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Development of an Optimal Diaphragmatic Hernia Rabbit Model for Pediatric Thoracoscopic Training

Eva M. PÉREZ-MERINO[1], Jesús M. USÓN-CASAÚS[1], Concepción ZARAGOZA-BAYLE[1], Ramón RIVERA-BARRENO[2], Carlos A. RODRÍGUEZ-ALARCÓN[2], Rupert PALME[3], and Francisco M. SÁNCHEZ-MARGALLO[4]

[1]*Department of Animal Medicine and Surgery, Veterinary Faculty, University of Extremadura, Avda. Universidad s/n, 10003 Cáceres, Spain*
[2]*Department of Medical Sciences, Institute of Biomedical Sciences, Autonomous University of Juarez, Anillo Envolvente del Pronaf s/n, 32310 Ciudad Juarez, Mexico*
[3]*Department of Natural Sciences, University of Veterinary Medicine, Veterinaerplatz 1, A-1210 Vienna, Austria*
[4]*Department of Laparoscopic Surgery, "Jesús Usón" Minimally Invasive Surgery Centre, Ctra N-521, 10071 Cáceres, Spain*

Abstract: Our objectives were to standarize the procedure needed to reproduce a similar surgical scene which a pediatric surgeon would face on repairing a Bochdalek hernia in newborns and to define the optimal time period for hernia development that achieve a realistic surgical scenario with minimimal animal suffering. Twenty New Zealand white rabbits weighing 3–3.5 kg were divided into four groups depending on the time frame since hernia creation to thoracoscopic repair: 48 h, 72 h, 96 h and 30 days. Bochdalek trigono was identified and procedures for hernia creation and thoracoscopic repair were standarized. Blood was collected for hematology (red blood cells, white blood cells, platelets, hemoglobin and hematocrit), biochemistry (blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and creatine kinase) and gas analysis (arterial blood pH, partial pressure of oxygen, partial pressure of carbón dioxide, oxygen saturation and bicarbonate) at baseline and before the surgial repairment. Glucocorticoid metabolites concentration in faeces was measured. Thoracoscopy video recordings were evaluated by six pediatric surgeons and rated from 0 to 10 according to similarities with congenital diaphragmatic hernia in newborn and with its thoracoscopic approach. Statistical methods included the analysis of variance, and comparisons between groups were followed by a post-hoc Tukey's test. Fourty -eight h showed to be the optimal time frame to obtain a diaphragmatic hernia similar to newborn scenario from a surgical point of view with minimal stress for the animals.
Key words: animal stress, congenital diaphragmatic hernia, rabbit model, thoracoscopy

Introduction

The development of specific surgical training models that mimic clinical pathologies that can be treated by minimally invasive surgery can enhance advanced laparoscopic skills and facilitate the development of surgical skills needed for rare presentation diseases. Specifically, thoracoscopy is a strong challenge for the skills and competence of the pediatric surgeon. Moreover, in most paediatric surgical departments, the number of patients of each disease that can require a thoracoscopic approach is limited: as an example, the congenital diaphragmatic

Table 1. Questionnaire and scores for DH-model assessment by surgeons

	Score
DH-model hernia had adequate similarities to newborn CDH	4.30 ± 0.67
Thoracoscopic repair difficulty in DH-model was similar to real scenario	4.10 ± 0.31
DH-model could improve thoracoscopic skills to resolve real CDH	4.70 ± 0.48
DH-model is useful into a training program in pediatric thoracoscopy surgery	4.50 ± 0.52
Need to use animal model for pediatric MIS training	5.00 ± 0.00

All items scored on a Likert- type scale (range 1–5 points). Values represent mean ± standard deviation.

hernia (CDH) with an approximate incidence of 1 in every 10,000 birth [2, 6]. The use of rabbits as training model for different endosurgical pediatric procedures has been well described [5]. However, despite rabbit popularity as a model to study CDH pathogenesis [8, 11], a realistic and easily reproducible diaphragmatic hernia (DH) model specific for advanced thoracoscopic training is lacking. To reproduce a surgical scene similar to which a pediatric surgeon would face on repairing a CDH, we need to determine the optimal time needed for hernia development and the size and location of diaphramatic defect to achieve an adequate presence of abdominal organs in thorax. Animal models must be designed following the principles of utility, availability and ethic. Therefore, our objectives were to assess DH-model usefulness, to standarize the procedure needed to cause a Bochdalek hernia similar to newborn-CDH and its thoracoscopic repair, and to establish the optimal time period for hernia development that achieve a realistic surgical scenario with minimimal animal suffering, in this order. In this paper, we describe how we mastered these procedures.

Materials and Methods

This study was performed in the University of Extremadura, Faculty of Veterinary, Animal Surgery Unit with permission from Laboratory Animals Ethical Committee of the University of Extremadura. Animal care complied with the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, USA and European Union's Directive 86/609 CEE.

Preliminary cadaveric study

Prior to *in vivo* procedures, rabbit cadavers were used to identify Bochdalek's triangle, and to determine the most accurate place to create the diaghramatic defect and the adequate trocars location and animal position.

Preliminary study to establish model usefulness

Diaphragmatic hernia was reproduced in three rabbits following the procedure described below. Six pediatric surgeons divided in two person teams performed the thoracoscopic repair after 72 h. Each surgeon also visualized video recordings corresponding to the remaining surgeries. All surgeons completed a questionnaire assessing the appropriateness of the model as a training tool (Table 1). Scoring for each question was determined using a five-point Likert-type scale ranging from 1 (strongly disagree) to 5 (strongly agree).

Surgical procedures

Diaphragmatic hernia induction: Twenty New Zealand white rabbits weighing 3–3.5 kg were divided in four groups each including four rabbits depending on the time elapsed since hernia creation to thoracoscopic repair: 48 h, 72 h, 96 h and 30 days. Four unoperated rabbits were used to control for the possible responses due to handling and housing conditions. Rabbits were premedicated with subcutaneous buprenorfine (0.050 mg/kg) and meloxicam (0.20 mg/kg). Anesthesia was induced with dexmedetomidina 0.015 mg/kg and ketamina (8 mg/kg) through an intravenous catheter placed in the marginal ear vein and maintained with propofol (1–2 mg/kg). A midline mini-laparotomy (4 cms) was performed. To create diaphragmatic defect, an incisión (3 cms) was made in dorsocostal area of the left diaphragm, at the small triangle formed between the *pars lumbalis* and *pars costalis* of the diaphragmatic musculature, and the *retractor costae* muscle. As a result, an oval-shaped defect was originated (Fig. 1). A chest tube was then placed through the defect and externalized through laparotomy, with a purse-string stitch around. Before anesthesia recovery pneumothorax was aspirated and stitch tied down after

Fig. 1. Recreation of diaphragmatic hernia procedure. Location of the diaphragmatic defect for diaphragmatic hernia development.

tube removal to ensure closure of the wound.

Thoracoscopic repair: Animals were premedicated with atropina (0.05 mg/kg) and midazolam (0.25 mg/kg) intravenous. Anesthesia was induced with propofol (10 mg/kg) intravenous. Animals were intubated under endoscopic guidance and anesthesia was maintained using mechanical ventilation with 1.5% isoflurane and fentanilo (0.01 mg/kg each 30 min). Rabbits were placed in the Trendelemburg reverse and right lateral decubitus position, elevated 20º from the horizontal. The 5-mm port for the optic was positioned in the fourth left intercostal space, under scapula caudal border. The left upper-limb was stretched toward the head and secured with adhesive strips to free as much chest surface as posible. Two 3.5-mm ports for the instruments were placed in the sixth left intercostal space, one 1.5 cm from the spine and the other 2 cm from the sternum. The left chest was insufflated with carbon dioxide at 2–4 mm Hg. During thoracoscopy, herniated abdominal organs were reduced within the abdomen and diaphagmatic defect repaired employing the intracorporeal suture technique with interrupted 5/0 glyconate suture and a laparoscopic needle. After the procedure the animals were sacrificed with a lethal dose of thiopental through the marginal ear vein.

Studies to determine optimal time frame

Clinical parameters: Pain and dyspnea were evaluated in all operated groups by two experts in laboratory animals using a visual analogic scale from 0–10, and compared with unoperated control group.

Analysis: Venous and arterial blood samples were obtained inmediatly before hernia induction and finishing survival time, before thoracoscopic repair. Baseline and end average values for each group were compared. The following hematological parameters were measured in venous blood from marginal ear vein (anticoagulant, EDTA-K3) using a semiautomatic hematology analyzer (Sysmex F-800, Sysmex Co, Hyogo, Japan): red blood cells, white blood cells, platelets, hemoglobin and hematocrit. In addition, separated plasma was subjected to blood chemical examination using a clinical chemistry analyzer (Saturno 100, VetCrony Instruments, Roma, Italy). Blood urea nitrogen, creatinine, alanine aminotransferase, lactate dehydrogenase and creatine kinase values were determined. Samples from central artery of the ear were subjected to arterial blood gas analysis: arterial blood pH, partial pressure of oxygen, partial pressure of carbon dioxide, oxygen saturation and bicarbonate [anticoagulant, heparin sodium; measuring instrument, blood gas analyzer Radiometer Copenhage NPT7 BG-OX, Radiometer, Copenhage, Denmark].

To determine acute stress response, glucocorticoid metabolites concentration (GMC) in faeces was measured in 48, 72 and 96 h groups. Rabbit pellets were collected daily (once a day) in the morning during the whole procedure, since a week before the first surgery (baseline) to the sacrifice (end). Immediately after collection, the samples were frozen at –60ºC and sent to Viena for its analysis with an established and validated technique [7, 9].

Surgeon evaluation: Rabbits were operated by well trained and experienced in thoracoscopy surgeons. Thoracoscopy video recordings were evaluate by six pediatric surgeons from diferent centers. Each evaluator rated model from 0 to 10 according to similarities of the surgical procedure with the thoracoscopic approach of a neonatal CDH. They were unaware of the group to which each video belonged.

Statistical analysis

All quantitative data are expressed as means ± standard deviation. Analized parameters changes over line-base were compared using repeated measures analysis

of variance (ANOVA), and comparisons between groups were analyzed by ANOVA followed by a post-hoc Tukey's multiple comparison test. Statistical analysis was performed using the SPSS 15.0 statistical software package (Chicago, IL). The significance level was set at $P<0.05$.

Results

Preliminay studies in cadaver determined correctly the adequated diafragmatic incision and ports location so that live animals number was minimized. Within the model pre-assessment, most values tend to be excellents with a mean score of 4.4 (Table 1). All the pediatric surgeons mostly agree with the similarity and usefulness of CD-model, and with the need to use animals in pediatric training.

Diaphragmatic hernia appeared in all animals with abundance of intestinal loops and stomach protruding into thoracic cavity (Fig. 2). Ports location was correct and organs were relocated to peritoneal cavity by pushing with two endoscopic graspers. Adhesions were just observed in the 30-days group. Diaphragmatic repair was achieved successfully in all animals using a thoracoscopic approach. The main complications of the procedure were hemorrhage, the fragile tissue and difficulty to replace organs back into the abdominal cavity using a minimally invasive technique. No anesthetic complications were seen and the average duration of procedures was 118 min.

Table 2 summarizes the values obtained for all studied parameters in the four groups. At the moment of the treatment, dyspnea and pain were significantly higher in all operated groups than in the control group, but only 30 days group showed significant difference with the rest of treated groups. The baseline values of analized parameters in blood and faeces did not show significant differences between the groups, so they were averaged to yield a single control value for each data. Plasma levels of alanine aminotransferase, lactate dehydrogenase and creatine kinase exhibited a significant increase from baseline values in all groups, and it was significant higher in 72-h group. Bicarbonate concentration increased significantly just in 30 days group. The rest of the blood parameters analyzed did not show significant changes from baseline values in any group. A clear pattern of increasing GMC was observed along the time. GMC in operated groups showed significant differences
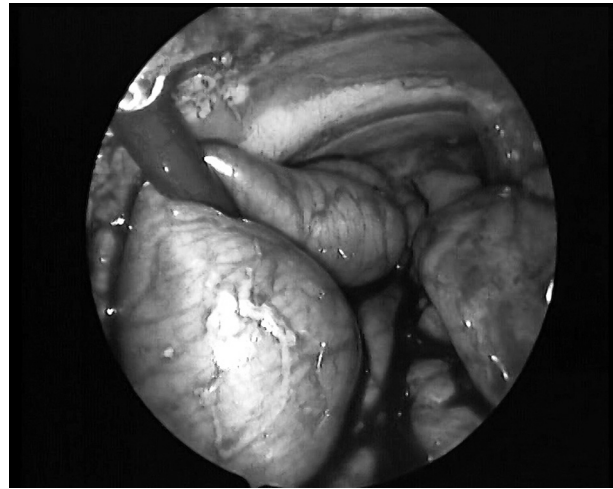

Fig. 2. Thoracoscopic view of a diaphpragmatic hernia in the rabbit model after 30 days.

with baseline values, but post-hoc comparison did not find significant differences between them.

Pediatric surgeons assessment was positive for most groups. 30-days group was rated significantly lower than the other three groups (5.46 ± 2.16, $P<0.05$). Although 72-h group obtained the highest score (9.04 ± 1.68) there was no significant difference between this group and 48-h (8.29 ± 1.53) and 96-h (8.46 ± 2.16) groups scores ($P>0.23$).

Discussion

Animal models are the most realistic, non-patient enviroment for MIS training. Artificial or virtual models teach basic techniques adequately, but only an animal model conveys specific intraoperative conditions mandatory for advanced endosurgery. Complicated procedures, ethical issues and costs associated with maintaining can hinder its use in training programs, so these parameters must be optimized because live models are an essential step before attempting minimally invasive techniques on the patient. The CDH-model has been used to measure different aspects of this pathology, however we have not found a specific DH-model for thoracoscopic training reported in the literature. Once model usefulness was shown, our main objective was to define objectively the most suitable time frame to obtain a diaphragmatic hernia similar to newborn scenario from a surgical point of view minimizing animal suffering. Although diaphragmatic hernia can be present since fetal stage to several

Table 2. Mean ± SD values for clinical parameters studied to determine optimal time frame for hernia recreation

	Baseline	48 h	72 h	96 h	30 days
Hematological data					
Hematocrit (%)	45.98	46.48	42.67	42.43	43.18
Hematoglobin (g/dl)	12.20 ± 0.61	12.15 ± 2.13	11.75 ± 0.87	11.07 ± 2.42	11.80 ± 2.75
Red blood cell (M/μl)	6.34 ± 0.32	6.70 ± 0.59	6.13 ± 1.52	6.14 ± 1.60	6.19 ± 0.96
White blood cell (K/μl)	6.03 ± 2.50	6.67 ± 4.18	5.97 ± 3.75	6.60 ± 2.06	5.83 ± 0.43
Absolute lymphocyte (K/μl)	4.48 ± 1.77	4.53 ± 2.26	4.16 ± 2.68	4.32 ± 1.01	4.09 ± 1.52
Absolute neutrophil (K/μl)	1.28 ± 0.77	1.31 ± 0.94	1.44 ± 0.97	1.07 ± 0.38	1.03 ± 0.30
Absolute eosinophilic(K/μl)	0.36 ± 0.27	0.32 ± 0.02	0.31 ± 0.25	0.38 ± 0.03	0.31 ± 0.09
Platelet count (K/μl)	275.00 ± 212.00	273.00 ± 131.00	279.00 ± 144.00	271.00 ± 195.00	269.00 ± 198.00
Blood Biochemistry data					
Alanine transaminase (U/l)	56.50 ± 13.37	70.25_a ± 22.27*	140.24_b ± 88.33*	75.12_a ± 25.24*	79.72_a ± 64.48*
Lactate Dehydrogenase (U/l)	117.50 ± 10.60	252.50_a ± 71.41*	568.45_b ± 506.99*	288.67_a ± 37.47*	298.16_a ± 60.15*
Creatine kinase (U/l)	128.5 ± 2.12	$1,045.50_a$ ± 381.37*	$1,864.65_b$ ± 702.15*	941.86_a ± 339.02*	$1,128.95_a$ ± 505.43*
Urea nitrogen (mmol/l)	7.92 ± 0.76	8.12 ± 0.48	8.75 ± 0.78	7.75 ± 0.18	8.02 ± 0.36
Creatinine (μmol/l)	76.91 ± 2.30	83.09 ± 5.13	85.75 ± 4.22	80.44 ± 5.28	78.67 ± 4.24
Blood Gas Analysis data					
Arterial blood pH	7.40 ± 0.01	7.25 ± 0.27	7.26 ± 0.29	7.44 ± 0.08	7.40 ± 0.09
PaO_2 (mmHg)	127.75 ± 57.10	130.75 ± 60.12	126.50 ± 43.56	129.75 ± 60.12	123.25 ± 33.31
$PaCO_2$ (mmHg)	35.75 ± 7.09	34.50 ± 8.43	35.45 ± 7.14	35.75 ± 6.40	40.75 ± 4.11
SaO_2 (%)	97.08 ± 2.13	96.80 ± 2.14	96.97 ± 4.20	93.35 ± 6.25	91.98 ± 6.45
HCO_3 (mEq/l)	20.65 ± 0.13	18.77_a ± 8.48	20.14_a ± 7.74	22.48_a ± 7.01	51.30_b ± 33.62*
Clinical Study					
Pain (VAS 1-10)	0.25 ± 0.05	1.57_a ± 0.61*	1.20_a ± 0.08*	1.17_a ± 0.30*	2.60_b ± 0.35*
Disnea (VAS 1-10)	0.25 ± 0.20	1.52_a ± 0.25*	1.42_a ± 0.14*	1.62_a ± 0.23*	4.32_b ± 1.93*
Glucocorticoid metabolites					
Fecal GMC (ng/g)	248.33 ± 29.93	918.00_a ± 304.33*	$1,036.63_a$ ± 607.03*	$1,177.50_a$ ± 414.60*	-

(*) Significant effect of time ($P<0.05$). (a, b) In the same file, values with different letter differ significantly. Tukey Test ($P<0.05$) PaO_2, partial pressure of oxygen; $PaCO_2$, partial pressure of carbon dioxide; SaO_2, oxygen saturation; HCO_3, bicarbonate; VAS, visual analogic scale.

days after birth, our study has showed that an extended period of time is not necessary to reproduce this pathology for surgery purposes. After the overall comparison of all studied parameters, 30-days group was discarded because the high level of stress for the animals added to a low realism. Surgical scenarios obtained after 48, 72 and 96 h were similar for evaluators rating, but animals showed more significant biochemical changes at 72 h and the highest levels of GMC at 96 h. Therefore, the model can be reproduced 48 h before its thoracoscopic repair with high realism and minimal stress for the animals. This fact is an advantage because improve its disponibility as the animals can be prepared only two days before the programmed training, saving problems derived from long housing (handling, costs, space, etc).

An area similar to Bochdalek's triangle was located in the dorsocostal area between the eleventh and twelfth ribs (Fig. 1). A diafragmatic incision 3 centimeters long was quite enough to cause herniation. In all animals, this wound became greater over the 72-h period due to progressive peritoneal organs herniation as seen in Fig. 2.

To reproduce diaphragmatic hernia, we agree with some authors that the surgical creation of a diaphragmatic defect, instead of nifrofen-induced models, is the best from a surgical therapeutic point of view [10]. Our model replicates exactly the Bochdalek's hernia classical picture and laparoscopic instrument size, ports placement and rabbit position are similar to those describe for newborns thoracoscopy [1, 3, 4]. With the thoracoscopic videos obtained, surgeons emphasize the similitude of the vision to a newborn CDH.

In conclusion, we have developed a high-reality and easily reproducible in the short term DH-model, for the refinement of thoracoscopic skills, complying with ethical and useful principles.

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References

1. Arca, M.J., Barnhart, D.C., Lelli, J.L., Greenfeld, J., Harmon, C.M., Hirschl, R.B., and Teitelbaum, D.H. 2003. Early experience with minimally invasive repair of congenital diaphragmatic hernias: Results and lessons learned. *J. Pediatr. Surg*. 38: 1563–1568. [Medline] [CrossRef]

2. Cho, S.D., Krishnaswami, S., Mckee, J.C., Zallen, G., Silen, M.L., and Bliss, D.W. 2009. Analysis of 29 consecutive thoracoscopic repairs of congenital diaphragmatic hernia in neonates compared to historical controls. *J. Pediatr. Surg*. 44: 80–86. [Medline] [CrossRef]

3. Gomes Ferreira, C., Reinberg, O., Becmeur, F., Allal, H., De Lagausie, P., Lardy, H., Philippe, P., López, M., Varlet, F., Podevin, G., Schleef, J., and Schlobach, M. 2009. Neonatal minimally invasive surgery for congenital diaphragmatic hernias: a multicenter study using thoracoscopy or laparoscopy. *Surg. Endosc*. 23: 1650–1659. [Medline] [CrossRef]

4. Keijzer, R., and Puri, P. 2010. Congenital diaphragmatic hernia. *Semin. Pediatr. Surg*. 19: 180–185. [Medline] [CrossRef]

5. Kirlum, H.J., Heinrich, M., and Till, H. 2005. Rabbit model serves as a valuable operative experience and helps to establish new techniques for abdominal and thoracic endosurgery. *Pediatr. Surg. Int*. 21: 91–93. [Medline] [CrossRef]

6. Nguyen, T.L., and Le, A.D. 2006. Thoracoscopic repair for congenital diaphragmatic hernia: lessons from 45 cases. *J. Pediatr. Surg*. 41: 1713–1715. [Medline] [CrossRef]

7. Palme, R. 2005. Measuring fecal steroids: guidelines for practical application. *Ann. NY Acad. Sci*. 1046: 75–80. [Medline] [CrossRef]

8. Roubliova, X.I., Deprest, J.A., Biard, J.M., Ophalvens, L., Gallot, D., Jani, J.C., Van de Ven, C.P., Tibboel, D., and Verbeken, E.K. 2010. Morphologic changes and methodological issues in the rabbit experimental model for diaphragmatic hernia. *Histol. Histopathol*. 25: 1105–1116. [Medline]

9. Touma, C., Palme, R., and Sachser, N. 2004. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. *Horm. Behav*. 45: 10–22. [Medline] [CrossRef]

10. van Loenhout, R.B., Tibboel, D., Post, M., and Keijzer, R. 2009. Congenital diaphragmatic hernia: comparison of animal models and relevance to the human situation. *Neonatology* 96: 137–149. [Medline] [CrossRef]

11. Vuckovic, A., Roublinova, X.I., Votino, C., Naeije, R., and Jani, J.C. 2012. Signaling molecules in the fetal rabbit model for congenital diaphragmatic hernia. *Pediatr. Pulmonol*. 47: 1088–1096. [Medline] [CrossRef]