Preventing Peridural Fibrosis with Nonsteroidal Anti-inflammatory Drugs

Presenter: Surachai Sae-Jung, MD

Faculty of Medicine KhonKaen University, Thailand
2011
International Short Course Training in Research Methodology & Biostatistics

Final Presentation on Critical Appraisal

Title

Preventing Peridural Fibrosis with Nonsteroidal Anti-inflammatory Drugs

Presenter: Surachai Sae-Jung, MD

2011
Course: Critical Appraisal

Module: How to read the clinical journal

Objective: To be able to critically appraise the published medical research article

Title: Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs

Authors: Sandoval MA, Hernandez-Vaquero D

Journal: European Spine Journal

DOI 10.1007/s00586-007-0580-y

Presenter: Surachai Sae-Jung, MD
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Clinical Scenario

I work as a spine surgeon in department of Orthopaedics, faculty of Medicine, Khon Kaen University.

Three months ago, I found a 35-year-old female who was previously operated on her low back for the L4-5 herniated nucleus pulposus that compressed to the left L5 nerve root 5 months ago. She had pain free in her back and leg for 4 months. In last month, the patient developed low back pain radiated to her left leg again. The physical examination showed tenderness at her lower back with progressive left L5 root deficit. She was investigated by the lumbosacral spine MRI and finally diagnosed as the epidural fibrosis around the L4-5 laminectomy and discectomy area. This epidural fibrosis also compressed the left L5 nerve root. The patient was operated in her low back again for adhesiolysis. The operation was complicated by the difficulty of adhesiolysis, the accidental dural tear and bleeding from the fibrosis. The postoperative period showed only some relieving in pain score (VAS 9 for preoperative and 7 for postoperative), so she was intravenously injected the NSAIDs as a pain killer and anti-inflammation (with the concept that reduction of inflammation can prevent the recurrence of epidural fibrosis).
Background and Rationale

Failed back surgery syndrome (FBSS) is a diagnosis given to patients who complain of pain symptoms after unsuccessful lumbar surgery. Many FBSS patients present for more surgery. The incidence of reoperation following lumbar spine surgery ranges from 4 to 19%\(^1,2\). An understanding of the common causes of FBSS can help prevent inappropriate surgery whenever possible. As with all failed surgeries, the most important task is recognizing the failure and elucidating the cause. A thorough history and physical examination, including obtaining appropriate diagnostic studies, can reveal the cause for FBSS in up to 95% of patients\(^3\). Common identifiable causes for FBSS include poor patient selection, incorrect initial diagnosis, incorrect or inadequate surgery, scarring, infection, and progressive disease\(^4\).

Epidural fibrosis or scarring is one of the common causes of failed back surgery syndrome. Fibrosis is a part of healing process, so the epidural fibrosis is inevitable when the epidural space is exposed such as an epidural fibrosis following the lumbar laminectomy procedure. The victims of epidural fibrosis are dural sac compression, nerve root tethering, interfere cerebrospinal fluid flow or compromise the nerve root vascular supply\(^5\). These cause a new onset of neurodeficit. The symptomatic epidural fibrosis occurs in 13 to 61% of patients who undergo back surgery\(^6\). Once the scar is form, there is no effective treatment and the scar revision surgery can also make a new scar\(^7\). The reoperation for scar had significantly poor result due to difficulty in operating the scar tissue. One of the modalities for epidural fibrosis is prevention or reduction fibrosis formation. Different substances that used to minimize inflammatory changes and fibrosis perioperatively are currently under development.

The nonsteroidal anti-inflammatory drugs(NSAIDs) are the examples of systemic chemical barrier. They present an advantage over the physical barriers of not introducing foreign bodies, which may increase the inflammatory reaction. Moreover, NSAIDs inhibit cyclooxygenase, which is responsible for synthesis of prostaglandins. The beneficial effect of the NSAIDs in the prevention of calcifications of the soft tissue, heterotopic ossifications and adherences is well known\(^8,9\).
References

Clinical Question

Can the nonsteroidal anti-inflammatory drugs reduce or prevent the epidural fibrosis?

Selection of Article

PICO model

P population: the laminectomized rabbits

I Intervention: NSAIDs

C comparator/control: Saline

O Outcome: degree of postoperative epidural fibrosis

• Searching Strategies:
  1. Firstly approach in Pubmed for Medline database
     The keyword of searching: NSAIDs AND epidural fibrosis (Fig. 1)
     Result: The search engine shows seven articles that match with the keywords. There is only one article that is specific to the clinical question (Can the nonsteroidal anti-inflammatory drugs reduce or prevent the epidural fibrosis?).
  2. The keywords was changed to diclofenac (that is one of the commonly prescribed NSAIDs) AND epidural steroid (Fig. 2)
     Result: The Pubmed shows two articles. The first article, named Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs, is the same as the previous searching.
  3. The keywords was changed to Ibuprofen (that is one of the commonly prescribed NSAIDs) AND epidural steroid (Fig. 3)
     Result: The Pubmed shows only one of unrelated article.
  4. The keywords was changed to NSAIDs AND peridural fibrosis (Fig. 4)
     Result: The Pubmed shows three articles. The first article, named “Comparison of Oxiplex and Gore-Tex effectivity in an experimental peridural fibrosis model”, is not related to NSAIDs. Only the second and the third ones are the related articles. But the third article has only abstract.
5. The searched term was changed to “Diclofenac AND peridural fibrosis” (Fig. 5)
Result: Only the article named “Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs” is found.

6. The database was alternated to Scopus with the searched term “NSAIDs AND epidural fibrosis” (Fig. 6)
Result: Two unrelated articles were found.

7. The keywords was changed to “NSAIDs AND peridural fibrosis” (Fig. 7)
Result: Only one unrelated articles was found.

According to the clinical question, PICO and the most recent study, the article entitle “Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs” is selected.

Fig. 1 The seven articles from Pubmed according to the keywords “NSAIDs AND epidural fibrosis”
Note: The fourth article is the only one which specific to clinical question and PICO. (Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs)
Fig. 2 The two articles from Pubmed according to the keywords “Diclofenac AND epidural fibrosis. Note: Title of the related article is “Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs”

Fig. 3 The article from Pubmed according to the keywords “Ibuprofen AND epidural fibrosis”
Fig. 4 The searched term “NSAIDs AND peridural fibrosis” found three articles.

Note: The most recent related article is “Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs”

Fig. 5 The searched term “diclofenac AND peridural fibrosis” found only one article.
Fig. 6 The searched term “NSAIDs AND Epidural fibrosis” found only unrelated articles.

Fig. 7 The searched term “NSAIDs AND peridural fibrosis” found only one unrelated article.
Summary of the article

Title: “Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs”

Authors: Sandoval MA, Hernandez-Vaquero D


DOI 10.1007/s00586-007-0580-y (impact factor: 1.994)

Objective of the study: to address was if a commonly used European NSAID, aceclofenac, administered from the time of intervention would inhibit fibroblastic proliferation after performing a laminectomy in experimental animals

Study design: experimental animal study

Study setting: Animal Research Laboratory, School of Medicine, University of Oviedo, Oviedo, Spain

Population: The 24 L4 laminectomized New Zealand white rabbits (with the Animal Research Committee approval)

Materials and Methods

- This animal study was approved from the Animal Research Committee.
- All of 24 New Zealand white (NZW) rabbits rabbits were anesthetized and received prophylactic antibiotics, a 4 cm posterior midline longitudinal incision was made along the L3 to L5 spinous process; the fascia and paravertebral muscles were subperiosteally detached from the spinous processes and laminas. The L4 spinous processess, laminas and corresponded ligamentum flavum were all removed with meticulous technique in order not to damage the spinal cord until achieving dural exposure of 4x8 mm. A drainage was used for 24 hours.
- The 24 laminectomized NZW rabbits were equally divided into 2 groups postoperatively.
1. Aceclofenac group: 5 mg/kg/day of aceclofenac was intramuscularly injected from the day of operation to the seventh postoperative day.

2. Control group: 1 cm$^3$ of saline was injected

- Six rabbits of each group were killed at 2 & 4 weeks postoperatively by means of overdoses of intravenous pentobarbital (60mg/kg).
- Immediately after killing, the spinal column was resected in bloc between L3 and L5 and this was fixed with 10% formalin for 24 hours and decalcified in formic acid for 3 weeks.
- Each of the decalcified spinal column was transversely resected in 4 sections and each section was stained with hematoxylin-eosin, Masson’s trichome and immunohistochemical methods. The streptavidin-biotin-peroxidase was used for the detection of antigens, and the enzymatic marking was made with horseradish peroxidase. The substrate was hydrogen peroxide and chromogen marks the fibroblasts in brown color (Fig. 8).

![Fig.8 The figure shows the fibroblasts as positive-vimentin cells in brown color (original magnification, x250)](image)

- In each section the following measures were made: the area of fibrous membrane (mm$^2$) (Fig. 9), the density of fibroblasts per square millimetre (Fig. 10) and the density of the inflammatory cells per square millimetre.
- The adherences were graded into 0 (no adherence was seen between the dura and fibrosis), 1 (adherence less than one-third of dural surface), 2 (between one and two-third) and 3 (greater than two-third).
• The 2 tissue examiners were blinded to examine the specimens.

![Fig. 9 Detection of area of fibrosis (false color)](image)

![Fig. 10 Automatic detection of fibroblasts(false color)](image)

**Statistical analysis**

• The analysis was made with descriptive and inferential statistics.
• As the sample size was small, the nonparametric Mann-Witney U test was employed for continuous quantitative variables (area of fibrous membrane, fibroblast density and inflammatory cell density) and $X^2$ for qualitative variables (fibrous adherence).
Results

- The histologic findings at 2 weeks
  - Control group: extensive hematoma was found in contact with the dura
  - Experimental group: a smaller adherence of the hematoma to the dura
- At 4 weeks
  - Control group: the hematoma was progressively replaced with fibrous tissue and in area near the resected lamina
  - Experimental group: the hematoma was progressively replaced with fibrous tissue and a metaplasia to the chondroid and bone were observed
- The mean fibrous surface was less (p<0.05) in the experimental group compared to the controls both at week 2 and 4 (Table 1).

<table>
<thead>
<tr>
<th>Table 1 Total fibrous surface area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (weeks)</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

SD standard deviation

- In more than 70% of samples the adherence of peridural fibrosis are at least grade 2 (between one and two-third) and at least 33% of samples the adherence are grade 3 (more than two-third). The adherence was less (p=0.04) in the experimental group (Fig. 11)
Fig. 11 A) Experimental group shows less adherence of peridural fibrosis than B0 control group (M spinal marrow, D dura and F fibrosis)

- A smaller number ($p=0.08$) of fibroblasts in the experimental group at week 2 (Table 2)

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Study group (mean ± SD)</th>
<th>Control group (mean ± SD)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2693 ± 415</td>
<td>3377 ± 802</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>1536 ± 930</td>
<td>2203 ± 1012</td>
<td>0.25</td>
</tr>
</tbody>
</table>

- In the study group, a smaller number of inflammatory cells than in the control group were seen. Without differences in the type of inflammatory cells (Table 3).
• No adverse event to all rabbits

Discussion

• The NSAIDs inhibit the inflammatory cell infiltration thereby reduce the fibroblastic activity. Aceclofenac is a widely used NSAID in musculoskeletal pathology due to its analgesic and anti-inflammatory properties and is well tolerated when used for a short periods. Its main action mechanism is inhibition of cyclooxygenase and prostaglandins synthesis. Its also modifies other cellular processes such as leukotrienes synthesis, superoxide generation, liposomal enzyme release, neutrophil activation and cellular membrane function.

• The authors chose the aceclofenac dosage upon the study of Brogden.

• The immunohistochemical techniques use the reaction between integral part of cell and corresponding antibodies, so this technique can facilitate the identification of fibroblat as a positive-vimentin cell.

• The study group found a smaller number of all inflammatory cells but different only in early stage.

• The mean of fibrous surface was less in the experimental group compared to controls at both week 2 and 4, but the difference was statistical significant only when the two time periods were combined, perhaps because of the small sample size.

Conclusion

• The aceclofenac can reduce the fibrous area and inhibit the inflammatory cell infiltration in fibrous scarring. The aceclofenac, when administered early, may be a useful drug for preventing the peridural fibrosis formation.
**Critical Appraisal on the article** (adapt from the CONSORT 2010 Statement: update guidelines for reporting parallel group randomised trials and the ARRIVE guidelines: Animal Research Reporting In Vivo Experiments)

**Citation**: Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs

**General**

- Is the clinical question clearly defined?  
  Yes
- What is the clinical question evaluating?  
  To address was if the aceclofenac, administered from the time of intervention would inhibit fibroblastic proliferation after performing a laminectomy in experimental animals
- What was the study design?  
  Experimental animal study: comparative study between aceclofenac group and control group
- Was there a clearly focussed clinical question and primary hypothesis?  
  Yes.  
  **Commentary**:
  The clinical question: Can the nonsteroidal anti-inflammatory drugs reduce or prevent the epidural fibrosis?
  The primary hypothesis:
  Null hypothesis: The effect of aceclofenac on peridural fibrosis is not different to the saline.
  Alternative hypothesis: The effect of aceclofenac on peridural fibrosis is different from the saline.
- Are there any declared conflicts of interest that may bias the results of the study?  
  No conflict of interest statement was declared.
Reliability & Validity

Methodology

• What was the sampling method?
  Convenient
  Commentary: The 24 rabbits were equally divided into aceclofenac and control
groups without the randomization & allocation concealment statement.

• Were the inclusion/exclusion criteria clearly defined?
  No
  Commentary: The inclusion/exclusion criteria should be defined clearly such as
the age, gender or body weight of the rabbits because these factors may
affect the inflammatory response or peridural fibrosis formation.

• Did the sample include a representative spectrum of subjects?
  Yes
  Commentary: All rabbits were operated in the same ways including the surgical
techniques except only the intervention that was the aceclofenac or
saline.

• How was the sample size determined?
  Arbitrary
  Commentary: Too small sample size. Based on the primary outcome
measurement that is total fibrous surface area of the control group
(because of the lack of previous study data), the total fibrous surface
area is 6.2116±3.2129 mm\(^2\) with the expected value of clinical
significance of the total fibrous surface area in study group should be
3.72696 mm\(^2\) (set the difference of fibrous surface area to 40%), \(\alpha\) 0.05
and 80% power, the sample size, calculated by the two-sample parallel
method with the null hypothesis states the equality between the
aceclofenac and saline groups, should be 27 rabbits in each group.
  Due to small sample size, the study may be underpowered
because of the potential type 2 error.
• Do the authors explain how selection bias was minimized?
  No
  Commentary:
  Selection bias not addressed.

• Was allocation of subjects to intervention and control groups concealed from the researchers?
  No
  Commentary:
  Allocation to group was not concealed (potential selection bias).

• Were the intervention group and control group similar at the start of the study?
  Yes
  Commentary:
  Equal number of rabbits in each group without the other important information of the animals such as weight, age, source of animals, housing or husbandry

• Were the group treated equally except for the receipt of intervention/placebo?
  Yes

• How were patients allocated to the treatment and control groups?
  At random
  Commentary:
  The authors informed only the rabbits were equally divided into 2 groups that look like random itinerary but lack of the declaration of the randomization statement.

• Were either the researchers or subjects blinded to the treatment?
  Blinded
  Commentary:
  The authors stated only outcome assessor blinding.
  The animals were blinded to the study automatically.
  The authors did not provide the information about the surgeon, the saline/aceclofenac administrators and outcome assessors were the same persons or not.

• Was the blinding process clearly explained?
Yes, only for the outcome assessors.

- Was an adequate placebo used in the control group?  
  Yes

- Were the clinical end points measured clearly stated?  
  Yes

- How was the clinical end point measured?  
  Directly to histology and immunohistochemistry of the peridural fibrosis

- Was a valid measurement of the clinical endpoint made?  
  Yes

- Were the endpoints assessed using validated measuring methods/instruments?  
  Yes

  **Commentary:**  
  The authors used the reliable scientific method in endpoint assessment.

- Was follow-up complete and of sufficient duration?  
  Yes

- Has an appropriate method of statistical analysis been chosen?  
  Yes

  **Commentary:**  
  As the sample size was small, the nonparametric Mann-Witney U test is proper for continuous quantitative variables (area of fibrous membrane, fibroblast density and inflammatory cell density) and \( \chi^2 \) is appropriate for qualitative variables (fibrous adherence).

**Results**

- Was the study analysed using an intention to treat analysis?  
  Yes

- How large was the treatment effect?  
  Due to the adherence of peridural fibrosis, the authors state that more than 70% of the samples had moderate to severe degrees of perisural fibrosis adherence and the adherences were less in the rabbits of experimental group significantly (p=0.04).
  So these data can provide information as followings.
Make a 2x2 table

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Peridural fibrosis</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease (moderate to severe)</td>
<td>No disease (none to mild)</td>
</tr>
<tr>
<td>Aceclofenac</td>
<td>A</td>
<td>b</td>
</tr>
<tr>
<td>Control</td>
<td>C</td>
<td>d</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>7</td>
</tr>
</tbody>
</table>

\[
a+c = 70\% \quad \text{therefore} \quad a+c = 17 \quad \text{and} \quad b+d = 7
\]

These outcomes were categorical data that the authors used the \(X^2\) to calculate the statistical significant \((p=0.04)\). According the \(p=0.04\) while the \(df=3\) (the adherence categories from 0-3), I calculate back the \(X^2\) (using the StatViz software) = 4.045.

The null hypothesis was \(p_1=p_2\).

The alternative hypothesis was \(p_1 \neq p_2\).

\(P_1\) was the probability of moderate to severe peridural fibrosis in experimental group \((p_1=0.5)\).

\(P_2\) was the probability of none to mild peridural fibrosis in control group \((p_2=0.5)\).

(assume the probability \(p_1=p_2=0.5\))

From the formular of a \(X^2 = \sum (O-E)^2 / E \)

\[
E=np = 0.5 \times 24 = 12 \ \text{in both groups}
\]

So \(4.045 = (a-12)^2 + (c-12)^2 / 12 \)

Finally \(a= 5, b=7, c=12 \) and \(d=0\)

The 2x2 table is as following.
### Peridural fibrosis

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Disease (moderate to severe)</th>
<th>No disease (none to mild)</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aceclofenac</td>
<td>5</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>7</td>
<td>24</td>
</tr>
</tbody>
</table>

EER = 5/12 = 41.67%

CER = 12/12 = 100%

ARR = 100 - 41.67 = 58.33%

RR = 41.67/100 = 41.67%

RRR = 100 - 41.67 = 58.33%

NNT = 100/58.33 = 1.71

- How precise was the estimate of treatment effect?
  95% CI of ARR = 58.33 ± 27.89 30.44 to 86.22%
  95% CI of NNT = -28.73 to 87.93
  A wide range of 95% confidence interval due to a small sample size.

The total fibrous surface area, cellular density of fibroblast and density of inflammatory cell the 95% confidence interval were calculated using the STATA (version 11) and summarized in the following tables.

#### Total fibrous surface area (mm²)

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Study group 95% CI</th>
<th>Control group 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.8058 5.0423</td>
<td>2.8399 9.5833</td>
</tr>
<tr>
<td>4</td>
<td>0.3010 2.4739</td>
<td>1.3434 5.2481</td>
</tr>
</tbody>
</table>
Cellular density of fibroblast (mm$^2$)

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Study group 95% CI</th>
<th>Control group 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2257.484 3128.516</td>
<td>2535.353 4218.647</td>
</tr>
<tr>
<td>4</td>
<td>560.0249 2511.975</td>
<td>1140.971 3265.029</td>
</tr>
</tbody>
</table>

Density of inflammatory cell (mm$^2$)

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Study group 95% CI</th>
<th>Control group 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>365.3036 564.6964</td>
<td>486.6991 669.3009</td>
</tr>
<tr>
<td>4</td>
<td>106.353 303.647</td>
<td>204.3912 357.6088</td>
</tr>
</tbody>
</table>

The 95% confidence interval of the total fibrous surface area, cellular density of fibroblast and density of inflammatory cell showed no difference between the experimental group and control group but the experimental group trend to have better results.

- Is there any adverse event in both the experimental and control groups?
  No, the authors reported no adverse event.

Applicability

- Is the treatment feasible in your setting?
  Yes
• Are your patients similar to the target population?
   No

Commentary:
This study was the phase 1 (animal study), so the study informed only the feasibility to use the NSAIDs in human to prevent/reduce the peridural fibrosis. They need further studies in human before generally using as the guideline in patients to prevent/reduce the peridural fibrosis.

Because of there are no evidence of NSAIDs uses in the prevention of peridural fibrosis that can conduct in human in a kind of ethical consideration when rebiopsy the patient’s spine to diagnose the peridural fibrosis. So this article is one of a few evidences to use the NSAIDs in prevention/reduction the peridural fibrosis.

• How the findings of the study are likely to translate to human?

“Virtually every medical achievement of the last century has depended directly or indirectly on research with animals”. Statements like this have been endorsed by many scientific bodies, including the US Public Health Service, the American Medical Association, and Britain’s Royal Society and its Department of Health. The researchers also use animals to learn more about health problems that affect both human and animals, and to assure the safety of new medical treatments. We have now an extending therapeutic potential in nerve cell for spinal cord damage, skin cells for wounds and repairing scar. So we have a role to confer the results of this study to use in human with the exception in a case of the stronger evidence such as randomised controlled trials in human.

Propose to translate the result to human, we have many spine operation that expose to the epidural space, so the peridural fibrosis is inevitable. The NSAIDs may have a role in peridural fibrosis prevention/reduction.
Conclusion

- This article was interesting because the research objective was directly to solve my question. Although this one is the animal study but it innovates the new treatment modality that never study in human before and because the ethical concerning in human when we assess the outcome in human by reoperation or rebiopsy the patient to measure the histology of patient’s tissue, so the animal research like this is only the basic evidence to adopt for human or further trials.

- The article showed inconclusive results, although the authors found the significant p-value in order to the fibrous surface area, cellular density of fibroblasts and inflammatory cell density, but the wide 95% confidence interval showed no significances due to the precision is low and the many reasons that reduce the power of the study such a potential type II error, the inclusion & exclusion criteria were not clearly defined, potential selection bias, no conflict of interest statement that may bias the study result.

Resolution of the scenario

- The clinical question in scenario needs the further study because the result of this article is inconclusive, but the NSAIDs seem to have a role in peridural fibrosis prevention.
References of the critical appraisal


vi Illman J. Stem cells-the master builders(2007). In Illman J. Editor, Medical advances and animal research (the contribution of animal science to the medical revolution: some case histories)(pp22-23).RDS.
## ARRIVE 2010 checklist for animal studies

<table>
<thead>
<tr>
<th>SECTION/TOPIC</th>
<th>ITEM No.</th>
<th>RECOMMENDATION</th>
<th>REPORTED ON page no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE</td>
<td>1</td>
<td>Provide as accurate and concise a description of the content of the article as possible.</td>
<td>451</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>2</td>
<td>Provide an accurate summary of the background, research objectives including details of the species or strain of animal used, key methods, principal findings, and conclusions of the study.</td>
<td>451</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>3</td>
<td>a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.</td>
<td>451</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study’s relevance to human biology.</td>
<td>451</td>
</tr>
<tr>
<td>Objectives</td>
<td>4</td>
<td>Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.</td>
<td>452</td>
</tr>
<tr>
<td>METHODS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethical statement</td>
<td>5</td>
<td>Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.</td>
<td>452</td>
</tr>
<tr>
<td>Study design</td>
<td>6</td>
<td>For each experiment, give brief details of the study design, including:</td>
<td></td>
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<tr>
<td></td>
<td>6</td>
<td>a. The number of experimental and control groups.</td>
<td>452</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>b. Any steps taken to minimize the effects of subjective bias when allocating animals to treatment (e.g., randomization procedure) and when assessing results (e.g., if done, describe who was blinded and when).</td>
<td>452-453</td>
</tr>
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<td></td>
<td>6</td>
<td>c. The experimental unit (e.g. a single animal, group, or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.</td>
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<tr>
<td>Experimental procedures</td>
<td>7</td>
<td>For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example:</td>
<td>452</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>a. How (e.g., drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s).</td>
<td></td>
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<tr>
<td></td>
<td>7</td>
<td>b. When (e.g., time of day).</td>
<td>N/A</td>
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<tr>
<td></td>
<td>7</td>
<td>c. Where (e.g., home cage, laboratory, water maze).</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>d. Why (e.g., rationale for choice of specific anaesthetic, route of administration, drug dose used).</td>
<td>452</td>
</tr>
<tr>
<td>Experimental animals</td>
<td>8</td>
<td>a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g., mean or median age plus age range), and weight (e.g., mean or median weight plus weight range).</td>
<td>No details of NZW rabbits</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug- or test-naive, previous procedures, etc.</td>
<td>N/A</td>
</tr>
<tr>
<td>Topic</td>
<td>Section</td>
<td>Details</td>
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<td>--------------------------------------------------</td>
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<tr>
<td>Housing and husbandry</td>
<td>9</td>
<td>Provide details of:</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>a. Housing (e.g., type of facility, e.g., specific pathogen free (SPF); type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish).</td>
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<td>b. Husbandry conditions (e.g., breeding programme, light/dark cycle, temperature, quality of water etc. for fish, type of food, access to food and water, environmental enrichment).</td>
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<td>c. Welfare-related assessments and interventions that were carried out before, during, or after the experiment.</td>
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<tr>
<td>Sample size</td>
<td>10</td>
<td>a. Specify the total number or animals used in each experiment and the number of animals in each experimental group.</td>
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<td></td>
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<td>b. Explain how the number of animals was decided. Provide details of any sample size calculation used.</td>
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<td></td>
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<td>c. Indicate the number of independent replications of each experiment, if relevant.</td>
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<tr>
<td>Allocating animals to experimental groups</td>
<td>11</td>
<td>a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done.</td>
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<td></td>
<td></td>
<td>b. Describe the order in which the animals in the different experimental groups were treated and assessed.</td>
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<tr>
<td>Experimental outcomes</td>
<td>12</td>
<td>Clearly define the primary and secondary experimental outcomes assessed (e.g., cell death, molecular markers, behavioural changes).</td>
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<tr>
<td>Statistical methods</td>
<td>13</td>
<td>a. Provide details of the statistical methods used for each analysis.</td>
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<td>b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron).</td>
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<td>c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.</td>
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<tr>
<td>RESULTS</td>
<td></td>
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<tr>
<td>Baseline data</td>
<td>14</td>
<td>For each experimental group, report relevant characteristics and health status of animals (e.g., weight, microbiological status, and drug- or test-naïve) before treatment or testing (this information can often be tabulated).</td>
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<tr>
<td>Numbers analysed</td>
<td>15</td>
<td>a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g., 10/20, not 50% ).</td>
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<td></td>
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<td>b. If any animals or data were not included in the analysis, explain why.</td>
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<tr>
<td>Outcomes and estimation</td>
<td>16</td>
<td>Report the results for each analysis carried out, with a measure of precision (e.g., standard error or confidence interval).</td>
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<tr>
<td>Adverse events</td>
<td>17</td>
<td>a. Give details of all important adverse events in each experimental group.</td>
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<td></td>
<td>b. Describe any modifications to the experimental protocols made to reduce adverse events.</td>
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<tr>
<td>DISCUSSION</td>
<td></td>
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<tr>
<td>Interpretation/scientific implications</td>
<td>18</td>
<td>a. Interpret the results, taking into account the study objectives and hypotheses, current theory, and other relevant studies in the literature.</td>
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<td>b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model and the imprecision associated with the results.</td>
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<td>c. Describe any implications of your experimental methods or findings for the replacement, refinement, or reduction (the 3Rs) of the use of animals in research.</td>
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<tr>
<td>Generalisability/translation</td>
<td>19</td>
<td>Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.</td>
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<tr>
<td>Funding</td>
<td>20</td>
<td>List all funding sources (including grant number) and the role of the funder(s) in the study.</td>
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Note: N/A is not available.
Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs

Manuel A. Sandoval · Daniel Hernandez-Vaquero

Abstract Peridural fibrosis is one of the more frequent complications of lumbar surgery. Nonsteroidal anti-inflammatory drugs inhibit the inflammatory and fibroblastic response. We performed lumbar laminectomies in 24 rabbits, divided into two groups. The experimental group received 5 mg/kg/day of aceclofenac for 7 days and the control group received 1 cm³ of physiological saline. The samples were stained using immunohistochemical methods. The cellular populations in the inflammatory reaction and the thickness of the fibrous membrane were quantified. The mean of the fibrous area was always less in the rabbits of the experimental group compared to controls (47% less at 2 weeks and 41% less at 4 weeks). We observed an 8% decrease in the number of fibroblasts with antivimentin monoclonal antibodies in the experimental group. In this model, aceclofenac inhibits the presence of inflammatory cells in the fibrous scar in the early stages and reduces the extension of adhesions without adverse reactions.

Keywords Prevention · Nonsteroidal drugs · Peridural fibrosis · Laminectomy · Epidural scar formation

Introduction

Peridural fibrosis consists of the formation of extradural fibrous tissue, which produces an adherence of the dura mater and the nerve roots to the erector muscles of the spinal column in the posterior part and to the disc and vertebral body in the anterior part. This scar formation may be compressive and at the same time restricts the mobility of the nerve root that is most vulnerable against new discal protrusions and favors the presence of a stenosis of the neural canal. Even today, the most accepted etiopathogenic theory is that proposed by LaRocca and MacNab [18], who suggest that the peridural fibrosis originates from the bleeding surface of the deep layer of the posterior paravertebral muscles. These authors baptized this layer as “laminectomy membrane” which would cover the defect created by the bone resection in an attempt to replace the empty space by means of the formation of fibrous tissue would aim to reconstitute the removed lamina and which would extend within the neural canal.

When faced with an established peridural fibrosis treatment is limited, no effective treatment is known to cure peridural fibrosis [5]. The prescription of analgesics, physiotherapeutic measures or anti-inflammatory medication has been considered only palliative for painful periods. There is no consensus on the effectiveness of surgery. Some authors rule out this possibility [4, 17, 23] and others recommend, such as, a last resort in patients with incapacitating pain [18, 20, 22].

Most authors believe the best way of avoiding the appearance of peridural fibrosis is to prevent its formation [2, 11, 12, 14]. Meticulous surgery, as a preventive intraoperative measure, has been suggested [6, 25] with careful hemostasis and the placement of physical [11, 21] and chemical [4, 9, 13] barriers between the paravertebral
musculature and the dura. The nonsteroidal anti-inflammatory drugs (NSAIDs) are the examples of systemic chemical barrier. They present an advantage over the physical barriers of not introducing foreign bodies, which may increase the inflammatory response. Moreover, NSAIDs inhibit cyclooxygenase, which is responsible for the synthesis of prostaglandins (biological mediators capable of triggering reactions of vasodilation and chemotaxis). The beneficial effect of the NSAIDs in the prevention of the calcifications of the soft tissues, heterotopic ossifications and adherences is well-known [10, 20].

The question we intended to address was if a commonly used European NSAID, aceclofenac (Almirall Prodesfarma, Barcelona, Spain), administered from the time of intervention would inhibit fibroblastic proliferation after performing a laminectomy in experimental animals.

Materials and methods

An experimental study was made in 24 New Zealand white (NZW) rabbits, in which, a laminectomy was performed in the lamina L4. The 24 animals were equally divided into two experimental groups, which were killed at weeks 2 and 4, respectively (six in each group), with their corresponding control groups. To the control group, 1 cm³ of physiological saline was injected and to the animals of the experimental group, 5 mg/kg/day of aceclofenac was injected intramuscularly, from the day of intervention to the seventh postoperative day. Approval was obtained from the Animal Research Committee before any animal studies were begun.

Under adequate anesthesia and antibiotic protection, a longitudinal incision of 4 cm was made in a posterior line between L3 and L5; the fascia was incised in order to expose the extreme of the spinous processes. The paraspinal muscles were detached subperiosteally from the spinous processes and the laminas, and retracted with an autostatic separator. Following a meticulous technique in order not to damage the spinal cord [23], the spinous processes of the caudal vertebra, the ligamentum flavum, inferior articular processes and the third distal part of the lamina of the cranial vertebra were resected until achieving an exposure of the dura of $4 \times 8$ mm. A drainage was used for 24 h.

The animals were killed at weeks 2 and 4 by means of an overdose of intravenous sodium pentobarbital (60 mg/kg). The spinal column was resected in bloc between L3 and L5. This was fixed with formalin at 10% for 24 h and decalcified in formic acid for 3 weeks.

We obtained systematically four histological sections of the entire transverse diameter of the spinal canal from each animal. Each section was stained with hematoxylin-eosin (H&E), Masson’s trichrome and immunohistochemical methods. The streptavidin-biotin-peroxidase was used for the detection of antigens, and the enzymatic marking was made with horseradish peroxidase. The substrate was hydrogen peroxide and the chromogen was DAB (diaminobenzidine). This chromogen marks the fibroblasts in brown color (Fig. 1). The monoclonal antivimentin antibody produced in rabbit (clone V9 of Biogenex®) was employed. In each section, the following measures were made: the area of the fibrous membrane (mm²), the density of the fibroblasts per square millimetre and the density of the inflammatory cells per square millimetre. The adherences were graded into 0 (no adherence was seen between the dura and the fibrosis), 1 (adherence less than one-third of the surface of the dura), 2 (between one and two-third) and 3 (greater than two-third).

For the quantification of the area of the fibrous membrane, the histological preparations stained with Masson’s trichrome were focused by a digital Leica DC 100 camera (Leica, Solms, Germany) and with a Micro-Nikon macro-lens of 55 mm. The samples were analyzed using a Leica QWIN image processor. The detection of the area of fibrosis was made according to the intensities of the green color of Masson’s staining, this being reflected in false color for better visualization (Fig. 2). A systematic selection was performed using an optic pencil for separating the regions of fibrosis from other areas that presented a tonicity similar to the green color, and proceeding finally to the quantification of the area of fibrosis in square millimetre.

In order to quantify the number of fibroblasts, the immunohistochemical preparations were digitalized with a Leica DC 100 camera connected to a Leica DMR-XA microscope and analyzed by means of a Leica QWIN image processor. The capture was performed by means of digitizing in real color, the mentioned regions of fibrosis, thus the range of color was established. At this time an

Fig. 1 This figure shows the fibroblasts as positive-vimentin cells in brown color (original magnification, $\times 250$)
automatic detection was made, according to the color, of all the cytoskeletal cells. The computer program interprets brown as a mixture of the basic red, blue and green colors in a range between 0 and 255 (Fig. 3).

Finally, we quantified the inflammatory cells (lymphocytes, polymorphonuclear cells and macrophages). The histological preparations were stained with H&E and Masson’s trichrome, and examined with a Reichert microscope and analyzed using an Olympus WH 10 × 3 reticle.

The histological and immunohistochemical examinations were performed in a blinded manner. Two persons making the examinations were unaware if the specimen was from study or control group.

The analysis of data was made with descriptive and inferential statistics. As the sample size was small, the non-parametric Mann–Whitney U test was employed for the continuous quantitative variables (area of the fibrous membrane, density of the fibroblasts and density of the inflammatory cells) and the $\chi^2$ for qualitative variables (fibrous adherence).

**Results**

No superficial or deep infection was seen. In no case, complications in the surgical wound were seen. At day 7 the skin sutures were removed.

The histological findings at week 2 in the control group consisted of an extensive hematoma that filled the laminectomy and was found in contact with the dura. A smaller adherence of the hematoma to the dura (adherence less than one-third of the surface of the dura) was seen in the experimental group at week 2, with similar cellular lines. In the control group at week 4, the hematoma cell infiltrate was progressively replaced with fibrous tissue and in the areas near the resected lamina, a metaplasia to the chondroid tissue and bone were observed in the experimental group at week 4, the hematoma had been progressively replaced by fibrous tissue.

The mean fibrous surface was less ($p < 0.05$) in the rabbits of the experimental group compared to the controls, both at weeks 2 and 4 (Table 1).

In more than 70% of the samples, the adherence of the peridural fibrosis to the dura was, as a minimum, of moderate size (between one and two-third of the surface of the dura) and at least one-third of the samples had considerable proportions (greater than two-third of the surface of the dura). Adherence was less ($p = 0.04$) in the rabbits of the experimental groups (Fig. 4). At week 2, statistically significant differences were seen in the adherence grade, between control and study groups.

We observed a smaller number ($p = 0.08$) of fibroblasts in the experimental groups with a progressive decrease ($p = 0.25$) over time between weeks 2 and 4 (Table 2).

In the study groups, a smaller number of inflammatory cells than the control groups were seen (Table 3). No differences were observed in the types of inflammatory cells.

**Discussion**

From the time of the first studies on peridural fibrosis by LaRocca and MacNab [18], different physical barriers have...
Some authors [1, 4, 17] have suggested a mechanical role for the epidural fat, which protects and facilitates the movement of the dural sac in the bone canal. However, this fat is not without complications, such as, infection and hematoma in the donor area [4]. Kuivila et al. [17] mention other inconveniences of the fat graft such as necrosis, atrophy and even compression on the cauda equina [27].

Other authors [20] studied some complications of the physical barriers, concluding in a prospective study, that the placement of fat barriers or gel foam on the roots and dura after a discectomy had no effect on the clinical result, functional status, or MRI findings. The interposition membrane produced no beneficial effects. Bellen [2] on the other hand found monoradicular motor paralysis produced by hematoma on the third postoperative day, this being attributed to the use of gelfoam as hemostatic agent. Le et al. [19] have published their experience with the use of ADCON-L. In four patients, it can be seen that this material exacerbated the leak of cephalorachidian fluid from microscopic durotomies unrecognized at the time of surgery.

Due to the controversial results of physical and mechanical barriers, attempts have been made to apply chemical barriers such as corticoids, activators of tissue plasminogen [2], mitomycin C, urokinase [9] and elastase. However, these are no longer used [2, 4, 5, 14].

The NSAIDs inhibit the synthesis of prostaglandins, thereby decreasing the permeability of the capillaries, inhibiting the inflammatory infiltration, and controlling fibroplastic hyperplasia and formation of granulation tissue. Aceclofenac is a widely used NSAID in musculoskeletal pathology due to its analgesic and anti-inflammatory properties and is well tolerated when used for short periods. It is a derivative of phenylacetic acid, with a structure similar to other NSAIDs. Its main action mechanism is the inhibition of cyclooxygenase and therefore of the synthesis of prostaglandins but it also modifies other cellular processes such as the synthesis of leukotrienes, generation of superoxides, release of enzymes by lysosomes, aggregation and adhesion of neutrophils, and the functions of the cellular membrane [16].

We chose the dose based upon the studies of Brogden [3]. Agreement exists that the appropriate dose is 5 mg/kg and that the route of administration has no clinical

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Cellular density of the fibroblasts (mm²)</th>
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<tr>
<td>Time (weeks)</td>
<td>Study group (mean ± SD)</td>
</tr>
<tr>
<td>2</td>
<td>2693 ± 415</td>
</tr>
<tr>
<td>4</td>
<td>1536 ± 930</td>
</tr>
</tbody>
</table>

Table 3 Density of inflammatory cells (mm²)

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Study group (mean ± SD)</th>
<th>Control group (mean ± SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>465 ± 95</td>
<td>578 ± 87</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>205 ± 94</td>
<td>281 ± 73</td>
<td>0.14</td>
</tr>
</tbody>
</table>

SD standard deviation
relevance [8]. This dose was administered for only 1 week in order to evaluate the early inhibitory effect of the fibrosis and to avoid possible adverse reactions.

The immunohistochemical techniques permit the detection and identification of biomolecular components, which are the integral parts of cells and tissues, by means of the study of the reaction between these components and the corresponding polyclonal and monoclonal antibodies. In our study, this technique facilitated the identification of the fibroblasts such as positive-vimentin cells.

The mean of the fibrous surface was less in the rabbits of the experimental groups compared to controls, at both weeks 2 and 4, but the difference was significant only when the two time groups were combined, perhaps because the sample size was small.

We have noticed a smaller number of all the inflammatory cells but different only in early stages. The types of inflammatory cells were similar in both groups, as He et al. [13] also affirm, after administering ketoprophene in rats.

We have shown that peridural fibrosis can be developed experimentally in the rabbit after lumbar laminectomy and that a NSAID is capable both of reducing the fibrous area and of inhibiting the presence of inflammatory cells in the fibrous scarring. The immunohistochemical techniques help to quantify them. We consider that aceclofenac, when administered early, may be a useful drug for preventing the formation of the peridural fibrosis.

References