An Alternative Technique for Heart Lesion in the Rat: A Step to Fetal Heart Implantation for Cardiac Tissue Repair Running Title: A New Technique of Heart Lesion in Rat
An Alternative Technique for Heart Lesion in the Rat: A Step to Fetal Heart Implantation for Cardiac Tissue Repair Running Title: A New Technique of Heart Lesion in Rat

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Abstract

Existing experimental models of cardiac lesion do not allow precise reproducible dimensions of injury with complete safeness of surrounding tissues and cannot answer the tremendous question for heart cell therapy: are the implanted cells unable to influence the heart healing process or do the conditions in the injured heart prevent their complete development and integration?

60 rats were used for elaborating an alternative technique of cardiac lesion. The anterior apical area of the heart was cauterized with a «Cautery high temperature fine tip» to create an injury of controllable, precise and reproducible dimensions (8×8 mm² surface, 1 mm depth).

To evaluate functional and morphological characteristics of the lesion, electrocardiography, pulse oximetry, echocardiography and optic microscopy were performed at different times (from day 0 to 230) after the operation. After technical adjustment a 100% survival of the last 15 operated animals was obtained. A sub epicardium “infarction” was documented: ECG mirror ST-modifications in 2 leads, stable ejection fraction significant decrease, necrotic alterations and fibrosis of the lesion area, with surrounding myocardium preserved.

The survival and the injury evolution suggest that the proposed technique could be used for studies concerning cardiac tissue repair.

Keywords: Electrocardiography; Heart disease; Cardiac surgery; Cardiac lesion imaging; Ventricles aneurysm

Introduction

Nowadays, because of the increasing of the prevalence and cost of cardiac insufficiency [1-6] and the difficulties to do more than slowing its natural evolution, the interest for cell therapy is rising. Many studies were provided in this direction, but it was reported that the injured heart remodeling was not always favorably influenced by stem cells or cultured cardiomyocytes implantation [7-11]. Such trials were performed either in infarct patients or in animals after coronary artery ligation. So the question remains: are the cells incompetent, unable to influence the heart healing process or do the conditions in the injured heart prevent the implant complete development and integration?

Conversely to stem cells cultures, ectopic implantation of fetal hearts in vivo has led to complete development of an adult-like functional organ [12,13]. But they have neither been implanted at the heart site nor tested on cardiac defect repairing yet.

So it seemed to be important to use an experimental model of cardiac lesion which could offer optimal conditions for the implant development.

Several experimental techniques of heart injury were elaborated for the study of cardiac infarction and its remodeling. Heart main artery obstruction was obtained by physical means: cold or heat injury, radiofrequencies [14,15], chemical methods: injections or application of chemicals on the myocardium [16-18] or surgical ones as vessel ligation [19]. Presently the most usual experimental model of creating a cardiac lesion remains the temporary or permanent ligation of a coronary artery [9-12,19-22] or other type of heart vessels occlusion [23]. The advantage of this model is its proximity with the injury caused by myocardial infarction in clinical practice. The disadvantages include the variability of the lesion extent due to the difficulty to obtain a precise location of the coronary artery (or one of its branches) ligation/occlusion and the variability of collateral vessel types [20-23]. Moreover, the myocardial ischemia around the infarction zone seems to be unfavorable for cells or tissue transplants development that explains the controversial results of such a procedure [7-11,20,23].

The purpose of this study is to provide an alternative technique of cardiac injury by creating a size and depth controllable myocardial lesion.

The benefits would be the reproducibility of the lesion, but also the opportunity to study the most favorable-time to practice cardiomyoblasts (fetal heart) grafting.

Methods

Surgical procedures

The experiments were performed on 60 rats. They were displayed
in 4 series: 1/ for testing the feasibility of the technique – 12 animals (Wistar and Fischer, males and females); 2/ for improvement and standardization of the technique, determination and correction of its complications – 18 animals (Wistar: 14 males, body weight (BW) 387-434 g and 4 females, BW 240-280 g); 3/ for testing the reliability of the technique – 19 animals (only Wistar males, aged 9.1+/−1.7 months, BW 427+/−28 g, among which 4 died during the operation from narcosis or ventilation problems, and were excluded); 4/ control series including 11 intact animals (only Wistar, males, same age because siblings, and BW 414+/−33 g).

Anesthesia and analgesia were provided as follows: induction by Isoflurane (4.5% for 1 min/100g BW), main procedure by 2 intraperitoneal injections of Natrium pentobarbitalum (Nembutal® - Ceva Santé animal- Brussels Belgium: 0.1 ml/100 g BW of a solution to 0.075 mg/dl) and of Buprenorphine hydrochloride (Temgesic® - Laboratoire Schering-Plough - Courbevoie France: 0.2 ml of a 0.05% solution). A subcutaneous injection of 0.2 ml (1%) solution of Atropine (atropine sulfate – Lavoisier- Paris-France) was performed at least 5 minutes before intubation to avoid the occurrence of a vagal shock. The tracheal intubation with a 14 G catheter was performed with the help of a laryngoscope (Mac0 blade-Heine Germany). The anesthesia-ventilation machine (Intermed–Penton, Sigma Delta, UNO-Netherland-USA) was used during the surgical procedure at a rate of 60 breaths/minute with a tidal volume at 12 ml/kg and ventilation pressure of 0 to 20 milliBars.

After longitudinal sternotomy and hemostomy, the heart was exposed.

We used the «Cautery high temperature fine tip» (Bovie Medical Corporation-USA) to induce, by several drop-contacts without any pressure – less than 1 second each - of the tip (standard temperature of 1200°C) with the surface of the heart, a myocardial lesion at the level of the anterior apical zone of the heart (including mainly left ventricle but also parts of septum and right ventricle). This localization was chosen because it is easy for access, relatively safe, as far as the left ventricle thickness is no less than 2 mm at diastole, and because the vessels of this zone are terminal, preventing an unexpected extension of the damage through major coronary branches lesions. The surface and the depth of the lesion depended on the total time of the tip contact with the myocardium. It was important to leave the internal muscular layer intact (ensured by visual control) in order to avoid immediate or delayed perforation (Figure 1). The extension of the damage was controlled by histology performed immediately after operation and during the follow up (see below). The thoracic wall wound was then sutured layer by layer with classic separate stitches using Vicryl 2°° for the skin. Suture 6°° for the skin.

During the operation and the early - first hours - follow-up, the impact of the lesion on the heart function was monitored by a “Pulse Oximeter” (Contec Medical systems Co, Ltd PRC – RP China; CMS-508 model) fixed to the hind left leg of the animal. Electrocardiogram (ECG – leads I, II and III), respiratory rate (RR) and rectal temperature were registered by a «Mouse Monitor» (Indus Instruments USA - UNO-Netherlands). ECG and RR were obtained from 4 needle electrodes subcutaneously inserted in the standard left - right axillar and groin sites, the rat being in supine position.

Later, daily observation of the animals was realized up to 8 months after the operation; the animal body weight (BW) was measured at days 2, 5, 7, 14 and, after the initial BW recovering, once a month.

Post operation investigations

Statistics: The results are given as Mean +/- Standard Deviation (M+/−SD). Differences were tested by Student's test versus control series (td–ct standard).

Resultes were considered significant for p < or =0.05.

Besides, the statistic evaluation concerned not only the mean data of each series but also the differences between the results obtained at days 10 and 30 in the same animal (paired results).

Variability (V) and reproducibility (R) of the results were also calculated (V=SD / M %; R=100-V %).

Animal's management: Before, during and after the experimentation animals were managed according to the international rules of Bioethics and the experimental protocol was agreed by the local Ethnic Committee (N°508N). The nutrition conditions were standardized all through the experiment with the use of “food for breeding” (AO4, "Safe"; France) and of fresh water as drink.

Figure 1: Per-operative macro photography of the myocardial lesion at the apex zone of the heart (arrow).
Results

The global results of our experiment are presented in Table 1. Their details are noted in Table 2.

The preliminary 12 experiments of series 1 have allowed determine the conditions for successful operations: 1/ appropriate assisted ventilation, 2/ cautery by repeated short contacts (<1 s providing an alteration of about 1 mm²/shot) of the cautery tip with the heart surface till the lesion demanded dimensions were reached.

The survival of 2 animals encouraged to continue improving the operative technique.

The next series 2 was devoted to the development of a standard, reproducible method: a necrotic lesion of 0.8 mm diameter area was visually checked, its depth was modulated by the duration of the cautery tip application on the surface of the heart. The survival was 50%. When this lesion was transmural, that is when the inner muscular layer of the ventricle was involved, it caused an immediate, uncontrollable and lethal bleeding (2 cases) or a late hemorrhage (1 case at day 37). When the injury only affected from 25 to 75% of the myocardium thickness, it was compatible with survival (9 cases - see Table 2 and Figure 1). Proper lesion was obtained with repeated short applications of the tip on the heart surface during less than 1 second each for a total duration no more than 50 seconds. Besides, the deep myocardial layers must be left “intact” (confirmed by macro and microscopic investigations).

Significant changes – QRS slight enlargement (up to 25 millisecond), ST alterations were already observed at the time of the lesion forming. Within 30-50 minutes after the operation significant elevation of ST (40-60 millivolts) in 2 leads (Figure 2A). Within 30-45 minutes after the lesion, the ECG has shown significant 30+/−5 millivolts mirror ST-modification in leads I and III, as well as slight enlargement (up to 20 millisecond) of QRS complex (Figure 2B). In late delays, typical for infarcted heart modifications of ECG were noted: deep Q wave (60-80 millivolts) and elevated ST (40-60 millivolts) in 2 leads (Figure 2C).

Control echocardiography provided on 11 different intact animals, as well as repeated on the same animal, has shown satisfying results considering either liability or reproducibility (Figure 3A). So they were used for comparison with those of operated animal investigations.

The results of the echocardiography provided on 12 animals of series 3 at days 10+/−2 and 30+/−5 and on the 11 intact controls (once each animal) have confirmed the functional significance of the lesion. Ejection fraction (EF) was significantly reduced (average EF decrease: 37.4%) in comparison with intact control (p<0.001), especially at the first delays 1-2 weeks (Figure 3B). Later some improvement but not complete normalization of the function seemed to occur (average EF decrease:27.3%) the difference with control remaining significant (p<0.01) (Table 3a,3b).

In late delays (2 months and more) the EF remained decreased (determined as about 50-60%). It is to be noted that the variability of the experimental results, especially at delay 10+/−2 days, was higher than the variability of control series datas.

The results of the heart wall thickness evaluation at the lesion level comparatively to the intact area and comparatively to the value obtained in the control animals are more difficult for interpretation because in early delays the wall limits determination is biased by tissue edema of the lesion zone. After 30 days, a non-significant tendency to reduction of the heart wall thickness in the lesion area was noted (Table 3c). In 2 cases it corresponded to aneurysm formation visually and histologically confirmed (Table 2 and Figure 4).

The histological evolution of the lesions was the following (Figure 4):

1) At day 0, within the first hours after operation, there was an important tissue edema, signs of myocytes suffering (shrunk nuclei, disappearance of muscle striation, intra-cellular edema, coagulation necrosis), activation of capillary endothelium and an inflammatory reaction start (Figure 4 a–4e).

2) During the following days 2, 4, 7, 10 both degenerative and inflammatory reactions increased. A coagulum indicated the place of cauteration.

3) At day 14: mild fibrosis was already present, and developed later at days 21 and 28 (Figure 4f).
### Series 1

| Date   | Sex/strain | Age (month) | BW (g) | lesion dim. (mm) | Operation time (min) | Issue | Observ. time | Complications            | Histo N° | investig |
|--------|------------|-------------|--------|-----------------|----------------------|-------|--------------|---------------------------|----------|----------|
| 12.11.09 | F/F       | nm          | 203    | 2x1             | 65                   | †     | D1           | Thoracic bleeding         | 1241     |          |
| 19.11.09 | F/F       | nm          | 243    |                 | †                    |       |              | Technic problems          | 0        |          |
| 19.11.09 | F/F       | nm          | 206    |                 | †                    |       |              | Technic problems          | 0        |          |
| 19.11/09 | M/F       | ~12         | 296    |                 | †                    | D0    |              | Technic problems          | 0        |          |
| 03.12.09 | M/W       | nm          | 397    | nm              | 30                   | †     | D37          | Thoracic bleeding         | 1248     |          |
| 03.12.09 | M/W       | nm          | 403    | nm              | 60                   | †     | D0           | Technic problems          | 0        |          |
| 14.01.10 | M/W       | nm          | 360    | 2x2             | 10                   | Euth. | D42          | Thoracic bleeding         | 1257     | Echo D33|
| 21.01.10 | M/W       | nm          | 340    | deep            | 10-15                | †     | D1           | Perforation               | 1246     |          |
| 09.02.10 | M/F       | nm          | 350    | nm              | 40                   | †     | D1           | Perforation               | 1256     |          |
| 17.03.10 | M/F       | 12          | 388    | large           | nm                   | †     | D0           | CRA                       | 0        |          |
| 05.08.10 | M/F       | nm          | 339    | nm              | 15                   | †     | D0           | CRA                       | 1277     |          |
| 05.08.10 | M/F       | nm          | 344    | nm              | 15                   | †     | D0           | CRA                       | 0        |          |

### Series 2

| Date N° | Sex/strain | Age (Mo) | BW (g) | lesion dim. | Oper. time | Issue | Observ. time | Complications | Histo | Investigation |
|---------|------------|----------|--------|-------------|------------|-------|--------------|---------------|-------|---------------|
| 14.05.13 | M/W       | 12       | 438    | 3x5        | 38         | †     | D0 3h       | CRA           | 1379  | EGG           |
| 07.05.13 | M/W       | nm       | 392    | 5          | 60         | Euth. | 7.5 mo      | 0             |       |               |
| 30.04.13 | M/W       | 12       | 394    | 5          | 40         | †     | D0 3h       | CRA           | 1377  | EGG           |
| 02.04.13 | M/W       | 14       | 464    | nm          | 20         | †     | D0 35min    | CRA           | 1368  | ECG           |
| 26.03.13 | M/W       | 13       | 452    | 1cm²       | 20         | Euth. | 6 mo        | 0             |       |               |
| 22.01.13 | M/W       | 10       | 434    | 8          | 30         | †     | D0 3h20     | Thoracic bleeding | 1366  | ECG           |
| 18.12.12 | M/W       | 16       | 462    | 5          | 23         | Euth. | D21         | 0             |       |               |
| 11.12.12 | M/W       | 18       | 421    | nm          | 27         | Euth. | D7          | 0             |       |               |
| 04.12.12 | M/W       | nm >12   | 434    | >5         | 20         | †     | D0 1 h.     | anesth        | 0      |               |
| 06.11.12 | M/W       | nm >12   | 387    | >5         | 20         | †     | D0 1h30     | CRA           | 0      |               |
| 16.10.12 | F/W       | nm       | 234    | nm          | 13         | †     | D0 1h30     | CRA           | 0      |               |
| 09.10.12 | F/W       | nm       | 245    | nm          | 20         | Euth. | D35         | 0             |       | 1358          |
| 13.09.12 | M/W       | nm       | 440    | 5x5        | 30         | †     | D0 1h40     | CRA           | 1355  |               |
| 28.08.12 | M/F       | 16       | 403    | nm          | 25         | Euth. | D35         | 0             |       | 0             |
| 31.07.12 | M/F       | nm       | 420    | 3x5 deep    | ~20        | Euth. | D2          | 0             |       | 1352          |
| 05.11.13 | F/W       | 8        | 250    | Trans mural | 17         | †     | D0          | Perforation          | ECG     |               |
| 12.11.13 | F/W       | 8        | 250    | 10x10x2     | 25         | †     | D0 4h       | CRA           | 1402  | Echo          |
| 03.12.13 | M/W       | 5        | 364    | 8x8x2       | 35         | Euth. | 8 mo        | 0             | 1487  | Echo D 52, 8 mo |

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Series 3

| Date N° | Sex /strain | Sex (mo) | BW (g) | lesion dim. (mm) | Oper. time (min) | Issue | Observ. time | Complication | Histo | Inv. tigations | Remarks |
|---------|-------------|----------|--------|-----------------|------------------|-------|--------------|--------------|-------|----------------|---------|
| 0312.14| M/W         | 4        | 364    | 9x9            | 35               | Euth. | 7 mo         | 0            | 1487  | Echo          | 2 & 5 mo |
| 18.02..14 | M/W       | nm      | 452    | 8x7            | 28               | death | D0 50 min    | CRA          | 1453  | Ventil.       | tech. probl. |
| 18.12..14 | M/W        | 8       | 461    | 8x7            | death            | J0 3 h | D0 3 h       | CRA          | 1454  | ECG           | Tech. probl. |
| 20.02..14 | M/W        | 7.5     | 395    | nm             | 30               | Euth. | 5 mo D141    | 0            | 1488  | Echo          | 5 mo |
| 25.02..14 | M/W        | nm      | 420    | 20             | death            | D0 2h | CRA          | 1455         | ECG   | Import. infarct |         |
| 28.02..14 | M/W        |         | 480    | <1 cm²         | Euth.            | D30   | 0            | Echo D48,30  |       |                |         |
| 04.03..14 | M/W        | 10      | 445    | Nm             | 20               | death | D0 1h        | CRA          | 1456  | ECG           | Ventil. tech. probl. |
| 11.03..14 | M/W        | 11      | 420    | 9x8            | 25               | Euth. | D30          | 0            | 1466  | Echo          | 10.30 |
| 18.03..14 | M/W        | 11      | 441    | 9x9            | 15               | Euth. | D30          | 0            | 1468  | Echo          | 10.30 |
| 25.03..14 | M/W        | 11      | 394    | <1 cm²         | 27               | Euth. | D30          | 0            | 1469  | Echo          | 10.30 |
| 01.04..14 | M/W        | 11.5    | 421    | 7x8            | 25               | Euth. | D41          | 0            | 1471  | Echo          | 10.30 Aneurysm |
| 08.04..14 | M/W        | 10      | 394    | 5x5            | 28               | Euth. | D34          | 0            | 1470  | Echo          | 10.34 |
| 15.04..14 | M/W        | 10      | 457    | 5.5x4         | 29               | Euth. | D29          | 0            | 1472  | Echo          | 8.29 |
| 06.05..14 | M/W        | 11      | 433    | 8x7           | 30               | Euth. | D24          | 0            | 1473  | Echo          | 8.24 |
| 20.05..14 | M/W        | 10      | 433    | 8x8           | 20               | Euth. | D31          | 0            | 1478  | Echo          | 10.31 Aneurysm |
| 27.05..14 | M/W        | 10      | 426    | 2x9           | 23               | Euth. | D30          | 0            | 1484  | Echo          | 0.7,30 |
| 26.01..15 | M/W        | 8       | 404    | 8x8           | 20               | living |              |              |       |                |         |
| 23.06..15 | M/W        | 7       | 434    | 7x8           | 15               | living |              |              |       |                |         |
| 24.07..15 | M/W        | 7       | 440    | 8x8           | 35               | living |              |              |       |                |         |
| Total 19 |             |         |        |                |                  |       |              |              |       |                |         |

NB: CRA – cardio-respiratory arrest; echo. – echography; Euth. - euthanasy; Histo – histology, tech probl – technique problems.

Table 3: Results of ultrasounds investigations (echocardiography) (EF: ejection fraction; RC: heart rate; LVWT: left ventricle wall thickness, s in systole, d in diastole. M+/SD on n=12).

4) After 1 month, the coagulum was practically phagocytized (Figure 4g); the fibrosis zone was well organized with oriented fibers, sometimes penetrating the healthy part of the myocardium (Figure 4 h–j). In several cases the fibrosis was transmural and an aneurysm was formed. In 2 cases formation of cartilage into the trabecular muscle was observed in the neighboring of the lesion (Figure 4k–4l). At a small distance from the injured zone and in the remained intact parts of the heart, the myocardium looked quite normal. Later up to 8 months marked fibrosis remained at the lesion area.

Other organs – lungs and liver – have shown no sign of evident pathology.

Discussion and Conclusion

These data suggest that an experimental technique and model of precise, reproducible, visually controlled lesion of the myocardium wall has been developed and specified. Precision was warranted by visual control of the technique, reproducibility was proved by standardization of the procedure. This has led to stable results of surgery itself, of functional follow up investigations data and of histological findings. It is to be noted that the success rate was low at the beginning of the research (16%) and much better during the last period of time (up to 100%), when the optimal experimental conditions were defined. Indeed, as well as coronary artery ligation, the procedure remained delicate, but was rather well tolerated, if the surgical technique and anesthesia conditions* were respected. It does not seem technically more difficult than interventions on the coronary arteries themselves, and it needed only usual equipment for cardiac surgery.

Another advantage of the proposed technique is the possibility of follow up with reliable functional evaluation of the damage by ultrasound investigation. In our experiments some difficulties of measurements were due to the high rate beatings heart of the rat that may be overcome by the use of a better resolution ultrasonic probe. Peculiarities of EF determination by Simpson method has to be lined: depending on localization of the ventricle section chosen for surface and volume calculation - through intact or injured part of the heart-, the results may differ. That was taken into account during the results analysis. Nevertheless echocardiography data have always shown a high correlation with anatomy and histology ones.

An interesting histological finding has also to be lined: the presence of cartilage in muscular cords of the left ventricle in some cases 1 or more months after lesion.

An interesting histological finding has also to be lined: the presence of cartilage in muscular cords of the left ventricle in some cases 1 or more months after lesion.
precursors, or that local and circulating stem cells were involved, perhaps under the influence of inflammatory cytokines, local hypoxia due to infarction or toxins and of the contractile function of the heart muscle, as assessed in literature [24-28]. Another explanation of the observed cartilage formation could be that fibroblast metaplasia could develop under the influence of mechanic and hypoxia conditions.

Anyway, the technique seems also to be a robust model of chronic cardiac insufficiency thanks to a long lasting EF decrease explained by

local myocardium fibrosis up to possibility of aneurysm formation.

Besides, whole heart histology has detected significant variations in thickness of the ventricle walls - kinds of crypts and trabeculae – between the bases of muscular part of the atrio-ventricular valves. Ultrasound investigation has also pointed a significant difference between systole and diastole ventricular thickness (Table 3). This justifies the necessity:

1. a) of controlling the lesion maximal deepness limit as no more than 1 mm.
b) of ensuring a visual control of the safeness of the myocardium inner layer during tip applications for avoiding perforation.

The last advantage of our technique is the rather strong limitation of injured area, confirmed by ultrasound and histological evaluations: the extension of the injured – shocked – zone was no more than 1 mm around the necrotic area (Figure 4b-4f); and the presence close to the lesion of healthy or slightly affected myocardium, the extension of inflammatory reaction and, later, fibrosis being limited (Figure 4d-4j). This may enhance the development of eventually grafted cells close to the lesion.

The negative aspects of the purposed technique may be the presence of a coagulum of burnt tissues in the lesion zone not only immediately after the operation but also within several weeks later, in fact as remnants only. The inflammatory reaction directed to the destruction and phagocytosis of this “foreign material” may be a danger for implanted in the neighboring area tissues, or an enhancing factor, as mentioned above, since vascularization of the zone may be increased. This must be verified in further experiments with stem or precursor cells implantation. Besides, knowledge of the different steps of the lesion healing allows study the most favorable moment for intervention by cell therapy.

In the other described models of cardiac lesion, for instance after coronary artery occlusion, the determination of the resulting injury extent needs special sophisticated techniques [21,22]. Ferric chloride use is not compatible with MRI imaging investigation, necessary to determine the lesion extent and evolution. Radiofrequency has been used recently to induce cardiac lesions through induction of intravascular coagulation, with promising results [16]. It represents a non-invasive coronary arterial occlusion with the same limits as mentioned above.

In conclusion, our technique is reproducible and, with experience, allows animals survival for a period, which is long enough to investigate the natural evolution of cardiac function after a localized lesion. It will be used to investigate the best modalities for fetal heart or cultured cardiomyoblast implantation to improve the injured heart remodeling and enhance tissue regeneration.

The early activation of regenerative processes after the thermic lesion of the heart may suggest that early cardiomyoblast implantation close to the lesion could have some chances of success.

Testing fetal heart implantation using our technique of local heart injury might be susceptible to answer to the above-mentioned question (concerning the cause of cell therapy controversial results) and give precious indications for reparative heart surgery. The first results obtained in our further investigation seem to support this hypothesis [24-28].

Some other conditions of success are: an adapted ventilation control, avoiding diaphragm and phrenic nerve lesion, accurate hemostasis, rectal temperature control (no less than 32.5°C) during anesthesia, operation duration no more than 30-40 min.

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| Control animal | Investigation number | RC (M+/−SD) | EF (M+/−SD) N=5 | EF (M+/−SD) N=12 | EF variability N=5 // N=16 |
|----------------|---------------------|-------------|----------------|----------------|---------------------------|
| Rat n°1        | 5 / 12              | 406 +/- 31  | 69.6 +/- 2.2   | 69.4 +/- 5.0   | 3.1% // 7.2%             |
| Rat n°2        | 5 / 16              | 382 +/- 26.6| 69.5 +/- 3.3   | 70.4 +/- 6.0   | 4.7% // 8.6%             |

No statistic difference between results (p>0.05) and acceptable variability (< 10%)

3a: Evolution of repeated EF evaluation in 2 control animals (for liability assessment)

| Delay (days) | N° of animals | RC (M+/−SD) | EF (M+/−SD) | % EF average decrease | Results variability % |
|--------------|---------------|-------------|-------------|-----------------------|-----------------------|
| 10 ± 2       | 11            | 421 ± 35    | 45.6 ± 13.4 | 37.4                  | 17.4                  |
| 30 ± 5       | 11            | 385.5 ± 50  | 56.3 ± 10.2 | 27.3                  | 15.4                  |
| Control      | 11            | 395 ± 30    | 72.9 ± 5.3  |                        | 7.3                   |

*p<0.01 comparatively to control

**p<0.001 comparatively to control

Acceptable variability, better in control than after lesion, especially in early delay

3b: Evolution of EF in operated and intact (control) animals (the same 11 operated animals being investigated at 2 delays).

| Series | systole normal wall | systole injured part | diastole normal wall | diastole injured part | Animal number |
|--------|---------------------|----------------------|----------------------|-----------------------|---------------|
| At day 10 | 3.28±/−0.26         | nm                   | 2.13+/−0.43          | nm                    | 9             |
| At day 30 | 3.47+/−0.33         | 3.07+/−0.38          | 2.66+/−0.26          | 2.2 +/− 0.42         | 9             |
| control  | 3.47+/−0.27         | -                    | 2.46+/−0.31          | -                     | 11            |

No statistic difference between injured part and normal wall at any moment (p>0.05).

3c: Results of left ventricle wall thickness (LVWT) measurements (M+/−SD).

Figure 4A: Histological aspects of heart high temperature lesion: Normal heart: Epicardium (1), myocardium (2), endocardium (3) covering the trabecular/muscular columns of the valve fibrous strings or chordae tendineae (hematoxilin eosin x5).

Figure 4B: Histological aspects of heart high temperature lesion: Day 0 after lesion: In the lesion center, epicardium and sub epicardial lesion in form of bubbles due to cauterization heat (hematoxilin eosin x5).

Figure 4C: Histological aspects of heart high temperature lesion: Day 0 (4 hours): Superficial area (1) shows a coagulation necrosis with loss of cardiomyocyte nuclei, intermediary area (2) shows a coagulation necrosis with cardiomyocytes vacuolization and inner area (3) with an apparently preserved myocardium (hematoxilin eosin x10).
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Figure 4D: Histological aspects of heart high temperature lesion. Day 0 (6 hours): Coagulation necrosis with neutrophil infiltration (hematoxilin eosin x40).

Figure 4F: Histological aspects of heart high temperature lesion. Day 14: Necrosis in superficial area (1), mild fibrosis: Inflammatory reaction and beginning fibrosis in the intermediary area (2) and preserved cardiomyocytes in deep area (hematoxilin eosin x20).

Figure 4E: Histological aspects of heart high temperature lesion. Day 0 (6 hours): Cardiomyocytes nuclei alterations focused (hematoxilin eosin x40).

Figure 4G: Histological aspects of heart high temperature lesion. Day 29: Resorption inflammatory reaction of coagulum remnants with giant cells in a small burnt sub epicardium area (hematoxilin eosin x20).

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Figure 4H: Histological aspects of heart high temperature lesion. Day 30: General view of the lesion area: heart apex (1), muscular columns of the valve tendineous strings (hematoxilin eosin x5).
1. Epicardium with inflammatory cells, 2. Large cicatricial zone (50 % of the thickness of the external wall), 3. Partially preserved muscular columns.

Figure 4I: Histological aspects of heart high temperature lesion: Day 34: The special coloration shows spider-like extension of the fibrosis. 1: epicardium, 2: fibrosis area, 3: intermediary area (Masson’s Green Trichroma, x 5).

Figure 4J: Histological aspects of heart high temperature lesion. Day 30: Dissociation of the cardiomyocytes by fibrosis (Masson’s Green Trichroma x 40).

Figure 4K: Histological aspects of heart high temperature lesion. Day 30: General view of one case of complete transparietal fibrosis (arrow) of the apex with pillar involvement and aneurysm forming (hematoxilin eosin x5).

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Figure 4L: Histological aspects of heart high temperature lesion: Day 30: Fibrosclerosis of the apex myocardium with chondrocyte metaplasia (Masson’s Green Trichroma x 5).