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Research Article

Once more about Syngeneic Foetal Organ Grafting and Teratoma Formation

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Abstract

Cases of teratoma development have been rarely reported after foetal organ transplantation into syngeneic adult animals. Recently during experiments on 68 Wistar Rats having survived either foetal heart graft to treat cardiac damage or implantation of foetal heart or intestine into different sites (including subcutaneous, thoracic and abdominal cavity locations), teratoma was observed in 4 animals. In one case malignancy was detected. The analysis of the experimental series from the point of view of the donor maturity has shown that in all the cases of tumour growth the body weight (BW) of the donor was equal or less than 1 g (correspondence to the second third of the foetus in utero growth). It represented 28% of the cases out of the 14 donors of the same category. When the BW of the foetal donor was higher than 2 g, no teratoma has developed. It is also to be noted that the 4 mentioned implants were fixed on the heart lesion by a degradable chitosan sheet.

These observations and literature reports probably indicate that different foetal organs implanted into different sites of syngeneic adult recipients may give growth to teratoma. The low degree of the foetal donor maturity presupposes an increased susceptibility of any grafted organ to include remaining important amount of pluripotent and even totipotent stem cells. The role of environmental conditions such as inflammation, presence of chemicals and - in experiments - the animal strain cannot be ruled out.

These considerations may help not only to define the conditions of a safe use of foetal organ grafts, but also to develop reliable models for the in vivo study of teratoma and terato carcinoma genesis.

Anyway teratoma growth from foetal organ implants can no longer be considered incidental and deserves further systematic investigation.

Keywords: Teratoma; Foetal Organ Development; Foetal Heart Grafts; Organoids; Stem Cells; Cell; Tissue and Organ Transplantation; Reparative Surgery

Abbreviations

BW: Body Weight; FHI: Foetal Heart Implant; FII: Foetal Intestine Implant; HES: Haematoxylin Eosin Saffron Staining; Ter: Teratoma; Tx: Transplantation.

Introduction

The problems caused by the donor deficit in the world stimulate search for alternatives to major organ transplantation and reinforce exploration of regenerative medicine pathways [1]. Possibilities of self-mobilization and development of local and distant autologous stem cells are studied and described [2-8].

But stem cells imported from outside origins are also used for engineering elaborated organ models [9-15]. Nowadays good results are mainly observed with tissue reconstruction, for instance bones, cartilage and even liver, when injected allogeneic stem cells may promote the reparation of the defect inducing local and general organism reaction [16-22].

Foetal organ Implantation in adult recipients is another way to obtain organoids [23-26] and to aim at organ insufficiency palliation [27-46]. Though the moratorium on research concerning foetal material (beginning in1970-ies and ending in 2009-2011) has slowed down the investigations in this area, some development perspectives exist especially after the published possibility to obtain "hypoimmunogenic" and so "invisible" for recipient allogeneic transplants [47-49].

However, it is well known that recipient immunosuppression increases the oncological risk. Does the use of foetal tissues and organs present a similar danger?

As a rule teratomas are obtained from embryonic cells and tissues [50-56]. In one case neural stem cell therapy has provoked a malignancy [57]. This is why some authors point not only the benefits but also the risks of stem-cell therapies and claim teratoma assays and evaluation of safe parameters for stem cell used for therapies in humans [55,58-60].

The formation of teratoma after foetal organ grafting was described as a casuistry but nevertheless a possibility [54,61].

So pursuing our investigations on foetal organ transplantation as an organ repair technique, particular attention has been given to cases of teratoma development cases, their frequency and their particularities. Their possible causes were analysed.

Material and Methods

The present work included 68 Wistar rats that have survived at least 7 days after foetal heart and/or foetal intestine transplantation at different sites into adult animals of the same strain (to avoid rejection reaction).

The following sites were used: (Table 1).

Site	Foetal organ	Animal number	Implant number	Recipient BW < 1g
Heart lesion total	Heart	23	30	9
+ feutre cover		10	12	3
+ chitosan cover		13	18	6
Total without heart lesion	Heart and digestive organs	45	72	5
Abdomen	Heart	13	20	3
Thorax	Heart	27	30	2
Neck	Heart and intestine, stomach	6	10	0
Ear	Heart and intestine	9	12	0
Intra or submuscular loge	Heart	9	12	0
Total	Heart and digestive organs	68	102	14

Table 1: Experimental cohort.

All the procedures were provided under anaesthesia. Initiation was ensured by Fluorotane® inhalation (4% 1min/100g BW) in the bow of the ventilation box of the apparatus for small rodents anaesthesia (Netherlands). For main anaesthesia and analgesia intra peritoneal injection of a Pentothal Natrium solution (0.1 ml/100 g BW, i.e.0.075mg/100g BW; Nembutal® Ceva Santé animal- Brussels Belgium), and 0.2 ml of 0.05% solution Buprenorphine hydrochloride (Temgesic® - Laboratoire Schering-Plough– Courbevoie France) were used. When intrathoracic surgery was required, subcutaneous Atropine Sulphate injection (1% 0.2ml) was added before intubation for preventing vagal reaction. Intubation was performed with a 14 G catheter and the help of a laryngoscope (Mac 0 blade-Heine Germany). Ventilation was conducted at a rate of 60/min, a tidal volume of 12 ml/kg and a pressure of 0 to 20 millibars (UNO Anaesthesia equipment for small rodents, Netherlands).

Donor preparation and graft procurement

The donors were foetuses aged 14-19 (±2) days in utero, with BW from 0.8g (14 cases) to 6 g (8 cases), mainly between 2g and 5 g (M ± SD = 3.3 ± 0.2 g).

The anaesthesia and analgesia of the gravid female was provided as described above but with a little overdosing allowing

anaesthesia of the foetus, proved by the absence of reaction during manipulation on the donors. The gravid female underwent a laparotomy, the uterus exposed and the foetuses extracted. After a wide thoracotomy and laparotomy the heart and/or the digestive tract were isolated and placed in a cup with saline at room temperature (22°C). Within 10-30 min it was transferred to the already prepared recipient.

NB. The assessment of the foetus age by the copulation date or the vaginal plug detection seems less accurate than measuring the foetal BW, based on the sum of the BW of the isolated foetus divided by the foetus number (no less than 6). BW seems to correctly reflect the development degree of the foetus.

Recipient surgical procedures

In the cases of heterotopic implantation of foetal organs into superficial layers (neck, ear pavilion, Marfan sub muscular space, intra muscular loge), a skin incision was provided, a subcutaneous loge was managed by blind tissue separation. The implant was placed into the pocket and skin was sutured by 2 or 3 separated stiches, except for ear where a Nobecutane® spray shut the incision line.

In 3 animals fetal heart implant after division by scissors was injected into the thoracic cavity through the thoracic wall by a thick needle (gauge 12).

In abdominal intervention, a longitudinal incision from sternum to pubis of the skin, white line and parietal peritoneum was provided. The omentum was found and a FHI inserted between its sheets. A loge was managed under the visceral mesentery layer at the level of the ileo-caecal junction and the implant introduced into the so made loge. An Ethylon 7°° stich closed the pouch. The peritoneal cavity was shut by a two-layer suture using 4 or 5°° Ethylon continuous suture.

In intra thoracic surgery, after longitudinal median sternal thoracotomy, the heart was exposed.

The "Cautery high temperature fine tip" (Bovie Medical Corporation-USA) was used to induce a myocardial lesion 7-9 mm in diameter, 1-105 mm in depth at the level of the anterior apical zone of the heart (including left ventricle and parts of septum and right ventricle) [45]. Directly after this preparation one or several fetal hearts (depending of the donor age and the organ volume) were placed on the myocardium wound in such manner to cover it. Then a flap of biocompatible artificial tissue of 10x10mm or a little more was fixed to the healthy part of the recipient heart using 8°° Ethilon purse shaped suture (Figure 1B and C). Feutre, Contegra, used in cardiac and vascular surgery, or chitosan known by previous experiments [46] were used to form the cover of the graft.

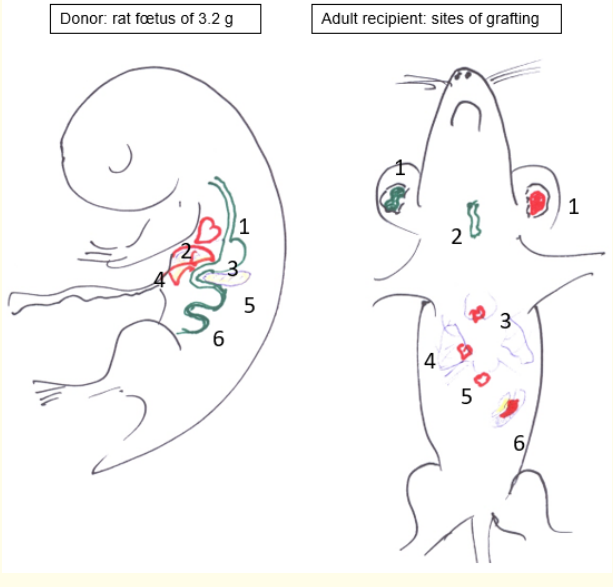

Figure 1: Schemas of different operations: a heterotopic implantation of foetal organs into different sites, b. Implantation of foetal heart at a heart lesion site. Donor: 1. oesophagus and stomach, 2. heart, 3. stomach, 4. liver, 5. pancreas, 6. small bowel. Recipient: 1. ear pavilion, 2. neck, 3. Thymus, , 4. lung hilum, 5. heart, 6. abdominal cavity.

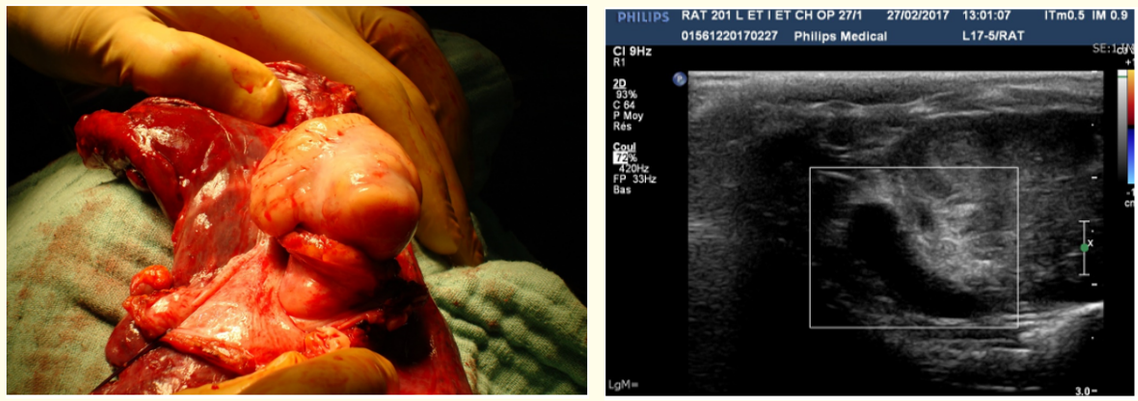

Figure 2: Macroscopic and ultra sound view of the teratoma (arrows and frame) grown on heart lesion repaired by foetal heart graft covered for fixation by a chitosan sheet.

The thoracic wall wound was then sutured layer by layer with classic separate stitches using Vicryl 2°° for sternum, 4°° for muscles and diaphragm (if necessary) and running suture 6°° for the skin.

Recipient follow up

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. Here are considered only the ultra sound system IU22 (Philips – Netherlands) with an ultrasonic probe (L17-5 MHz) and histological investigations (10% formalin fixation, paraffin embedding, hematoxylin eosin saffron staining) provided through 10, 30, 30 days and later monthly till 1 year.

All the experiments were conducted according to the European rules for animal welfare and allowed by the Local Ethic Committee under Number 508N and 690N.

Results

Among the 68 animal having survived to their respective implantation of foetal heart or digestive organs, absence of development considered as a failure was observed in the 7 cases of foetal heart intrathoracic injection, most of implantations into abdominal sites as well as implantation into thoracic organs, whereas subcutaneous location of the grafts was more successful (Table 2).

Series (implantation sites)	Observation number	Implant development	Development failure	Teratoma, growth
Heart lesion	23	19	4	4
With feutre cover	10	9	1	
with chitosan cover	13	10	3	4
Total				
Thorax cavity	7	0	2 remnants	0
Thorax organs (thymus, lung hilum, pericardium)*	30	4	26	0
Abdominal cavity (omentum, ileocaecal mesenterium)	13	5	8	0
Ear pavilion subcutaneous pouch*	18	17	1	1
Neck	6	5	1 remnants	0
Intra or sub muscular loge	9	0	4 remnants	0
TOTAL	106	50		5

Table 2: Grafting results depending on the implantation site.

*Several sites could be involved in one animal (for instance: both ears, thymus and lung hilum), so the observation number does not correspond the animal number mentioned in table 1.

As table 2 shows, implant development, documented by macro and microscopic investigations, was globally observed in 50% of the cases but with different frequency depending on the implantation site. The best results that is the formation of adult-like organ were obtained in the ear and neck sites, as well as in the heart lesion site. Development of teratoma and even teratocarcinoma was noted in the heart site (4 cases) and the ear pavilion site (1 case). It represents 4.7% of the cases. But the 5 cases are related to donors with a BW < 1 g and this represents 35.7% of the cases. The typical characteristics of a a teratoma were present in every case – mixture of different tissues and organs from the 3 embryonic layer origin – and in 1 case, malignancy was patent in a part of the tumour (Figure 3).

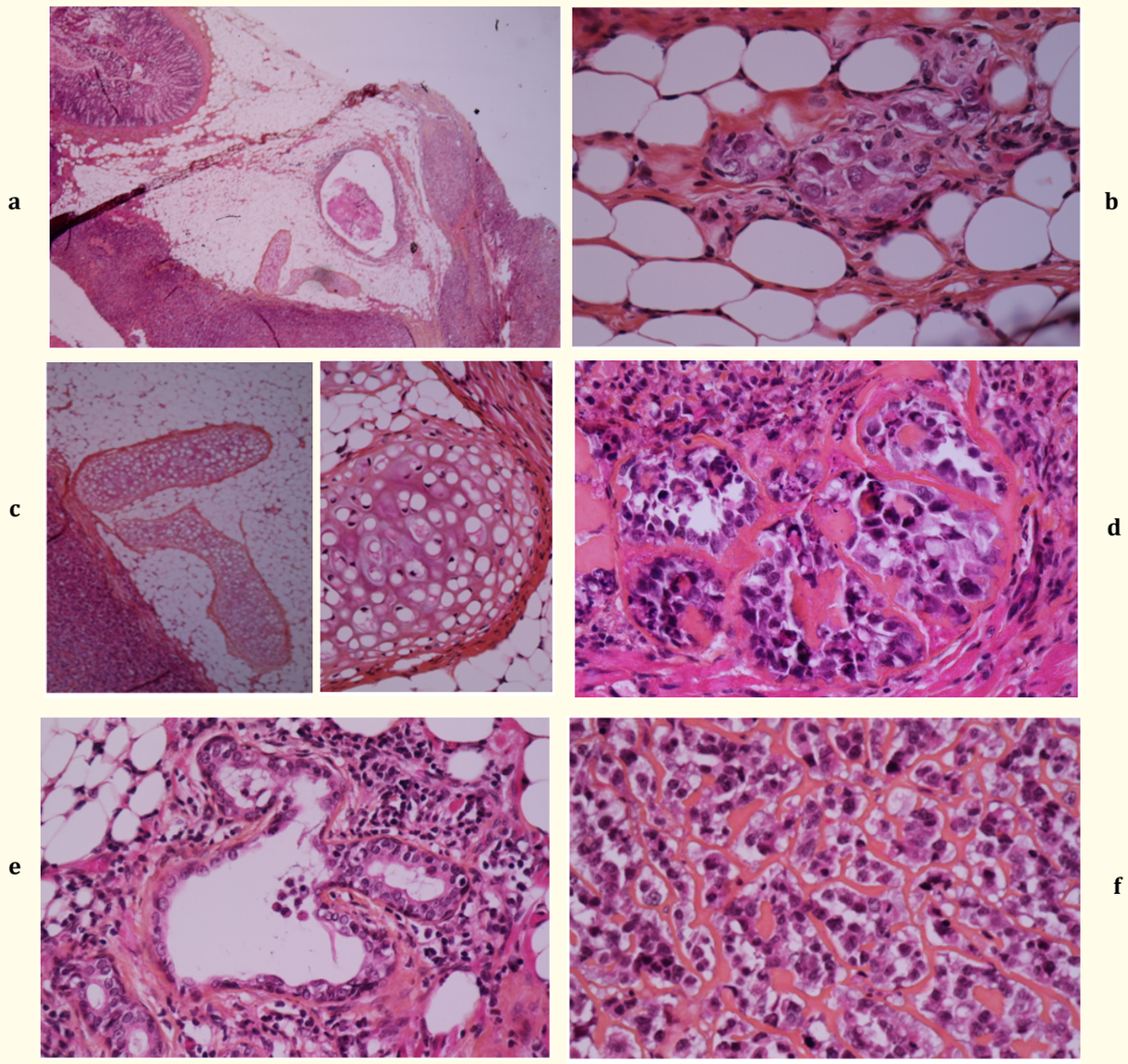

Figure 3: Microscopic views of the partly malignanced teratoma.(Hematoxylin eosin saffron stained).

a. Benign digestive gtube within adipose tissue (X5)
b. Neuron into fat (X 40)
c. Benign cartilago (X10 and X20)
d. Tumoral gland (X40)
e. Benign glands (X40)
f. Adenocarcinoma(X40)

Discussion

It is well known, that the human spontaneous teratoma develops mainly from germinal cells of ovarium and testicle, but the experimental one is obtained mainly from chorion, embryonic and stem cells *in vivo* and *in vitro* [50-56]. At the same time stem cell therapy occupies more and more place in bone and cartilage reconstruction, but also in organ engineering and treatment of diseases due to organ cell function deficit such as diabetes, Fabri disease, retinopathy, degenerative changes in the central nervous system and other hematopoietic defects after oncology treatment [10-12,15]. So some authors already think about tumour derive prevention when stem cell therapy is forecasted [58-61].

Up to now foetal organ transplantation seemed to be safer than stem cell injections. But the analysis of the present results and comparison with literature data show that in certain conditions the risk may reach the 35%. It is true than in most experimental series with a sufficient follow up the tumour did not develop or but in a weak proportion - 4% or even less if we consider the whole experience during many years (out of 206 implants - 7 teratoma including 1 with partial malignancy, i.e. 2.4%).

The summary of different authors experience allows to figure some factors susceptible to enhance the teratoma incidence. First of all probably the presence of totipotent or pluripotent cells in the graft that is waited for in early embryos or young foetus. Their presence may be maintained later depending on the organ, for instance in the liver, as in our previous observation [57] or bones. Second, local circumstances may have an influence on the graft development, such as inflammatory reaction which is known to accompany the chitosan sheet degradation [62,63] and was observed in our experiences with heart lesion repair. It is to be noted that in series with oesophagus defect repair and the preliminary investigation of the biocompatibility between chitosan sheets and foetal digestive organ, only 1 teratoma has developed [46]. The influence of the graft initial ischaemia, are not to be taken into account here because the graft ischemia duration was the same and did not overcome 15 min. The same may be said as well about the role of the heart lesion remodelling process, because the lesion dimensions were standardized. At last, in the present work as in the previous one, the teratoma growth was observed only in Wistar recipients and was absent in the Fischer ones used in comparable number. This seems to be confirmed by the results of former series performed in the 1980-ties when the prevalence of tumour development was rather high, all other conditions being similar, the rat strain apart: a crossing between Wistar and August strains [33]. Have the Wistar animals a genetic weakness enhancing tumour growth? And by extension, has the recipient condition to be also tested in the frame of a teratoma assay in the case of stem cell therapy?

Anyway the present results are not a pretext to refuse foetal organ grafting. First, it remains safer than the stem cell use, because it is possible to choose the donor in such a way that the grafted organ will contain mainly precursor cells giving growth only to the target organoid. This corresponds to the observations of the different authors working in this area [28,30,34,37,38]. Moreover foetal organ grafting possibilities seem important when creation of immunologically "invisible" allogeneic graft with reduced antigenicity has become a reality [47-49]. In fact, the necessary genetic and other manipulations would be easier to perform with a foetal organ graft, which at first undergoes "dedifferentiation" or simply regression, and which cells are then isolated and particularly accessible to external actions before further growth, organization as structures and integration to the adult recipient organism.

At last foetal organ grafting may be used as an interesting model for systematic experimental study of benign and malignant tumour growth at "the crossroad of foetal- and onco- development" [56].

Conclusion

1. Teratoma development after foetal organ grafting cannot be considered anymore as "incidental".
2. Several factors seemed to play a significant role in the process: donor weak maturity allowing the presence of pluripotent or even totipotent stem cells in the foetal graft, local inflammatory processes or presence of organic products due, for instance in our observations, to chitosan degradation, peculiarities of the recipient strain.
3. Above mentioned circumstances encourage foetal organ grafting from donors in the last third or even last quart of in utero development (in rats or mice: 16-19 days).
4. The possible development of teratoma and terato carcinoma from foetal organs as an experimental model of tumour genesis is worthwhile further systematic study.

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