transition of 435.1→415.0. For further TA confirmation, additional transitions (435.1→171.4 and 435.1→397.1) were also monitored. The assay exhibited excellent

linearity (R2 value of 0.9917) over a TA concentration range of 100–600 ng/mL.

Statistical analysis

The RSA and RSC in the lumbar vertebrae were compared between the extracted upper and lower segments. These measurements were also compared with respect to the day of harvest. To this end, all comparisons were performed using the linear mixed-effects model, which accounts for two-way measurements per rabbit. For the mixed-effects model, the level of the segments (upper or lower) and the day of harvest (3, 7, or 15) were used as fixed effects, and rabbit identification was used as a random effect. Wilcoxon's signed-rank test was used as the *post hoc* test for comparing RSA and RSC on each harvest day. Statistical analysis was performed using the software Stata 14.0 (StataCorp LLC, College Station, TX, USA); $p < 0.05$ was considered statistically significant.

Results

The 12 rabbits in the experimental groups were able to bear weight, and exhibited normal gait after the ESI procedure; the rabbit used for obtaining baseline data was not assessed as it was sacrificed immediately after ESI.

The collected spinal segments weighed 48.6 ± 9.07 mg (mean ± standard deviation). Mean RSA/RSC values in the immediately collected tissues were 1.3 mg/19.4 ppm for the injected lower segment and 0.93 mg/20.9 ppm for the adjacent upper segment.

The RSA values in the injected area versus the adjacent area (mean, mg) on each day of sacrifice, and the percentage normalized to the immediately collected tissue, were as follows: 17×10^{-2} (13%) vs. 4.7×10^{-2} (5.0%), 7.9×10^{-2} (6.0%) vs. 2.4×10^{-2} (2.6%), and 2.5×10^{-3} (0.25%) vs. 0.65×10^{-3} (0.070%) for sacrifice after 3, 7, and 15 days, respectively.

The RSC values in the injected area versus the adjacent area (mean, ppm) on each day of sacrifice, and the percentage normalized to the immediately collected tissue, were as follows: 3.3 (17%) vs. 1.0 (4.8%), 2.1 (11%) vs. 0.56 (2.7%), and 0.048 (0.25%) vs. 0.015

(0.071%) for sacrifice after 3, 7, and 15 days, respectively (Table 1). The RSA and RSC values in the injected segment were significantly greater than those in the adjacent segment ($p < 0.001$, both). However, RSA and RSC significantly decreased over time ($p = 0.009$ and $p = 0.016$, respectively).

Discussion

In this study, we developed a rabbit model that was used to assess the amount of steroid remaining after fluoroscopy-guided lumbar ESI. As expected, the RSA and RSC of the injected segments were significantly larger than those of the adjacent segments; these results assure the reliability of this rabbit ESI model. We also found that the injected TA remained in the harvested tissue for 15 days after lumbar ESI. The amount and concentration of the remaining steroid decreased over time, and only 2.5×10^{-3} mg of TA remained in the injected segment by 15 days after ESI. This suggests that the injected steroid is dispersed or degraded relatively rapidly. These results are compatible with previous reports that ESI shows robust effectiveness in providing short-term symptom relief to patients with disc herniation (4) and those with spinal stenosis exhibiting neurogenic claudication or radiculopathy (7). However, for long-term effectiveness, the evidence was conflicting or less reliable for both patient groups (4, 7). In this study, TA, a particulate steroid, was used instead of a nonparticulate steroid, such as dexamethasone. Particulate agents have been shown to be superior to nonparticulate agents in terms of

the duration of pain relief (6, 8, 9); however, there are reports on serious adverse events after particulate steroid administration, such as spinal cord infarction or cerebellar infarction (10, 11), presumably due to particle embolization. The current guideline of the US Food and Drug Administration (12) recommends against using particulate formulations in ESI. We believe that animal studies are crucial for the development of longer-lasting and safe particulate steroid formulations with more pain relief, and therefore, used TA in the development of the rabbit ESI model.

The epidural space is encased between the dura mater and the walls of the vertebral canal, with intermittent attachment via connective tissue. There is a thin layer of areolar tissue embracing the vertebral venous plexus and epidural adipose tissue, which lies in a recess between the dura mater and the ligament flava (13, 14). Drugs injected into the epidural space are absorbed and stored in the epidural fat, particularly lipophilic drugs, such as steroids. Redistribution of these drugs is affected by lipophilicity, tissue permeability, local blood supply, and the surface area involved (15). Madsen et al. reported that 1.0 mL of contrast agent (Omnipaque), which was injected through an epidural catheter at the level of the

6–7th lumbar vertebrae, is distributed to the level of the 8–9th thoracic vertebrae in a rabbit model (16). Our study also showed similar distribution of the contrast agent, and only 1.3 mg of injected TA (40 mg) remained at the injected segment. However, the amount of steroid retained in the injected level was significantly larger than that in the adjacent level. This finding could be explained by the characteristics of steroids. In contrast to water-soluble contrast agents, which might spread promptly to other spinal levels through the potential epidural space, lipophilic steroid agents might be dissolved in the epidural adipose tissue of the injected level. This could imply that targeted injection at the specific spinal level may be more effective.

The first documented steroid injection into the epidural space for managing lumbar radicular pain was performed in 1952 (17). Since then, the epidural administration of steroids has been one of the best-studied subjects in interventional pain management. Although Beall et al. reported the tissue distribution of clonidine after intraforaminal implantation of biodegradable pellets (18), a methodology for investigating the presence of corticosteroid residue in the tissue after ESI has not been reported. In the present

study, we designed a rabbit model to measure the quantity of drug remaining after ESI, over time. This rabbit model will make it possible to compare the residual amount of concentration of various steroid formulations.

There are several limitations to our experimental study. First, the number of rabbits in each group and the total number of animals used were relatively small. Further studies with larger sample sizes would strengthen our results. Second, the measurement method could not differentiate between residual amounts of steroid in specific tissues, such as the spinal cord, dura mater, epidural fat, or bones. However, the mechanism of the lumbar pain relief provided by steroids is complex (19), and investigating the RSA in specific tissues might be meaningless. Third, we only used the midline approach at the lowest lumbar level for the ESI, for experimental convenience. Other experiments with different approaches, such as transforaminal routes or different injection levels, are warranted.

In conclusion, we verified that retention of injected steroids can be quantitatively assessed using the rabbit ESI model; the duration of retention and spread of the injected steroid can also be analyzed. Thus, this model could be useful not only for comparing current

steroid medications, but also for developing new, better steroid formulations.

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Table 1. Comparison of residual steroid amount and concentration

	RSA (mg)			RSC (ppm)		
	Upper (Mean ± SD)	Lower (Mean ± SD)	*p*-value	Upper (Mean ± SD)	Lower (Mean ± SD)	*p*-value
3 days	$4.7 \times 10^{-2} \pm 3.1 \times 10^{-2}$	$1.7 \times 10^{-1} \pm 1.6 \times 10^{-1}$	0.002*†	1.0 ± 0.63	3.3 ± 3.2	0.005*†
7 days	$2.4 \times 10^{-2} \pm 2.6 \times 10^{-2}$	$7.9 \times 10^{-2} \pm 6.3 \times 10^{-2}$	0.002*†	0.56 ± 0.64	2.1 ± 2.0	0.002*†
15 days	$0.65 \times 10^{-3} \pm 0.69 \times 10^{-3}$	$2.5 \times 10^{-3} \pm 2.7 \times 10^{-3}$	0.002*†	0.015 ± 0.016	0.05 ± 0.05	0.002*†
LMM			0.009*			0.016*

RSA, residual steroid amount; RSC, residual steroid concentration; SD, standard deviation; LMM, linear mixed model

*$p < 0.05$

†Results of Wilcoxon signed rank test

Figure legends

Figure 1.

Flow diagram of the experimental design

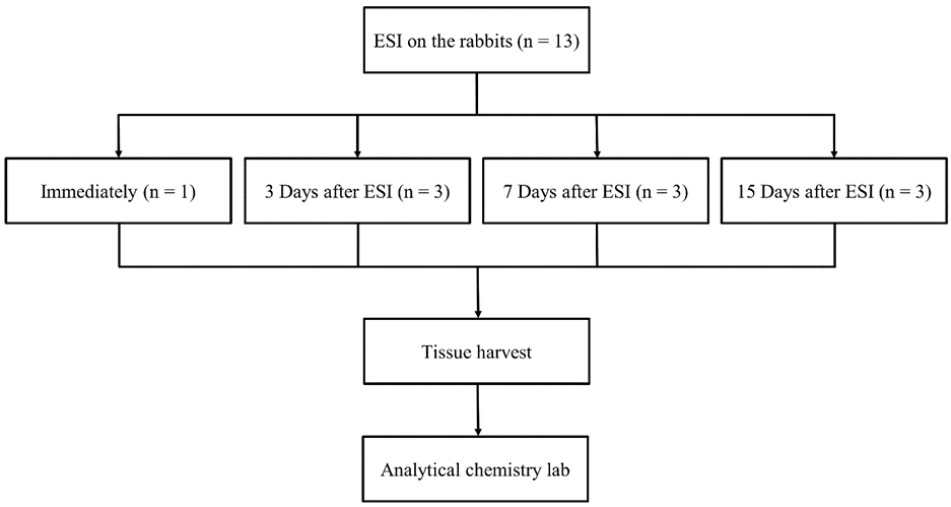
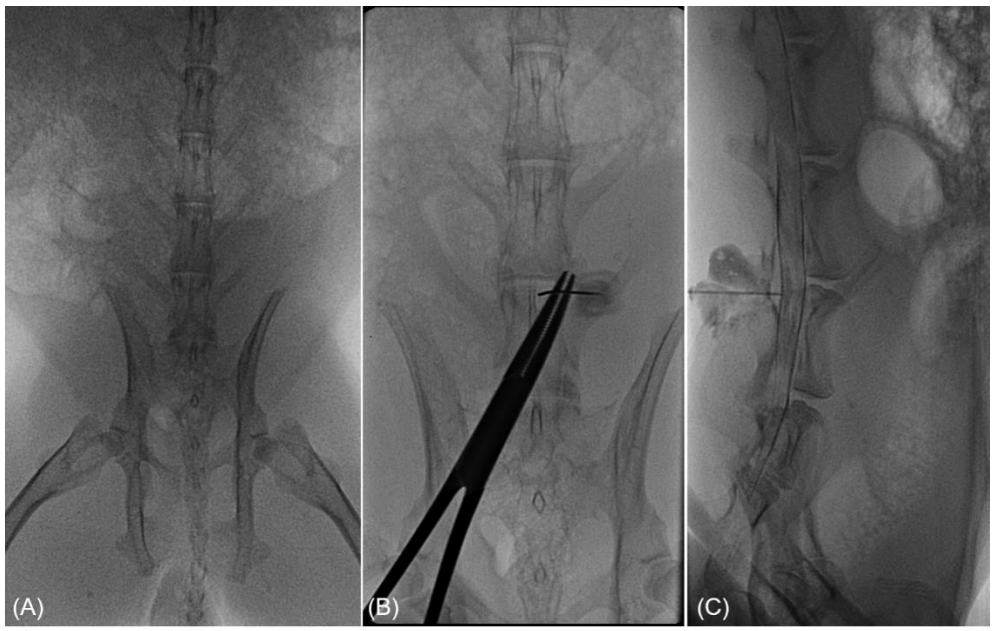


Figure 2.

Epidural injection of triamcinolone acetonide through the interlaminar route.



(A) Spine antero-posterior radiograph of the prone-positioned rabbit. (B) Interlaminar approach with a 25-gauge spinal Quincke needle. (C) Appropriate needle tip placement confirmed by the injection of a contrast agent

Figure 3.

Representative image showing the tissue being harvested. Two dorsal sections of the rabbit including the lumbar spine were excised en bloc: including the lowest two (level of epidural steroid injection) lumbar vertebrae and the two vertebrae immediately above them

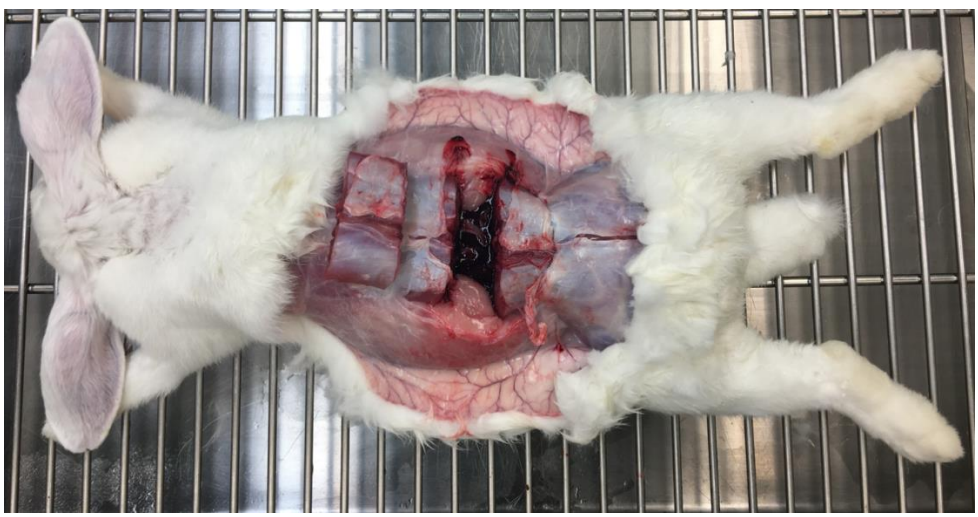
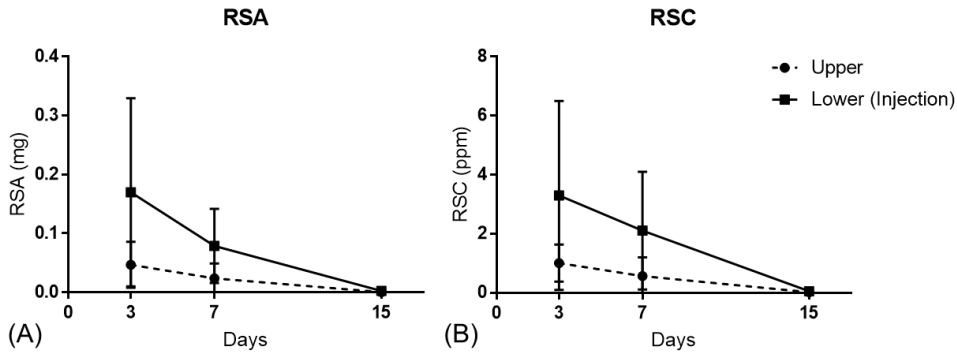


Figure 4.

Line graphs of residual steroid measurements in the harvested tissue.



(A) Residual steroid amount (RSA). (B) Residual steroid concentration (RSC)

논 문 초 록

목적: 경막외 스테로이드 주사(Epidural steroid injection, ESI) 후 스테로이드 잔류량을 분석하는 토끼 모델을 만들고, 위치 및 시간에 따른 스테로이드 잔류 정도를 평가하고자 한다.

대상 및 방법: 40 mg 의 트리암시놀론 아세토니드를, 토끼의 가장 아래의 두 요추의 추궁판간(interlaminar) 경로를 통해, 투시 유도(Fluoroscopy-guided) 방법으로 경막외 공간에 주사하였다. 13 마리의 암컷 뉴질랜드 흰 토끼(New Zealand white rabbit)를 주사 후 각각 4 마리씩 3, 7, 15 일에 조직을 얻었다. 또한 한 마리는 경막외 주사 후 즉시 조직을 얻었다. 조직을 얻을 시, 가장 낮은 두 개의 요추를 포함한 분절과 그 바로 위의 두 개의 요추를 포함한 분절, 두 개를 추출하였다. 얻어진 분절은 분석 연구실을 통해 잔류 스테로이드의 양(Residual steroid amount, RSA)과 농도(Residual steroid concentration, RSC)를 측정하였다. 선형 혼합 모델 (Linear mixed model)을 통해 약물이 주입된 분절과 인접한 분절 사이의 잔류 스테로이드 양과 잔류 스테로이드 농도를 비교하고, 조직 추출 시간까지의 일 수에 따른 차이를 비교하였다.

결과: 주사 부위의 RSA 와 RSC, 모두 인접 부위의 RSA 와 RSC 보다 높았다 ($p < 0.001$, 모두). RSA 와 RSC 는 시간에 따라 유의하게 감소하였다 (각각 $p = 0.009$ 와 $p = 0.016$).

결론: 개발된 토끼 ESI 모델에서 직접 주입한 분절에서의 스테로이드 잔량은 인접한 분절보다 유의하게 컸고, 시간에 따라 잔량은 감소하였다. 토끼 ESI 모델은 주입된 스테로이드의 잔량을 분석함으로, 다른 스테로이드 약제에 따른 잔량을 비교할 수 있을 뿐 아니라, 이를 통해 새로운 스테로이드 약제를 개발하는 것에 도움이 될 수 있을 것이다.

주요어: 척추, 경막외 주사, 동물 실험, 투시
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