ORIGINAL ARTICLE: REPRODUCTIVE BIOLOGY

Testicular cell transplantation into the human testes

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Objective: To translate spermatogonial stem cell (SSC) transplantation towards a clinical application.
Design: Mouse green fluorescent protein (GFP)-positive testicular cells were labeled with ^{99m}technetium and microbubbles. These labeled cells were injected into the rete testis of isolated human testes under ultrasound guidance. Three different conditions were tested: 1) 800 μL of a 20 million cells/mL suspension; 2) 800 μL of a 10 million cells/mL suspension; and 3) 1,400 μL of a 10 million cells/mL suspension. After injection, the human cadaver testes were analyzed with the use of single-photon-emission computerized tomography (SPECT) imaging and histology.
Setting: Laboratory research environment.
Patient(s): Cadaver testes, obtained from autopsies at the pathology department.
Intervention(s): Ultrasound-guided injection of mouse GFP-positive testicular cells.
Main Outcome Measure(s): Presence of radioactive-labeled cells in the human cadaver testes and GFP-positive cells in the seminiferous tubules.
Result(s): In all of the experimental groups, GFP-positive cells were observed in the seminiferous tubules, near and far from the rete testis, but also in the interstitium. On SPECT, significant difference was seen between the group injected with 800 μL of a 20 million cells/mL suspension (1,654.6 $\pm$ 907.6 mm^3) and the group injected with 1,400μL of a 10 million cells/mL suspension (3,614.9 $\pm$ 723.1 mm^3). No significant difference was reached in the group injected with 800 μL of a 10 million cells/mL suspension.
Conclusion(s): Injecting cells in the human cadaver testis is feasible, but further optimization is required. (Fertil Steril® 2013;■:■–■. ©2013 by American Society for Reproductive Medicine.)
Key Words: Translation, spermatogonia, human, testis, transplantation

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In Belgium, one out of three men develops cancer before the age of 70 years. Although most cancers occur in older people, in 2006 0.6% of all cancers were diagnosed in children (1). Luckily, their prognosis has improved substantially over the past years. In the period 1999–2006, an 81.4% survival was seen in an all-site study for children and adolescent cancers in the USA (2).

However, due to chemo- or radiotherapy, spermatogonial stem cells (SSCs) can be lost. This loss is an important cause of male infertility. Low-dose chemotherapy can deplete the progenitor-type A_{pale} spermatogonia and the differentiating B spermatogonia, leading to temporary oligozoospermia or azoospermia. When the testis is severely damaged, the SSCs go into apoptosis and/or the Sertoli cells are unable to support the SSCs, eventually leading to a depletion of the SSC pool causing permanent infertility (3).

In case of adult men, a semen sample can be stored to prevent infertility. Prepubertal boys do not have this option, because spermatogenesis has not started yet. During the past 20 years, research has been done to find a suitable strategy to overcome this problem. One of those techniques is SSC transplantation (SSCT), a technique introduced by Brinster et al. (4). Efficacy of the SSCT technique has been demonstrated by our group (5) and by Kubota et al. (6). Although the homing efficiency of SSCs after SSCT is only 12% (7), recently it has been shown that even after long-term cryopreservation of SSCs, these cells are capable to regenerate complete spermatogenesis and produce fertile offspring after transplantation (8). The safety of SSCT has also been investigated, and it has been proved that after SSCT, offspring did not show morphologic differences nor major alterations on (epi)genetic level (8–11).

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Xenogeneic transplantations have also been studied. Injection of rat testicular cells in immunodeficient mice has led to rat spermatogenesis in nearly all recipient testes (12). In that study, normal rat spermatozoa were also found in the epididymis (12). In more distant species, such as rabbit and dog, both fresh and cryopreserved testicular cells were able to survive and proliferate in busulfan-treated Swiss nude mice but failed to differentiate (13). Xenotransplantation of germ cells from porcine, equine, or bovine testis cells were able to colonize recipient mouse testes. Although the porcine testis cells survived even for a year, none of the germ cells differentiated in the immunodeficient recipient (14). Nagano et al. (15) have studied xenotransplantation of testicular cells from baboons, who are closely related to humans. Baboon germ cells are able to colonize the recipient mouse tubules for at least 6 months after transplantation. However, differentiation did not occur (15). This same group has also transplanted human cells into mouse testes and have seen proliferation of the human cells during the first month after transplantation. Six months after transplantation, human cells were still present but no differentiation was seen (16).

As in the mouse model, rat testes could be efficiently injected via the efferent duct (17). However, this technique is not feasible in animals with larger testes, such as cows and monkeys (18). Schlatt et al. have tried to microinject cells into the efferent ducts and seminiferous tubules of isolated bull and monkey testes with a glass needle (30–40 μm luminal diameter). Intratubular injections were more difficult to perform owing to the large volume-to-surface ratio, the more resistant lamina propria, and the more convoluted seminiferous tubules. Efferent duct injections were also difficult, owing to surgical preparation, burdensome localization, and cannulation of the efferent ducts (18). Rete testis injections proved to be the most promising injection site, because the rete testis can be localized by ultrasonography thus avoids open surgery. Studies in other large animals, such as pigs (19) and goats (20), have also demonstrated the usefulness of an ultrasound-guided injection. Recently, autologous and allogeneic transplantations in rhesus macaques were performed successfully (21). Donor cells were injected via ultrasound guidance under constant pressure and chased with saline. Although in 60% of prepubertal recipients who underwent an autologous transplantation donor sperm was found in the ejaculate after maturity, fertilization studies were not possible owing to low efficiency of marking SSCs. However, fertilization studies performed after allogeneic transplantations in adult recipients resulted in fertilized oocytes after intracytoplasmic sperm injection, showing the functionality of the sperm.

The first attempt to inject cells in human testes was made in 1999 (18). The full length of the rete testis could be filled with the use of an ultrasound-guided injection, although the extent of tubular infusion was variable. Unfortunately, the concentration of cells in the injected suspension was not mentioned. In a subsequent study, single injections were compared with multiple infusions in the rete testis of isolated human testes with the use of trypan blue dye. The infused testes were examined both macro- and microscopically, and they showed that 55% of the testes could be filled (22). However, it was not clear whether the dye was injected in the seminiferous tubules or in the interstitium. Recently, our research group has shown that a single ultrasound-guided infusion of 800 μL in the rete testis may be a promising method to transplant human SSCs in a clinical setting (23), because ink particles were observed in the lumen of the rete testis and in tubules both close and distant from the rete testis. However, in that study only a mixture of Chinese ink, microbubbles, and computerized tomography (CT) contrast was injected, which may not mimic the clinical situation of cells being injected. Because in these earlier studies the human testes were either infused with an unknown concentration of injected cells or only with dye, the aim of the present study was to define the parameters needed for a good infusion of isolated human cadaver testes with a cell suspension.

MATERIALS AND METHODS

Supplemental Materials and Methods is available online at www.fertstert.org.

Tissue Source

The cadaver testes (n = 18) used in these experiments were obtained from autopsied bodies at the Department of Pathology of UZ Brussel. The cadaver testes were kept in physiologic solution and refrigerated until use. The time elapsed between cardiac arrest and time of infusion, also known as the ischemia time, was on average 27 ± 13 hours (Table 1).

Approval for this study was given by the Ethical Committee of UZ Brussel (approval no. 2011-278) and the Animal Care and Use Committee of the Vrije Universiteit Brussel (approval no. 2012-216-4).

Injection of Cells in Human Testis

Donor cells were obtained from mice bred in the animal centre of the Vrije Universiteit Brussel. In this study, adult male GFP^+ F1 hybrids were used (n = 10). After decapsulation, the testes were enzymatically digested to a cell suspension according to the previously described method (5) (Supplemental Materials and Methods). The obtained cell suspension was then labeled with ^{99m}technetium–hexamethylpropylene amine oxime (^{99m}Tc-HMPAO; Ceretec N159; GE Healthcare) to visualize the cells via single-photon-emission computerized tomography (SPECT) imaging. Microbubbles (Optison; Mallinckrodt Medical) were added in a 1:1 ratio to visualize the cells during ultrasonography. Cell viability and cell clumping were tested in a pilot experiment. Cell viability was measured twice by adding trypan blue dye to the radioactive cell suspension. A viability of 98.5 ± 0.7% was obtained. Large cell clumps were not observed.

In a first set of experiments, cadaver testes were injected with a 23-G needle via the rete testis under ultrasound guidance with 800 μL of ^{99m}Tc-labeled GFP^+ testicular cells (20 million cells/mL). This concentration was chosen as initial concentration because of its proven efficacy in mouse studies