Paracetamol and ibuprofen block hydrothorax absorption in mice

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Abstract

OBJECTIVES: Non-steroidal anti-inflammatory agents (NSAIDs) and paracetamol alter pleural permeability, hindering pleural fluid recycling. The aim of this study was to investigate the effect of different analgesic and anti-inflammatory agents on fluid recycling in an induced hydrothorax model in mice.

METHODS: Hydrothorax was induced in C57BL/6 mice by injecting 500 μl phosphate-buffered saline–bovine serum albumin 1% isosmotic in the right hemithorax. Paracetamol (1 g/kg), ibuprofen (250 mg/kg) and parecoxib (2 mg/kg) were administered systematically by intraperitoneal injections. Each drug group included eight mice, which were sacrificed at 2 h and 4 h, respectively, after injections. The remaining hydrothorax volume and total cells contained were determined.

RESULTS: Regarding the paracetamol and ibuprofen groups, the remaining hydrothorax volume was greater than in the control group (350 ± 61, 348 ± 62 and 270 ± 51 μl, respectively, P = 0.042) when mice were sacrificed within 2 h. Similar observations were made in groups sacrificed after 4 h (202 ± 45 and 198 ± 44 vs 107 ± 56 μl, respectively, P = 0.002). In the parecoxib group, the remaining hydrothorax volume was 122 ± 53 μl (P = 0.038 versus paracetamol and ibuprofen, P > 0.05 versus control group). At the same time, the absorption rate in the paracetamol and ibuprofen groups was lower than in the parecoxib and control groups (P = 0.033). In the parecoxib group, the absorption rate was lower than that in the control group after 2 h (P = 0.042). In the paracetamol and ibuprofen groups, the total cell count and the macrophage and the neutrophils counts were increased, compared with the control and parecoxib groups (P = 0.025, 0.028 and 0.032, respectively).

CONCLUSIONS: Paracetamol and ibuprofen acutely hinder pleural fluid recycling by lowering the fluid absorption rate (higher remaining hydrothorax volume), while they increased total white cell counts. COX-2s presented lower remaining hydrothorax volume without acutely increasing the absorption rate. These findings could present some relevance to the administration of painkillers in patients with pleural effusion after thoracotomy.

Keywords: Hydrothorax • Mice • NSAIDs • Paracetamol • Absorption • Pleural

INTRODUCTION

Non-steroidal anti-inflammatory agents (NSAIDs) are considered to play an important role in the progression of exudates by inducing alteration of their volume, leucocyte migration and pleural vascular permeability, and are consequently involved in their treatment [1–4]. Additionally, NSAIDs are widely used in thoracic surgery postoperatively, along with other regimes, in order to achieve analgesia.

However, it is widely accepted that, apart from their analgesic and anti-inflammatory features, NSAIDs also interfere with epithelial permeability, i.e. in kidneys and lungs mainly via prostaglandin production alteration [5–7], implicating in this way a possible role in fluid recycling and transportation across tissues. Additional data show that in pleura, dexamethasone itself produced an increase in hydrothorax absorption in a mice model, whereas histamine, an inflammatory hormone, was previously shown to interfere with pleural fluid recycling mainly via H1 receptors [8, 9]. NSAIDs were shown to hinder pleural permeability in vitro, by inhibiting prostaglandin synthesis and eventually blocking the Na+/K+ pump activity normally operating in pleura [10].

From all the aforementioned, it is evident that NSAIDs can cause the alteration of pleural fluid recycling, an event that is
extremely important for postoperative fluid balance between the pleural cavity and systemic circulation.

The aim of the present study was to investigate in vivo the effect of different analgesics usually used in clinical practice (NSAIDs and paracetamol) in a hydrothorax absorption model induced in mice.

**MATERIALS AND METHODS**

Hydrothorax was induced in 8- to 10-week old C57BL/6 mice, which were acclimatized in the laboratory for 1 week, receiving food and water ad libitum. The mice were anaesthetized with an intraperitoneal injection of 0.67 mg/ml xylazine and 1.34 mg/ml ketamine mixture. The right hemithorax was shaved and prepared with povidone–iodine solution 1%. A small incision was performed over the mid of the right hemithorax at the mid-axillar line. An aliquot of 500 μl of phosphate-buffered saline-bovine serum albumin (PBS–BSA) 1% isosmotic was injected in the hemithorax by puncturing the superior aspect of the rib, but avoiding puncture of the lung. The incision was closed with a 4/0 nylon suture (Fig. 1A). A small area of each mouse abdomen was also shaved and accordingly prepared. Regarding the control animals, 1 μl of PBS was injected intraperitoneally, and 1 μl of each drug (paracetamol 1 g/kg, ibuprofen 400 mg/kg and parecoxib 2 mg/kg) was similarly injected in the drug groups (Fig. 1B). These drugs were chosen based on previous investigations to include at least one representative of each analgesic drug commonly used in clinical practice [10].

After the injections, the animals were returned to their cages. Half of the animals from each control and drug groups were randomly euthanized within 2 h, and half within 4 h after the initiation of each experiment, by sevoflurane overdose.

Each group (control and drug) included eight animals, and therefore 32 experiments were conducted in total. In each group, the amount of hydrothorax volume remaining in the hemithorax after euthanasia of the animals was determined. Total white blood cell numbers were evaluated under a microscope. Cytospins were also prepared and differential cell counts were evaluated upon May Gruwald Giemsa staining.

All procedures were approved by the Veterinary Administration Bureau of the Prefecture of Athens, Greece, and were conducted in accordance with European Union Directives for animal research (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm).

**Statistical analysis**

Statistical analysis was performed using SPSS for Windows version 17.0 (Chicago, IL, USA). ANOVA (Bonferroni’s post hoc) was used for comparison among groups. A P-value of less than 0.05 was accepted as significant.

**RESULTS**

**Effect on hydrothorax volume**

The hydrothorax volume in control mice sacrificed within 2 h was lower than in the paracetamol and ibuprofen groups (270 ± 51, 350 ± 62 and 348 ± 58 μl, respectively, P = 0.042, Table 1), as opposed to the parecoxib group where it presented similarity (300 ± 55 μl, P = 0.688 versus control and P = 0.198 versus paracetamol and ibuprofen groups, Table 1).

In control mice sacrificed 4 h after the initiation of experiments, again the hydrothorax volume was lower than that referring to the paracetamol and ibuprofen groups (107 ± 56, 202 ± 45 and 198 ± 44 μl, respectively, P = 0.002, Table 1). The remaining hydrothorax volume in the parecoxib group was lower than the measurement received from the paracetamol and ibuprofen groups (122 ± 53 μl, P = 0.038, Table 1) and similar to the one regarding the control group (P = 0.983).

**Table 1: Volume of hydrothorax found after mouse sacrifice, 2 and 4 h post intrapleural injection of the fluid**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Hydrothorax volume (μl, mean ± SEM)</th>
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<tbody>
<tr>
<td></td>
<td>2 h</td>
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<td></td>
<td>4 h</td>
</tr>
<tr>
<td>Control</td>
<td>270 ± 51</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>350 ± 62*</td>
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<tr>
<td>Ibuprofen</td>
<td>348 ± 58*</td>
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<tr>
<td>Parecoxib</td>
<td>300 ± 55</td>
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<td>107 ± 56</td>
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<td></td>
<td>202 ± 45*</td>
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<td>198 ± 44*</td>
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<td>122 ± 53**</td>
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</tbody>
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*P < 0.05 versus control.

**Figure 1:** Hydrothorax induction by injecting PBS–BSA 1% within the pleural cavity (A). PBS (control group) and drugs (paracetamol, ibuprofen and parecoxib) injected intraperitoneally (B).

**Table 1:** Volume of hydrothorax found after mouse sacrifice, 2 and 4 h post intrapleural injection of the fluid

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*P < 0.05 versus control.

**SEM:** standard error of the mean.
**Table 2:** Rate of hydrothorax absorption at 2 and 4 h

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Absorption rate (μl/h, mean ± SEM)</th>
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<tbody>
<tr>
<td></td>
<td>2 h</td>
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<tr>
<td></td>
<td>4 h</td>
</tr>
<tr>
<td>Control</td>
<td>118 ± 9</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>75 ± 8**</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>76 ± 9**</td>
</tr>
<tr>
<td>Parecoxib</td>
<td>95 ± 8***</td>
</tr>
</tbody>
</table>

*P < 0.05 versus control rate at 2 h.
**P < 0.05 versus control and parecoxib.
***P < 0.05 versus control. SEM: standard error of the mean.

**Effect on absorption rate**

The hydrothorax absorption rate calculated in 2 h was higher in the control group (118 μl/h) when compared with the parecoxib, paracetamol and ibuprofen groups (95, 75 and 76 μl/h, respectively, P = 0.048, Table 2). The hydrothorax absorption rate calculated in 2 h was also higher in the parecoxib group than in the paracetamol and ibuprofen groups (P = 0.042, Table 2).

The hydrothorax absorption rate calculated in 4 h was of similar measure in paracetamol and ibuprofen groups, but not in the parecoxib and control groups, which presented a higher rate (74, 75, 94 and 90 μl/h, respectively, P = 0.033, Table 2). The absorption rate in the 4-h sacrifice parecoxib group was similar to the control absorption rate (P = 0.168, Table 2).

Absorption rates between 2 and 4 h in all drug groups, showed no differences. The same did not apply to the rate measurements regarding the control group, where a decrease in rate was observed in the 4 h control group when compared with the 2 h control group (P = 0.042, Table 2).

**Effect on white cell migration**

The total white cell count in the control group (122 × 10⁵ cells/ml) was similar to the parecoxib group (102 × 10⁵ cells/ml, P = 0.648) and lower than those of the paracetamol and ibuprofen groups (205 × 10⁵ and 199 × 10⁵ cells/ml, respectively, P = 0.025, Fig. 2).

Macrophages were identified in similar percentages in all groups (P = 0.866, Fig. 2), whereas the neutrophil percentages were higher in the paracetamol and ibuprofen groups in comparison with the control and parecoxib groups (P = 0.028) in which, however, lymphocyte percentages were increased (P = 0.032, Fig. 2) compared with paracetamol and ibuprofen groups.

**DISCUSSION**

The main finding of the present study states that NSAIDs and paracetamol, except parecoxib, limited the absorption of hydrothorax in vivo, by reducing the acute and late absorption rate and thus the volume of the hydrothorax eventually absorbed. Parecoxib resulted in higher hydrothorax volume absorbed, and although it did not acutely increase absorption rate, maintained a higher absorption rate in comparison to the rest of the drugs tested. NSAIDs and paracetamol, except parecoxib, additionally induced increased white cell migration within the pleural cavity and in the effusion, altering the percentage of the macrophages/ neutrophils/lymphocytes and suggesting the initiation of an acute inflammatory response of the remaining hydrothorax, rather than blocking it.

Cellular transportation and tissue permeability via various ion channels has been shown to be influenced by different NSAIDs in the literature. In the kidneys, the absorption of sodium was increased by ibuprofen leading to an overall decrease in the secreted sodium [5, 11]. Ibuprofen also inhibited transmembrane current when added in the basolateral side of Xenopus A6 cell lines [11, 12]. In small dorsal root ganglion cells, ibuprofen and celecoxib suppressed the up-regulation of sodium channels [13]. Celecoxib interfered with ion currents in rat retinal neurons [14] while indomethacin decreased the permeability increase induced by lung adenocarcinoma cells [6]. In previous studies, NSAIDs (acetylsalicylic acid, ibuprofen, diclofenac and lornoxicam) and paracetamol, but not parecoxib, were shown to acutely decrease the permeability of the human parietal pleura effect mediated by the blockage of Na⁺ channels and Na⁺/K⁺ pumps normally operating in pleura [10]. Furthermore, dexamethasone was shown to acutely increase hydrothorax absorption in a mice model [9]. Concurrently, the results of the present study show that the non-selective NSAIDs hinder the pleural fluid absorption in vivo.

COX-2 selective inhibitors are considered to induce less and weaker side effects compared with other non-selective NSAIDs [15]. These COX-2 selective inhibitors offer a better inflammatory pleural response during pleurodesis [16] when compared with the decreased quality of pleural adhesions induced by the non-selective NSAIDs [17-19]. Ketoprofen induced lower exudate volumes and decreased vascular permeability in pleurisy models, administering a more potent role in exudate progression when compared with the non-selective NSAID ibuprofen [2]. In previous studies, parecoxib was shown not to alter the pleural permeability or hinder the Na⁺ channel and Na⁺/K⁺ pump function, which means that it does not affect the pleural fluid recycling ability of
the mesothelium. Results from the present study prove in vivo the beneficial effect of COX-2 selective inhibitor parecoxib on pleural fluid removal ability, which is preserved, in contrast with the non-selective NSAIDs and paracetamol.

The fluid redistribution between the pleural space and the systemic circulation is altered in thoracic surgery, since the operation itself involves the thorax. The pleural ability to reabsorb fluids is therefore significant for postoperative recovery. Results from this study suggest that clinicians should consider the effects of each type of analgesic drug used postoperatively. In cases of an unexplained postoperative persistent effusion, in cases of prolonged chest drain output or in cases where a fast chest drain removal is planned, a thorough review of the analgesics administered should be performed and possibly changed to analgesics that do not hinder pleural fluid absorption. Relative to this, unexplained pleural effusions in patients receiving non-selective NSAIDs have been reported to resolve after the discontinuation of these drugs [20]. The findings of the present study may also prove useful in the treatment of inflammatory pleural effusions where NSAIDs are usually selected for their ability to suppress inflammatory response, as per the aforementioned.

A limitation of the study is that it was performed using animals in which the pleurisy was not inflammatory, but simple hydrothorax. However, in inflammatory pleurisy conditions, different types of cyclooxygenases (COX-2 more specifically) are expressed, the vascular permeability is different and numerous inflammatory factors are involved (i.e. histamine, leukotrienes). Consequently, the effect of NSAIDs may be different from the effect shown in this study [1]. In exudates, NSAIDs are shown to decrease extravasation of plasma and chemotactic activity [3], to decrease exudate volume and leucocyte migration [2] and, in general, induce decreased vascular permeability through which all the above changes are mediated. Accordingly, the leucocyte profile in this study’s non-inflammatory hydrothorax model findings was different from that expected in inflammatory exudates; in non-selective NSAIDs groups, more leucocytes migrated in the hydrothorax, with most of them being neutrophils, suggesting the initiation of an acute inflammatory response of the remaining hydrothorax. In the COX-2 selective group, the leucocytes were reduced and leucocyte populations were similar to the ones in the control group. It is therefore indicated that, with the use of COX-2 selective drugs, an acute inflammatory response in the pleural space, shown to initiate with the administration of non-selective NSAIDs and paracetamol, is suppressed and consequently any fluid left in the pleural space may not progress to an exudate, affecting postoperative recovery.

The use of a mouse model may constitute an additional limitation of the study. The mouse pleura morphologically and functionally differs from that of larger animals, i.e. animals with thick visceral pleura in which the pleural fluid recycling is different, as for instance in the case of humans [21]. Moreover, mice do not have a separate mediastinum, and so the two pleural spaces communicate. On this basis, the results of this study should be interpreted with caution as for their precision in mimicking clinical conditions or extrapolating technical thoracic surgery issues.

Finally, the pleural pressure imbalances induced by surgery is another factor that may impact the postoperative fluid recycling. This factor was not taken under consideration in this model, because open thoracotomy and lung resection was not performed. Consequently, the observations on fluid absorption made in this study might not absolutely reflect physiological alterations occurring in a pleural cavity after thoracotomy.

In conclusion, non-selective NSAIDs and paracetamol, except COX-2 selective parecoxib, limited the absorption of hydrothorax by reducing the acute and late absorption rate and the overall volume eventually absorbed. Additionally, they induced increased white cell migration within the pleural cavity with a higher percentage of neutrophils noted, indicating the initiation of an inflammatory response in the remaining hydrothorax. These findings should be considered in exudate treatment and analgesia management after thoracic surgery.

ACKNOWLEDGEMENTS

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Conflict of interest: none declared.

REFERENCES

APPENDIX A. CONFERENCE DISCUSSION

Dr D. Mathisen (Boston, MA, USA): Were there any differences in the dose of the agents you used or did you just pick this one dose and study that?

Dr Kouritas: These are actually the initial results of the study, which is ongoing. Actually these are the levels used in humans but lowered, but we haven’t looked at other agents. We are going to do that later on.

Dr T. Grodzki (Szczecin, Poland): We are giving drugs because of complaints of pain or suffering. So in accordance with your results we should stop it, but nevertheless patients continue to suffer pain. So should we switch to opioids? Did you make any comparison?

Dr Kouritas: Of course, we don’t propose stopping them. We are just saying that if we find out with our supposition that something cannot be explained, maybe then we have to turn to other things.