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Optimal Access to the Rat Heart by Transverse Bilateral Thoracotomy with Double Ligature of the Internal Thoracic Arteries

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Rats are used widely in ischemia–reperfusion and other heart experiments, but current protocols for thoracotomy have serious shortcomings. Median sternotomy causes bleeding from sternum itself and the internal thoracic arteries, whereas left thoracotomy requires exteriorization of the heart and its reintroduction after completion of the procedure and often is complicated by traction or torsion of the cardiopulmonary bundle and atelectasis in the left lung. Here we describe a new, terminal procedure that minimizes blood loss and allows wide access to the heart without disturbing its anatomic position. Transverse thoracotomy, preferably through the fifth intercostal space, is performed after double ligature of both internal thoracic arteries 1 intercostal space above and 1 below the incision. Blood loss is minimal and occurs mainly with dissection of deep pectoral muscles and intercostal muscles, and the animal is better ventilated than with conventional protocols. We believe that our procedure is superior to existing techniques because it minimizes blood loss during intervention, does not disturb the anatomic position of the heart, and allows wide access to the organ for experimental manipulation.

Rats are used widely in terminal ischemia–reperfusion and other heart experiments because of their wide availability and researchers' familiarity with the anatomy of this species.[8] Median sternotomy[5] is one of several current approaches for providing experimental access to the rat heart, but bleeding from the sternum and internal thoracic arteries limits the applicability of this method. Left thoracotomy[7,9] is performed frequently but requires exteriorization of the heart and its reintroduction after completion of the procedure. Further, left thoracotomy usually is complicated by traction and torsion of the cardiopulmonary bundle, potentially leading to atelectasis in the left lung and perturbations in pulmonary gas exchange. In addition, both median sternotomy and left thoracotomy are associated with marked mortality, ranging from 13% to 35%.[3] A third option involves an abdominal approach through an upper median laparotomy to the xyphoid, which is retracted. A T-shaped incision then is made in the diaphragm, and with the help of a retractor, the thoracic cavity is accessed.[4] The subsequent visibility of the operating field and freedom of movement for the surgeon's hands is less than optimal with this protocol. Although bilateral transverse thoracotomy might seem to be the best approach, transecting the sternum inevitably would damage the internal thoracic arteries, causing massive hemorrhage and compromising the experiment.

Here we describe a new method of opening the rat thorax and exposing the heart without its exteriorization or significant blood loss. Our technique involves bilateral thoracotomy with transverse sternotomy[2] but includes the essential addition of prior ligature of both internal thoracic arteries 1 intercostal space cranial and 1 caudal of the site of the transverse sternotomy. The double ligature of the internal thoracic arteries thus prevents bleeding from these arteries, such that our technique affords maximal access to the rat heart without disturbing its normal anatomic position.

Materials and Methods

All the animal experiments described herein comply with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.[1] The experimental protocol was reviewed and approved by the Committee on Ethics of the County Hospital Timisoara. We used male Sprague–Dawley rats weighing 250 to 300 g that were housed under conditions of controlled temperature (22 °C), a 12:12-h light:dark cycle (lights on, 0700 to 1900), and ad libitum access to standard rat chow and water. The animals were obtained from a nearby facility (Pius Branzeu Center for Laparoscopic Surgery and Microsurgery, Timisoara, Romania) that certified them to be free of *Mycoplasma* spp., adventitious viruses, respiratory and enteric bacteria, and ecto- and endoparasites. The animals were anesthetized with ketamine (60 mg/kg IP) and xylazine (6 mg/kg), placed in dorsal recumbency, and then intubated and ventilated throughout the procedure with 100% humidified oxygen at 90 strokes/min and 10 ml/kg tidal volume.[6] An extra dose of anesthetic cocktail (20% of the initial dose) could be administered as needed at 1.5 h after the first dose to prolong anesthesia (a typical ischemia–reperfusion experiment lasts about 3.5 h). For invasive arterial pressure and blood gas measurements, the carotid artery was cannulated.

The surgical procedure is shown schematically in Figure 1 A and is presented in detail below. The animal is anesthetized and mechanically ventilated as described above and then the carotid artery is cannulated. With the animal in dorsal recumbency, the skin on the ventral thorax is shaved and disinfected and then is incised transversely at the level of fifth intercostal space, extending about 2 cm on either side of the sternum. Carefully dissect the underlying pectoral muscles to expose the ribs and intercostal muscles, so that intercostal spaces 4, 5,

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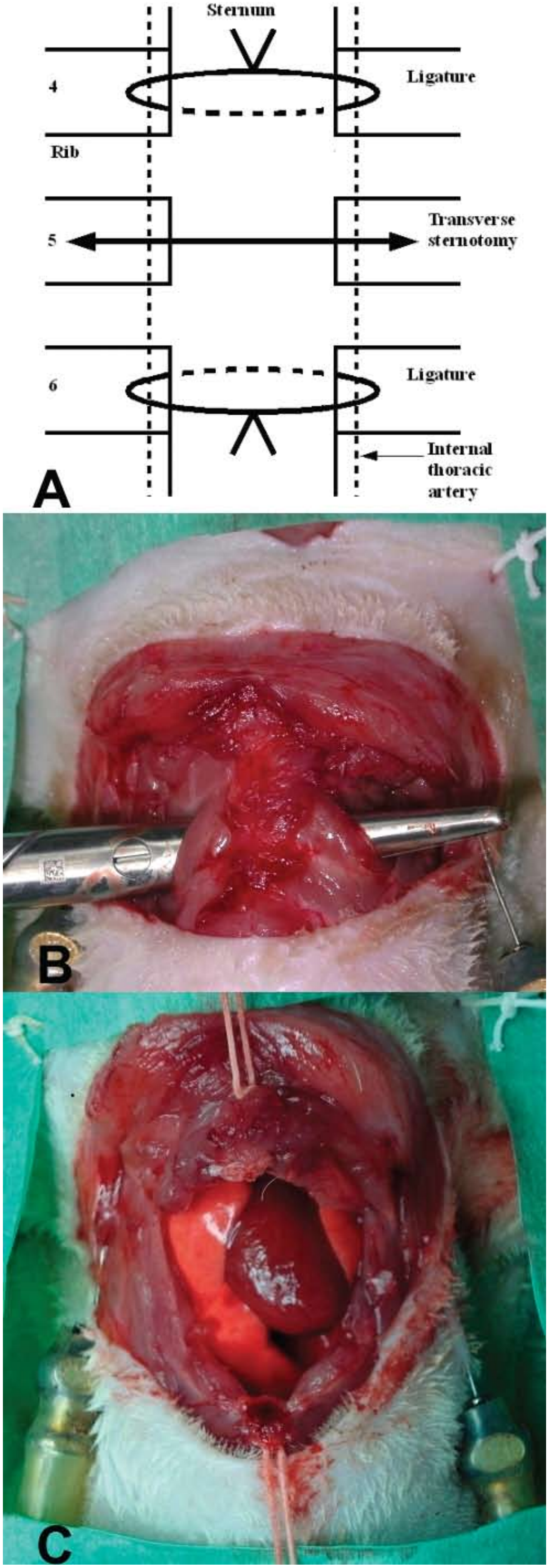

Figure 1. Stages of the Procedure A) Overall scheme of our technique. The numerals 4, 5, and 6 indicate the respective intercostal spaces. (B) Surgical scissors pass underneath the sternum and through the fifth intercostal space bilaterally, lifting up the rib cage. (C) The thorax is opened by pulling sideways on the surgical strings, thus providing convenient access to the heart.

and 6 are accessible. Incise the intercostal muscles of the fifth intercostal space on both sides of the sternum, starting about 3 mm from the edge of the sternum (to avoid injury to the internal thoracic arteries, which pass behind the sternocostal joints) and continuing sideways for about 1 to 1.5 cm. Gently insert blunt-ended surgical scissors through the fifth intercostal space on one side, passing under the sternum and emerging through the fifth intercostal space on the other side of the sternum while slightly raising the rib cage (Figure 1 B). Raising the rib cage is necessary to prevent injury to major vessels underneath in the next step. Using 3-0 Vicryl surgical string, ligate the internal thoracic arteries at the level of the fourth intercostal space. To do so, puncture the intercostal muscles about 4 mm from the side of the sternum, go under the sternum, and emerge on the other side of the bone. Tighten the knot as much as possible (Figure 1 A, C) to compress the arteries against the sternum. Repeat the ligature of the internal thoracic arteries for the sixth intercostal space. The internal thoracic arteries are thus ligated in 2 places (the fourth and sixth intercostal spaces). Transect the sternum at the level of the fifth intercostal space. If both thoracic arteries have been ligated completely 1 intercostal space cranial and caudal to the site of transection, no hemorrhage should occur. The bleeding from the 2 ends of the sternum is negligible and can be controlled easily. Pull the strings of the 2 ligatures apart or use a separator to keep the 2 parts of the rib cage apart and get generous access to heart (Figure 1 C). The procedure is not technically demanding, and a researcher accustomed to surgical procedures on rats needs about 6 to 8 min from incision of the skin until exposure of the heart. At the end of the experiment, rats were euthanized by injection of 1 ml 1 M KCl into the carotid artery, and the hearts were harvested for further examination.

We also evaluated comparatively the blood oxygenation in animals that underwent our procedure, left thoracotomy (in which atelectasis in the left lung may lead to alterations in the gas exchange), and intubation and anesthesia only. A total of 30 rats was allocated into 3 groups of 10 animals each; all 30 animals were anesthetized, intubated, and ventilated under similar conditions (100% humidified oxygen; 90 strokes/min; tidal volume, 10 ml/kg) and underwent carotid artery cannulation. One group served as controls (anesthetized, intubated, and ventilated only); another underwent standard left thoracotomy; and the remaining group underwent bilateral transverse thoracotomy according to our procedure. After surgery was completed, animals were allowed a 20-min recovery period before the partial pressure of the oxygen in the arterial blood was measured (AVL Blood Gas Analyzer, AVL Scientific Corporation, Roswell, GA). Five independent experiments were performed, each one involving two rats from each group and the data is displayed as mean ± SEM.

Results

This procedure was performed on 20 rats used for ischemia–reperfusion experiments. At the end of the experiments, the hearts were harvested for further evaluation. Of the 20 rats, 2 died from hemorrhage due to incorrect ligature of the thoracic arteries; both of these animals died during the early stages of developing the procedure.

We measured also the arterial oxygen partial pressure in the three groups of rats 20 min after completion of surgery in order to compare the blood oxygenation during left thoracotomy and our procedure. The results show that the partial pressure of the oxygen in the arterial blood is significantly higher($P < 0.05$, Student *t* test) in the animals that underwent bilateral transverse thoracotomy (300,1 ± 7,77* mmHg) than in those that underwent left thoracotomy (128,9 ± 7,2* mmHg), approaching the levels seen in animals intubated and ventilated only (439,5 ± 12,2). These findings confirm that our procedure better preserves gas-exchange capacity of the lungs than does the conventional left thoracotomy.

Discussion

The current approaches for gaining access to the rat heart for experimental manipulation (such as ischemia–reperfusion) have several shortcomings. Median sternotomy is accompanied frequently by hemorrhage due to damage of the internal thoracic arteries and from the resected sternum itself. With left thoracotomy heart must be exteriorized and replaced manually, a process that leads to traction and torsion of the large vessels; the left lung typically is repositioned incorrectly, causing atelectasis and loss of gas exchange surface area. A third method, the abdominal approach is less traumatic but does not offer good visualization of the operating field, and operator access to the heart is somewhat limited by the thorax. Our method is technically simple and gives wide access to heart without disturbing its anatomic position, in a procedure that prevents clinically significant blood loss that otherwise might result from the opening of the chest. Double ligature of the internal thoracic arteries, cranial and caudal of the incision site, makes transverse sternotomy without bleeding possible. This double ligature is the crucial step in our procedure: a loose knot causes massive and fatal hemorrhage. Therefore, we stress that the ligature knot should be as tight as possible. Applying a retractor or simply pulling the strings sideways and sticking them to the table with duct tape keeps the thorax open, allowing access to heart in its anatomic position. Our protocol is intended to be a terminal procedure; we euthanized our rats and harvested their hearts for further examination. We believe that our transverse sternotomy technique offers optimal access to the rat heart, minimizes the blood loss during the intervention, and is not difficult to perform.

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