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Full Length Research Paper

An experimental model to study pneumothorax in Rats

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Researchers have used various techniques to form pneumothorax in animals, but to date, no standard method or rate of pneumothorax has been defined. We tried to develop a standard and a safe method of pneumothorax model in this study. 12 female Wistar Albino rats weighing 200 - 220 g were used. Two groups (6 rats in each) were formed. Open pneumothorax was formed by 0.5 cm incision through the right, 5th intercostal space. The first group was held in open pneumothorax position for 1 min and the second group, for 2 min and the incisions were sutured. After 48 h, computed tomography of the rats were obtained and the pneumothorax rates were measured. The mean pneumothorax value was greater in Group II (13.89%) than in Group I and it was statistically significant ($p < 0.05$). The above mentioned method produces low rates of pneumothorax (10 - 15%), without injuring lung tissue. It should be useful for researchers, who plan to study lung physiology with low rates of pneumotohorax.

Key words: Model, pneumothorax, rat, experimental pneumothorax.

INTRODUCTION

Pneumothorax is a common clinical entity in thoracic surgery practice (Fry and Paape, 2005). Various studies including animal experiments have been conducted to investigate pneumothorax (Hill et al., 1995).

Literature review shows that studies with animal models of pneumothorax do not describe a standard method of pneumothorax formation. In some studies, a catheter was inserted into the pleural space of rabbits and 5 - 20 ml air was pumped through, while in others, intrapleural space was penetrated and the pleural pressure was measured (England et al., 1998). However, the method or degrees of pneumothorax to be formed have not been clearly described.

In this study, we tried to find a simple method. The method should be standard, safe and applicable. Also, the method should be feasible because almost all kind of animal laboratories lack high technology. The safety of previous methods is also questinable, especially methods using “percutaneous” techniques. The injury of underlying lung is inevitable by blind techniques. However in our method; the pneumothorax, which is created under direct visualization do not harm the pulmonary parenchyma.

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MATERIALS AND METHODS

Before the study was conducted, the study protocol was presented to the Animal Ethics Committee of the University, and the approval of the committee was obtained (Date: 02/15/2007; Number: 50/169). The study was performed in Animal Experimentation Laboratory of the University, Medical School. The study involved Wistar Albino rats with a weight of 200 - 220 g, which were reproduced in the laboratories of the university.

A pilot study was conducted to determine the incision length and the duration (min) of pneumothorax that would be inflicted.

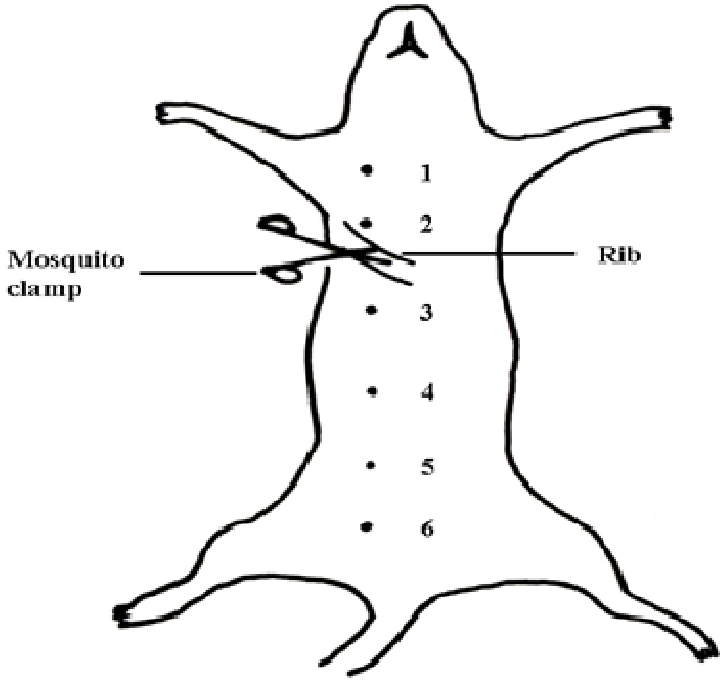

Figure 1. Schematic appearance of a rat in a state of open pneumothorax. The nipples on the right are numbered between 1 and 6.

Pilot study

Four rats were used. The animals were anesthetized before the procedure and were given painkillers after the procedure. In two of the animal, 1 cm incision was made and in the other two animals, 2 cm incision was made. The animals' number 1 and 3 were performed 3 min of open pneumothorax and number 2, 4 and 5 min of open pneumothorax, and then the thorax of the animals were closed. While in the animals with 1 cm thoracotomy, mild mediastinal flatter was observed, in those with 2 cm thoracotomy, severe mediastinal flatter was noted. One animal with severe mediastinal flatter died. Computerized tomography (CT) of the thorax was performed for survived three animals forty eight hours after the procedure. The rates of pneumothorax were calculated.

The highest rate of pneumothorax was observed in the animals with 1 cm incision and 3 min open pneumothorax. Based on these results, to reduce mediastinal flatter and enhance the amount of pneumothorax, it was determined that the incision would be smaller and the duration would be shorter. To ensure safety of the animals, the duration of pneumothorax was planned to be limited to 1 min in the first group and to 2 min in the second group, and the incision was planned to be 0.5 cm.

The animals were anesthetized with Xylazine HCl 3 mg/kg IM and Ketamine HCl 90 mg/kg IM before the procedure. The thoraxes of Group I (n = 6) and Group II (n = 6) were penetrated through the area under the second nipple the right 5th intercostal (Figure 1). During the procedure, two animals in Group I and one animal in Group II died due to mediastinal flatter. The animals that died during the procedure were replaced with new ones.

After the procedure, Seftriakson Na (Rocephin, 500 mg, Roche, Sweeden) 30 mg/kg/day IM, were given to the animals. Carprofen (Rimadyl, 20 ml, Pfizer, England) 4 mg/kg/day, S.C was also used as an analgesic for 3 days. After 48 h, the animals were sedatized with Xylazine HCl 3 mg/kg IM and Ketamine HCl 90 mg/kg IM. Then, thorax CT of each animal was obtained in Radiology Department by Philips Brilliance 16 detector CT. CT images were transferred into Philips Extended Brilliance Workspace program. In evaluations, Cavalieri principle was used in volume calculations (Emirzeoglu et al, 2005).

$V = t \times (a1+a2+\ldots\ldots\ldots\ldots +an) \times 100\ cm^3$

Where:

V = Volume,
t = slice thickness,
a = Cross-sectional area.

The pneumothorax rates of the groups were compared.

Statistical evaluation

In this study, we only examined pneumothorax ratio of rats. The data of the study were evaluated with SPSS (ver 14.0) program. Kruskal-Wallis test, Mann-Whitney U test and correlation analysis were used. The data were expressed as the mean ± standard deviation. The value $p < 0.05$ was considered to be significant.

RESULTS

The highest pneumothorax value was obtained in Group

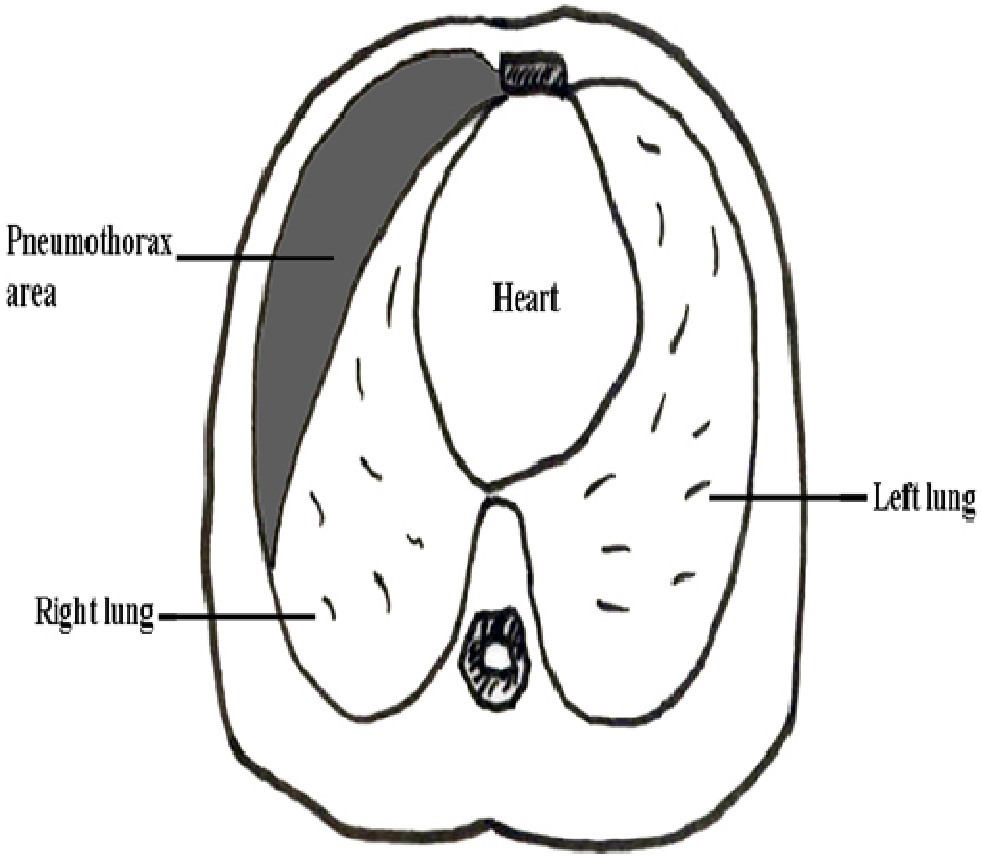

Figure 2. In Group II, the area of pneumothorax obtained in subject no 3 is shown as a marked area on the schematic thorax-CT.

Table 1. The pneumothorax values of the both groups

Rat number	Group I*(%)*	Group II (%)
1	13.53	7.28
2	7.99	9.72
3	8.99	24.92
4	7.46	16.24
5	12.20	13.71
6	20.06	11.47
Average	11.7	13.89

II and the mean pneumothorax rate was 13.89% (Figure 2). The pneumothorax values of Group I and Group II are presented in Table 1.

Comparisons of the study groups for pneumothorax rates were made through Kruskal-Wallis test, and the difference between the groups was statistically significant ($p < 0.05$). Positive correlation coefficients were determined between duration and pneumothorax percentage. However, the correlation was not statistically significant ($p > 0.05$).

In this study, a total of 19 animals were used including the animals in the pilot study. One of the animals died in the pilot study, and three of the animals died in the main study. The other 15 animals were returned to the animal laboratory after the study was completed and all were alive.

DISCUSSION

Various experimental models of pneumothorax have been developed, for treating of pneumothorax, however, no standard method of forming pneumothorax has been described (Xie et al,1998).

In this study, we attempted to describe a standard pneumothorax model. Some researchers have formed pneumothorax by using a catheter rather than opening the pleura (Cetin et al., 2005). However, the risk of pleural injury has been overlooked. Others have determined

whether the pleural space was penetrated by observing the absorption level of the fluid applied on the tip of the catheter or the pleural pressure waves obtained from the catheter attached to a transducer (Akopov et al., 2005). In our study, intercostals space and pleura were opened with a clamp under direct visualization and thus the lungs were not injured.

Hill, in a study to achieve resolution of experimental pneumothorax, used New Zealand albino rabbits and 20 ml of room air was pumped into the pleural spaces of the animals, through which 100% pneumothorax was obtained. Similarly, England used similar animals and similar technique and pumped 15 ml room air and 100% pneumothorax was obtained (Hill et al., 1995). This indicates that description of a standard model is difficult even when similar methods and subjects are used. The rate of pneumothorax (28%) obtained in the pilot study, however was not obtained in the main study. Comparisons of Group I and Group II for the rates of pneumothorax showed a positive correlation between time dependent pneumothorax rates ($p > 0.05$). In open pneumothorax, as the waiting time prolonged, the rate of pneumothorax increased. However, the mediastinal flatter in the animals increased in the mean time and consequently, some animals died. This is affected by the size of thoracotomy. Despite prolonged endurance of the animals with small incisions, these findings could not be statistically evaluated in our study. The size of hemithorax in the rats used in our study was nearly 4.5 cm on upper-lower axis. For open pneumothorax, an incision of 0.5 cm was inflicted. In other words, a thoracotomy of 1/9 of the size of hemithorax was performed. This was easily tolerated by the animals. In the light of this finding, animals that will be used in future studies on pneumothorax may tolerate an incision of this size more easily. Because it was not possible to obtain thorax CTs of the animals during the procedure, CT was performed 48 h after the procedure. Absorption of the pnumothorax area by 1.25% of the hemithorax volume in 24 h was a disadvantage (Don et al., 1995). This may have caused lower pneumothorax rates in our study.

Nevertheless, it was shown that a rat that was inflicted with open pneumothrax without any injury to the lung parenchyma survived for 48 h, which may prove helpful for future researchers.

Conclusion

The highest rate (14%) of pneumothorax was determined in Group II. Comparisons of the study groups for pneumothorax rates, the difference between the groups were statistically significant. In future studies on absorption, pleurodesis and respiratory physiology, the rate of pneumothorax obtained in Group II may be sufficient.

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