Brief report

Reproductive capability in dogs with canine leukocyte adhesion deficiency treated with nonmyeloablative conditioning prior to allogeneic hematopoietic stem cell transplantation

Tanya H. Burkholder, Lyn Colenda, Laura M. Tuschong, Matthew F. Starost, Thomas R. Bauer Jr, and Dennis D. Hickstein

Nonmyeloablative conditioning regimens are increasingly replacing myeloablative conditioning prior to allogeneic hematopoietic stem cell transplantation (SCT). The recent advent of these conditioning regimens has limited the assessment of the long-term effects of this treatment, including analysis of reproductive function. To address the question of reproductive function after nonmyeloablative transplantation, we analyzed a cohort of young dogs with the genetic disease canine leukocyte adhesion deficiency that were treated with a nonmyeloablative dose of 200 cGy total body irradiation followed by matched-littermate SCT. Five males and 5 females entered puberty; all 5 males and 4 females subsequently sired or delivered litters following transplantation. We demonstrate that fertility is intact and dogs have uncomplicated parturitions following nonmyeloablative conditioning for SCT. These results are encouraging for children and adults of childbearing age who receive similar conditioning regimens prior to allogeneic transplantation. (Blood. 2006;108:1767-1769)

Introduction

Hematopoietic stem cell transplantation (SCT) represents the only definitive treatment for a number of hematologic diseases, including leukemia,1 hemoglobinopathies,2 and immunodeficiencies, such as leukocyte adhesion deficiency.3 Due to the success of SCT many patients are now living increased life spans free from their primary disease. Thus, late complications of transplantation are increasing in importance.

One complication of transplantation, reproductive failure, has a high incidence and is a well-characterized late complication of SCT in both children and adults.4 The conditioning regimens used for SCT, including myeloablative dosages of chemotherapy and/or total body irradiation (TBI), are responsible for many of the late complications. In children, gonadal damage from irradiation can result in delayed onset or absence of puberty, failure to achieve menarche in girls, and infertility in both sexes.4,5 A large, retrospective survey of 19 412 allogeneic and 17 940 autologous transplantation patients showed an overall pregnancy rate of 0.6%, which was 10-fold lower than the crude birth rate for the control population.6

Recently, nonmyeloablative conditioning strategies have been developed to reduce the complications of SCT and to extend the possibility of transplantation to older individuals, those with comorbid medical conditions, and children with nonmalignant hematologic disease. Because of the limited period of observation following nonmyeloablative transplantation in humans, the relationship of these reduced-intensity conditioning regimens to fertility remains unclear.

The canine model is a well-recognized model for developing new SCT regimens and for identifying complications following SCT.7 We have described the successful use of matched-littermate transplantation following nonmyeloablative conditioning with 200 cGy TBI in puppies with the immunodeficiency disease canine leukocyte adhesion deficiency (CLAD).8,9 We now describe the preservation of reproductive function and pregnancy outcomes in male and female puppies treated with this nonmyeloablative conditioning regimen followed by matched-littermate allogeneic transplantation.

Study design

Dogs

Animals in this study are all members of a breeding colony housed by the Division of Veterinary Resources at the National Institutes of Health. All procedures performed were approved by the Institutional Animal Care and Use Committee of the National Cancer Institute.

Transplantation regimen

CLAD dogs were conditioned with TBI at a nonmyeloablative dose of 200 cGy delivered from a 60Co source on the day of transplantation. The source of the hematopoietic stem cells, bone marrow cell infusion, and posttransplantation immunosuppression have been described.9 All of the dogs were treated before 4 months of age.

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Fertility assessment and breeding

Estrus was detected by visual examination for vaginal discharge and tumescence and confirmed with vaginal cytology prior to breeding. Dogs were bred either by natural cover or by artificial insemination using fresh semen on days 1, 3, and 5 of estrus. At the end of the study, all puppies with birth defects and dogs that had received transplants were submitted to the Pathology Section of the Division of Veterinary Resources for full gross and histologic analysis. Semen was collected from conscious dogs and analyzed using standard methods by an independent evaluator (Lee Jones, International Canine Semen Bank–Mobile Delaware, Hockessin, DE) experienced in canine semen evaluation.

Results and discussion

Five male dogs in our study entered puberty and achieved normal spermatogenesis by 1 year of age following nonmyeloablative conditioning with 200 cGy TBI prior to SCT. This is the same reproductive milestone seen in normal dogs not receiving irradiation. All 5 have sired at least 1 litter (Table 1). Only 2 of 65 puppies from litters sired by the males that had received transplants had congenital malformations. This incidence of birth defects is not significantly different than the incidence of 1 of 69 puppies born to untreated carrier animals in our colony.

Five female dogs in our study had spontaneous onset of estrus by 1 year of age. The average interestrus interval of 227 days was within normal limits for dogs (Table 2). Four of these 5 females subsequently delivered litters of healthy puppies by natural parturition. Conception rates and litter sizes after SCT treatment were similar to the rate observed in normal dogs. The natural parturition. Conception rates and litter sizes after SCT in canine semen evaluation.

Table 1. Semen quality, paternity, and number of offspring produced by 5 male CLAD dogs after nonmyeloablative conditioning for SCT

<table>
<thead>
<tr>
<th>Dog</th>
<th>Source of HSCs</th>
<th>Time after SCT, y</th>
<th>Sperm count, x 10⁴</th>
<th>Motility, %</th>
<th>Morphology, % normal</th>
<th>Litters sired after SCT (no. puppies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1*</td>
<td>BM</td>
<td>1/4</td>
<td>1143/1348</td>
<td>70/90</td>
<td>87/92</td>
<td>2 (15)</td>
</tr>
<tr>
<td>M2</td>
<td>BM</td>
<td>2</td>
<td>High concentration</td>
<td>Good</td>
<td>High percentage</td>
<td>1 (6)</td>
</tr>
<tr>
<td>M3</td>
<td>PBSCs</td>
<td>3</td>
<td>543</td>
<td>75</td>
<td>92</td>
<td>1 (6)</td>
</tr>
<tr>
<td>M4</td>
<td>BM</td>
<td>3</td>
<td>585</td>
<td>90</td>
<td>93</td>
<td>2 (11)</td>
</tr>
<tr>
<td>M5</td>
<td>CD34⁺ BM</td>
<td>3</td>
<td>High concentration</td>
<td>Good</td>
<td>High percentage</td>
<td>3 (28)</td>
</tr>
</tbody>
</table>

The conditioning regimen was 200 cGy TBI for each dog.

HSCs indicates hematopoietic stem cells; M, male; BM, bone marrow; PBSCs, peripheral-blood stem cells; BM CD34⁺, CD34⁺ fraction of bone marrow.

Table 2. Estrus cyclicity and parity in 5 female CLAD dogs after nonmyeloablative conditioning for SCT

<table>
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<tr>
<th>Dog</th>
<th>Source of HSCs</th>
<th>Time after SCT, y</th>
<th>Interestrus interval, d</th>
<th>No. of cycles</th>
<th>Pregnancies after SCT (puppies)</th>
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<tbody>
<tr>
<td>F1*</td>
<td>BM</td>
<td>3</td>
<td>249</td>
<td>4</td>
<td>1 (12)</td>
</tr>
<tr>
<td>F2*</td>
<td>BM</td>
<td>3</td>
<td>337</td>
<td>3</td>
<td>1 (7)</td>
</tr>
<tr>
<td>F3*</td>
<td>PBSCs</td>
<td>3</td>
<td>227</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>F4*</td>
<td>PBSCs</td>
<td>3</td>
<td>213</td>
<td>4</td>
<td>1 (11)</td>
</tr>
<tr>
<td>F5</td>
<td>BM</td>
<td>2</td>
<td>235</td>
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The conditioning regimen was 200 cGy TBI for each dog.

F indicates female; other abbreviations are explained in Table 1.

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The conditioning regimen was 200 cGy TBI for each dog.

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F1 and F3 did not conceive or had an early loss of the pregnancy after their first breeding. The sires used were a male conditioned with busulfan and a normal dog. F1 conceived on her second breeding and delivered a healthy litter. F3 has not been rebred.

Litters sired by males that had received transplants, producing 100% affected progeny.

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*Two sets of data are provided for this dog because semen was evaluated at 2 time points after SCT.

These results suggest that pregnancies in patients after nonmyeloablative conditioning with 200 cGy TBI do not carry the increased risk of complications that has previously been reported for myeloablative conditioning in dogs and women.

The germinal epithelium of the testis is more vulnerable to the effects of radiation-induced damage than either the ovary or the Leydig cell. In 2 large studies, following conditioning that includes TBI, most men failed to regain spermatogenesis, although testosterone levels were generally normal. Several studies have shown that following conditioning with TBI during childhood, approximately two thirds of boys experienced germ-cell damage, although most had adequate testosterone concentrations and entered puberty spontaneously.

In females, the age of the patient at the time of transplantation appears to be as important as the conditioning regimen in predicting return of fertility. Twenty-one of 32 girls treated prepubertally achieved menarche after conditioning with chemotherapy and a hyperfractionated TBI dose of either 13.75 or 15 Gy. The median age at transplantation for girls in this study who did not achieve menarche was 8.6 years, versus 6.1 years for those who achieved spontaneous puberty. These findings suggest that in girls, the younger the age at transplantation the greater the potential for normal ovarian function later in life, which is likely because their ovaries contain a greater number of oocytes. The treatment age of the puppies in our study is analogous to very young prepubertal girls, which may have been a positive contributing factor in their preservation of fertility.

While the ovary is less prone to damage during youth, the growing uterus and uterine blood supply are more susceptible to radiation-induced damaged resulting in a reduced uterine volume, a subsequent increased risk of miscarriage, and low-birth-weight babies later in life. All of the pregnancies conceived by 5 women who were treated prepubertally with chemotherapy and 10 to 15.75 Gy TBI resulted in spontaneous abortion. In 1 study of SCT in dogs, fertility was reported in 4 of 4 male and 5 of 7 female animals after treatment with 7.5 Gy TBI administered at 8 to 18 months of age. However, 12 (55%) of 22 of the neonatal puppies died due to prolonged delivery times as a result of secondary uterine inertia. From this study it was not clear whether female dogs treated at a younger age experienced a greater percentage of complications.
The results from the canine model indicate that fertility is retained following nonmyeloablative conditioning for SCT consisting of 200 cGy TBI and matched-littermate allogeneic transplantation in a genetic immunodeficiency disease. These results demonstrating intact fertility and uncomplicated parturitions after nonmyeloablative conditioning and transplantation in dogs suggest that children and adults of childbearing age receiving this regimen might be expected to retain fertility as well during their subsequent reproductive years.

Acknowledgments

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