ORAL DISEASES

Oral Diseases (2011) 17, 670–676 doi:10.1111/j.1601-0825.2011.01821.x

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ORIGINAL ARTICLE

Long-term salivary function after conditioning with busulfan, fractionated or single-dose TBI

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OBJECTIVES: Does conditioning with fractionated total body irradiation (fTBI) or busulfan (Bu) causes less salivary dysfunction compared with single dose (sTBI) after hematopoietic stem cell transplantation (HSCT).

PATIENTS AND METHODS: A total of 74 adolescents below 13 years of age received allogeneic HSCT and conditioning with either: sTBI, fTBI or Bu. The unstimulated (USSR) and stimulated (SSSR) whole salivary secretion rates were measured at 15 years of age.

RESULTS: Irrespective of conditioning type, there were no significant differences in USSR or SSSR between groups. Girls had a significantly lower SSSR, 0.7 ± 0.3 ml per min compared with 1.1 ± 0.4 ml per min in boys ($P < 0.001$). A significant correlation between age at HSCT and SSSR at 15 years of age ($P = 0.02$) in children conditioned with sTBI was found as well as an inverse correlation between the plasma area under curve (AUC) of Bu and SSSR. In the multivariate model, only female sex was significantly correlated with low SSSR at 15 years of age (OR 3.93, 95% CI 1.21–12.79; $P = 0.021$).

CONCLUSION: No differences in long-term whole salivary function after HSCT in adolescents receiving conditioning with sTBI, fTBI or Bu were found. Total systemic exposure to Bu was negatively correlated with stimulated salivary secretion.

Oral Diseases (2011) **17**, 670–676

Keywords: busulfan; cancer; children; oral; pharmacokinetics; saliva

Introduction

Hematopoietic stem cell transplantation (HSCT) is increasingly used to treat a variety of malignant and non-malignant disorders in children (Ringdén and Le Blanc, 2005). HSCT is the transplantation of multipotent hematopoietic stem cells derived from bone marrow, peripheral blood, or umbilical cord blood from a donor and given to the patient. Allogeneic HSC donors must have a tissue human leucocyte antigen (HLA) type that is compatible with the patient. Even if there is a good match, the patient will require immunosuppressive medications to mitigate graft-versus-host disease (GVHD). Immediately before transplantation, the patient is given chemotherapy or irradiation or both together with immunosuppressive therapy. After several weeks of growth in the bone marrow, expansion of HSC and their progeny is sufficient to normalize the blood cell counts and reinitiate the immune system (Garthon and Ringdén, 1997).

As a result of advances in treatment, almost 80% of children and adolescents diagnosed with cancer become long-term survivors. It is also evident that damage to organs and tissues caused by chemotherapy and radiation therapy may not become clinically evident for many years after treatment. In a study of 10 397 childhood cancer survivors, 62% had at least one chronic health condition and 28% had a severe or life-threatening condition. The cumulative incidence of a chronic health condition was 73%, 30 years after cancer diagnosis (Oeffinger *et al*, 2006).

Several factors may contribute to the delayed effects after HSCT including the primary disease process itself, the transplant immune biology or the pretransplantation regimens (Ringdén *et al*, 1996; Ringdén and Le Blanc, 2005). Delayed effects are found in 78% of childhood cancer survivors treated with HSCT (Ness *et al*, 2005). Symptoms such as cataracts, renal dysfunction, diabetes mellitus, growth hormone deficiency, osteoporosis, delayed puberty, developmental delay and muscle weakness are highly specific for children who received HSCT.

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Received 28 February 2011; Revised 8 May 2011; accepted 22 May 2011

With regard to oral and dental health, childhood cancer survivors treated with HSCT are a major risk group. Children treated with HSCT have significantly lower salivary secretion rate and disturbances in dental and craniofacial development compared with children treated with chemotherapy protocols only (Dahllöf *et al*, 1988, 1994).

Efforts have been made to reduce the toxicity of conditioning therapy. Dose fractionation of total body irradiation (fTBI) is performed to spare the non-hematopoietic tissues and to allow sublethal DNA repair in cells of non-hematopoietic origin (Cosset *et al*, 1990). Busulfan (Bu) is an alkylating agent that can be combined with cyclophosphamide (Cy) to avoid the use of TBI in children (Hassan *et al*, 2000).

In this study, we investigated whether conditioning with fTBI or Bu will result in less salivary dysfunction compared with single-dose TBI (sTBI) and whether other known risk factors for low salivary secretion rate after HSCT also contributed.

Patients and methods

Patients

This study included 74 adolescents at 15 years of age who were recipients of allogeneic HSCT and grafted between January 1980 and December 2006. The age at HSCT varied between 0.5 and 13 years. During this period, a total of 309 children received allogeneic HSCT at Karolinska University Hospital, Huddinge. Of these children, 129 died before the age of 15, and 23 children were not available for evaluation since they were only in Sweden for the transplantation. Thirty-three children received other types of conditioning that did not include radiotherapy or Bu and were excluded from this study. Another 50 children were not available for follow-up because of other reasons.

The children belonged to one of three groups: those receiving sTBI, fTBI or Bu in combination with other chemotherapeutic agents depending on diagnosis. At our centre, fTBI was not used before 1998. Baseline patient characteristics are shown in Table 1. The protocol for this study was approved by the ethical committee at Karolinska University Hospital, Huddinge.

Conditioning

Single-dose TBI, 7.5–10 Gy with the lungs shielded to receive no more than 7–9 Gy, in combination with cyclophosphamide (Cy) 60 mg kg^{-1} for two consecutive days was administered to 33 patients (Ringdén *et al*, 1999). One patient received the sTBI/Cy protocol with the addition of Vepecide (VP) 300 mg m^{-2} and one with the addition of Fludarabine 30 mg m^{-2} for 3 days.

Fractionated TBI 4×3 Gy and Cy 60 mg kg^{-1} for 2 days were given to 11 patients, with the addition Cytarabine (Ara-C) in 1 and VP in one patient. Fludarabine 30 mg m^{-2} for 3 days and fTBI 2×3 Gy and Cy 60 mg kg^{-1} for 2 days was given to three patients (Ringdén *et al*, 2007).

Twenty-two patients were conditioned with busulfan, 1 mg $kg^{-1} \times 4$ per day for four consecutive days in combination with Cy (Ringdén *et al*, 1999). Another three patients received the Bu/Cy protocol with the addition of VP.

Patients transplanted using unrelated donors, HLA-mismatched donors or patients with a non-malignant disorder were also given antithymocyte globulin (ATG) 2 mg kg^{-1} per day for 3–5 days (Remberger *et al*, 1999) (Table 1).

Donor matching

Among donors, there were 45 HLA-identical sibling/related donors, 22 HLA-A-, B- and DR-matched unrelated donors (MUD) and four with an allele or antigen MUD, and in three cases, mismatched related donors were used (Table 1). Tissue typing for genomic HLA classes I and II were used (Schaffer *et al*, 2003).

Stem cell source and cell dose

The majority of patients in all groups received bone marrow as stem cell source. For detailed information about stem cell source and cell dose, see Table 1. The stem cells were given to the patient, after completed conditioning, as an intravenous infusion. In case of peripheral stem cells, the yield was boosted with daily subcutaneous injections of granulocyte-colony stimulating factor (GCS-F).

Immunosuppressive prophylaxis and treatment of graft-versus-host disease

The majority of patients received metothrexate (MTX) or cyclosporine A (CsA) or both as prophylaxis against GVHD (Storb *et al*, 1988; Ringdén *et al*, 1995). Treatment of acute GVHD consisted of steroids, 2 mg kg^{-1} per day during the first week and 1 mg kg^{-1} per day for the following week (Ringdén *et al*, 1995). In more severe cases, ATG, methylprednisolone, MTX, psoralene and UV light (PUVA) or mesenchymal stem cells were used. Treatment of chronic GVHD consisted of steroids and CsA (Table 1).

Viral serology and prophylaxis

Serology prior to HSCT of the four most common herpes viruses, [*Herpes simplex virus 1* (HSV), *Cytomegalovirus* (CMV), *Varicella zoster virus* (VZV) and *Epstein–Barr virus* (EBV)], was performed in patients and donors. In the sTBI group, 20 patients (59%) were seropositive for 3–4 herpes viruses and 14 (41%) for 0–2 viruses before HSCT. In the fTBI group, the corresponding values were 10 patients (71%) positive for 3–4 herpes viruses and 4 (29%) for 0–2 viruses. In the Bu group, 14 patients (58%) were positive for 3–4 herpes viruses and 10 (42%) for 0–2 viruses. In the sTBI and BU groups, serology for the herpes viruses was not performed on one patient.

In case of herpes simplex virus' IgG titres ≥10 000 or close recurrence of symptoms of herpes virus infection, the patient received oral acyclovir 200 mg × 4 per day as prophylaxis from start of conditioning until neutrophil blood cell count $> 0.5 \times 10^9$ L^{-1} (Lundgren *et al*, 1985). In case of severe oral lesions, the treatment consisted of 5 mg $kg^{-1} \times 3$ per day acyclovir intravenously.

Table 1 Baseline characteristics of 74 hematopoietic stem cell transplantation recipients who received single-dose total body irradiation (sTBI), fractionated total body irradiation (fTBI) or busulfan (Bu)

Variable	*sTBI group (n = 35)*	*fTBI group (n = 14)*	*Bu group (n = 25)*
Age at cond (x, SD)	7.2 ± 33	8.8 ± 4.0	7.7 ± 3.7
Range	1–13	1–13	1–13
Male/Female	17/18	9/5	13/12
Underlying disease			
ALL	18	4	3
AML	8	3	6
MDS	3	0	0
CML	1	1	2
Lymphoma T cells	0	1	0
SAA	0	4	1
FHL	0	0	3
CGD	0	0	2
Metabolic disease	5	1	8
Donor matching			
Human leucocyte antigen-identical related	23	3	19
Mismatched	2	3	2
Matched-unrelated donor	10	8	4
Stem cell source			
Bone marrow	35	11	19
PBSC	0	3	4
CB	0	0	2
Conditioning regimen			
sTBI/CY	33	0	0
sTBI/CY + Other	2	0	0
fTBI/CY	0	11	0
fTBI/CY + Other	0	3	0
BU/CY	0	0	22
BU/CY + Other	0	0	3
ATG	11	11	10
Graft-versus-host disease (GVHD) prophylaxis			
MTX	5	0	1
CsA + MTX	18	10	20
CsA + MTX + TcD	0	1	0
CsA + Pred	0	0	2
Sirolimus + Prograf	0	3	1
CsA	11	0	0
CsA + MMF	0	0	1
No prophylaxis	1	0	0
Acute GVHD			
Grade 1	22	4	12
Grade 2–4	5	5	3
Chronic GVHD			
Yes	9	6	6
No	26	8	19

ALL, Acute lymphoblastic leucemia; CY, Cyclophosphamide; AML, Acute myeloblastic leucemia;CsA, Cyclosporine A; MDS. Myelodysplastic syndrome; MTX, Methotrexate; CML, Chronic myelogenous leucemia; TcD, T-cell-depleted graft; SAA, Severe aplastic anaemia; Pred, Prednisolone; FHL, Hemophagocytic lymphohistiocytosis; MMF, Mycophenolate mofetil; CGD, Chronic granulomatous disease (Cell-cept); PBSC, Peripheral blood stem cells; CB, Cord blood.

Busulfan concentration determination

For each patient, the concentration of Bu in plasma was determined, and the area under the plasma concentration time curve (AUC) for the first and the last dose of Bu was calculated according to one compartment open model using Win Non Lin (ver 5.1; Pharsight corporation, Mountain View, CA, USA) (Hassan *et al*, 2000).

Unstimulated and stimulated whole salivary secretion rate

The unstimulated and stimulated whole salivary secretion rates were measured at 15 years of age. At this age, the secretion rate has reached normal adult values (Crossner, 1984). All examinations were performed at the Department of Pediatric Dentistry, Karolinska Institutet, Huddinge.

The patients were instructed not to eat, drink, put anything in their mouth, or brush their teeth for at least one hour before the examination. The saliva was collected in a quiet examination room. The aim was to do the examination in the morning, preferably before lunch.

Unstimulated whole saliva (USSR) was collected over 10 min. The patients were asked to sit in a passive position and to drool or spit into a collecting vessel. Immediately before the test, they were instructed to swallow any remaining saliva in the mouth. The saliva

was allowed to accumulate in the mouth and was collected in the vessel approximately once a minute. The volume was recorded, and the USSR was expressed as ml per min. A USSR of ≤0.1 ml per min was considered low (Dahllöf *et al*, 1997).

After a short break, paraffin-stimulated whole saliva (SSSR) was collected for 5 min. The patient was asked to chew a standard piece of paraffin without swallowing and to spit the saliva into a collecting vessel. Before the collecting procedure started, the paraffin wax was chewed for 1 min, whereupon the patient was instructed to swallow any saliva that might be in the mouth. The volume was recorded, and SSSR was expressed in ml per min. An SSSR of below or equal to 0.5 ml per min was considered low (Dahllöf *et al*, 1997).

Risk factors

Possible risk factors for low salivary secretion rate after HSCT such as gender, age, GVHD, seropositivity for herpes viruses, sTBI, fTBI and Bu were tested in a multivariate analysis against unstimulated and stimulated whole saliva secretion, respectively (Dahllöf *et al*, 1997).

Statistical analyses

The logistic regression model was used in univariate analyses of possible risk factors that may contribute to a low salivary secretion rate. A stimulated whole salivary secretion rate below or equal to 0.5 ml per min was regarded as the event. Significant variables at the 5% level from the univariate analyses were included in a multivariate logistic regression analysis. Comparisons between groups of patients were performed using Fisher's exact test and the Mann–Whitney *U*-test.

Results

The results of this study demonstrate that irrespective of the type of conditioning, there were no significant differences in unstimulated or stimulated salivary secretion at 15 years of age (Table 2). In the three groups, 47% (16/35) of children conditioned with sTBI had a stimulated salivary secretion rate less than or equal to 0.5 ml per min and the corresponding values were 47% (7/15) in the fTBI group and 42% (10/24) in the Bu/Cy group. When analysing salivary secretion in the entire cohort, girls ($n = 35$) had a significantly lower stimulated salivary secretion rate at 15 years of age, 0.7 ± 0.3 ml per min compared with 1.1 ± 04 ml per min in boys ($n = 39$) ($P < 0.001$). The unstimulated secretion rate was also significantly lower in girls 0.3 ± 0.2 ml per min compared with 0.5 ± 0.4 ml per min in boys ($P < 0.05$). Among girls, 66% (23/35) had a stimulated salivary secretion rate less than or equal to 0.5 ml per min compared with 25% (10/39) in boys ($P < 0.001$). As shown in Figure 1, a significant correlation between age at stem cell transplant and stimulated salivary secretion rate at 15 years of age ($P = 0.02$) was found in children conditioned with sTBI/Cy. The younger the patient was at conditioning the lower salivary secretion rate at 15 years of age. This correlation was not seen in children conditioned with fTBI/Cy ($P = 0.35$) or Bu/Cy ($P = 0.35$).

Figure 2a illustrates the distribution of plasma AUC of Bu, expressing the total exposure to Bu in the patient group. AUC varied between 3301 and 8986 ng ml^{-1} per h. We found a significant inverse correlation between the plasma AUC of Bu and stimulated salivary secretion rate measured at 15 years of age (Figure 2b).

There was no difference in salivary secretion between patients with ($n = 21$) or without cGVHD. Unstimulated salivary secretion was in patients with cGVHD: 0.36 ± 0.21 ml per min and in patients without cGVHD: 0.36 ± 0.22 ml per min ($P = 0.93$).

Stimulated salivary secretion in patients with cGVHD: 0.84 ± 0.40 ml per min and without cGVHD: 0.91 ± 0.52 ml per min ($P = 0.90$).

Recipient seropositivity of 0–2 or 3–4 herpes viruses before transplantation was not associated with a low degree of stimulated salivary secretion at 15 years of age. None of the viruses examined, HSV, EBV, CMV or VZV, was individually correlated with salivary dysfunction.

In the univariate analysis, the variable female sex (OR 4.89, 95% CI 1.76–13.61; $P = 0.002$) was associated with low salivary secretion rate at 15 years of age, i.e. ≤0.5 ml per min, while a female donor to male recipient transplantation (OR 0.23, 95% CI 0.06–0.95; $P = 0.039$) was associated with a higher salivary secretion.

In the multivariate model, only the variable female sex remained significantly correlated with low stimulated salivary secretion rate (OR 3.93, 95% CI 1.21–12.79; $P = 0.021$).

Discussion

In the present investigation, we have examined the long-term effects on salivary function in children after stem cell transplantation using three conditioning regimens. The results add three novel findings regarding the effect of chemotherapy and radiation therapy on salivary

Table 2 Salivary secretion rates at 15 years of age in 74 allogeneic stem cell recipients with different conditioning regimens, mean ± SD

Variable	*sTBI group* (n = *35*)	*fTBI group* (n = *14*)	*Bu group* (n = *25*)
Unstimulated			
Salivary secretion rate	0.34 ± 0.23	0.34 ± 0.22	0.40 ± 0.19
Stimulated salivary			
Secretion rate	0.86 ± 0.44	0.93 ± 0.60	0.92 ± 0.50

sTBI, Single-dose total body irradiation; fTBI, Fractionated total body irradiation; Bu, Busulfan.

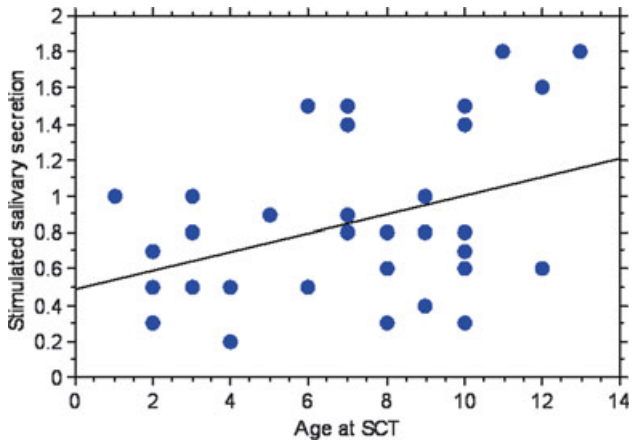

Figure 1 Correlation between age at stem cell transplantation and stimulated salivary secretion rate at 15 years of age in children conditioned with sTBI and CY ($P = 0.0228$). SCT, Stem cell transplantation

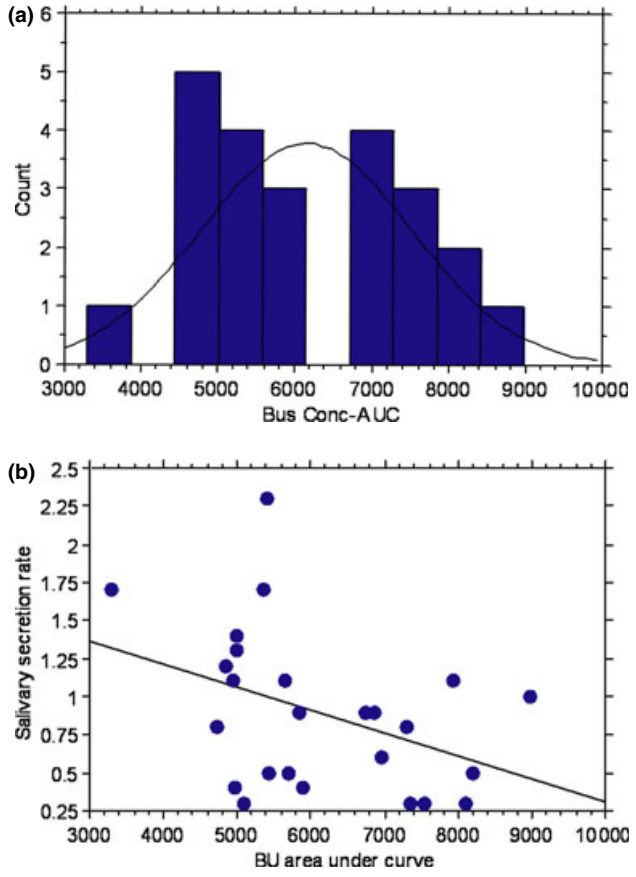

Figure 2 (**a**) Distribution of busulfan AUC in 25 children conditioned with busulfan and cyclophosphamide. Count: Number of children. Bus Conc – AUC: Total systemic exposure (area under curve [AUC]) of busulfan in plasma. (**b**) Correlation between total systemic exposure (AUC) of busulfan and stimulated whole salivary secretion rate at 15 years of age

secretion in children. First, we found no significant difference with regard to the three conditioning regimens on long-term salivary function. Secondly, we demonstrate a negative correlation between age at conditioning with sTBI and salivary function; this correlation was not seen using fTBI or Bu. We also found that salivary dysfunction after conditioning with Bu correlates with the total systemic exposure to Bu.

Previous studies comparing the intensity of the conditioning regimens show that reduced intensity regimens (RIC) are associated not only with lower transplant-related mortality but also with higher relapse risk. The balance of these two factors has resulted in similar overall survival with RIC and myeloablative regimens (Adkins and Dipersion, 2008). Chronic GVHD in long-term survivors of childhood cancer involve multiple organ systems and have a wide variety of severity. It is also clear that damage to the organ systems of children caused by chemotherapy and radiation therapy may not become evident for many years after treatment. The incidence of health conditions reported in this group of patients increases with time and does not appear to plateau (Oeffinger *et al*, 2006).

We recently reported that children conditioned with fTBI have less salivary dysfunction 1 year after Stem cell transplantation (SCT) than those conditioned with sTBI or Bu (Garming Legert *et al*, 2011).In the present study, with a mean follow-up time of 8 years after SCT, there were no significant differences between the three conditioning regimens. Dose fractionation of TBI is used as hematopoietic cells are less capable of DNA repair than other tissue cells as well as a reduced risk for late organ toxicity (Aschan, 2007). It was not expected to find that 8 years after SCT, the level of salivary function does not differ between children conditioned with either fTBI or sTBI.

It has also been reported that the addition of concomitant chemotherapy, in this study Cy, is associated with an increased acute toxicity, in particular mucositis, and there is some evidence to suggest an increased incidence of salivary gland dysfunction when compared to radiation alone (Kosuda *et al*, 1999). It is also possible that fTBI can cause damage to progenitor secretory cells that in long-term lose their capacity to multiply.

The level of salivary output in this group of long-term survivors after SCT is lower than levels reported in healthy teenagers. Among the children, in this study, the stimulated salivary secretion rate at 15 years of age varied between 0.2 and 2.2 ml per min, and about 45% had a stimulated secretion rate below or equal to 0.5 ml per min. Crossner (1984) showed that the salivary output increased up to 15 years of age and that a mean stimulated salivary secretion rate in boys and girls was 1.9 ± 0.7 and 1.6 ± 0.7 ml per min, respectively. As girls have a significantly lower salivary gland secretion than boys, because they are smaller, there is a higher probability that they will have a secretion rate ≤0.5 ml per min after conditioning. In the multivariate analysis, the variable female sex was the only remaining risk factor. This is in agreement with a previous report from our group (Bågesund *et al*, 2000).

Busulfan and Cy are an alternative conditioning regimen used to avoid the detrimental effects of radia-

tion on growth and central nervous system development. A significant problem with oral Bu is the wide interpatient variability of pharmacokinetics depending on unpredictable intestinal absorption, age and metabolism (Hassan *et al*, 1996). By examining Bu plasma concentrations, a high AUC correlates with increased toxicity, mainly hepatic veno-occlusive disease and seizures, and a low AUC results in a higher risk of graft rejection or relapses (Ljungman *et al*, 1997). Young children have a lower systemic exposure compared with adults given identical Bu doses based on body weight (Schuler *et al*, 1994). In agreement with previous reports on other side effects, salivary dysfunction in children conditioned with Bu was significantly correlated with the total systemic exposure of Bu. This is most probably due to that busulfan is equally distributed to saliva as to plasma, and hence, salivary glands are exposed to high concentrations of an alkylating agent during 4 days (Hassan *et al*, 1991). Monitoring blood levels of Bu followed by dose adjustment to achieve a targeted steady-state concentration is important to reduce toxic side effects.

Stem cell-related therapies are widely viewed as offering promise for people suffering from various types of tissue damages (Zander *et al*, 2006) Studies have shown that gender-mismatched bone marrow transplant recipients may serve as natural populations for generation of mature bone marrow-derived epithelial cells in the liver, lung, skin and gastrointestinal tract (Mattsson *et al*, 2004; Krause, 2005) The mechanism is not yet fully understood, and although bone marrow-derived nonhematopoietic cells can perform tissue-specific functions, their number becomes a limiting factor. There is also evidence that tissue injuries promotes recruitment of primitive multipotent cells residing outside that tissue to participate in tissue repair (Lagasse *et al*, 2000). It is possible that circulating donor stem cells may differentiate into epithelial cells in the salivary gland, but further determination of the roles of resident and circulation populations of stem cells is needed for enabling the progress towards the repair process of salivary gland damages.

In young adults, xerostomia is associated with reduced oral health-related quality of life (Thomson, 2005). In adults receiving conditioning for HSCT, salivary gland hypofunction can be associated with lower quality of life because of a significant influence of other major complications of cancer therapy as soft tissue destruction, taste loss, carious destruction and GVHD. Domains such as senses, speech, sleep, eating, swallowing; social contact, dyspnoea and need for nutritional support are affected. A systematic review by Jensen *et al*, 2010; found no studies addressing quality of life in relation to salivary gland hypofunction or xerostomia as sequelae of conditioning with total body irradiation or chemotherapy for HSCT. In adults, it is reported that a more than a 50% reduction in salivary flow is accompanied by xerostomia (Dawes, 2004). We have also previously reported that in paediatric long-term survivors of cancer that received SCT as treatment, 79% express xerostomia (Bågesund *et al*, 2000).

In the univariate analysis, female donor to male recipient was significantly correlated with higher salivary secretion. This is most likely an effect of boys having a higher salivary secretion rate from the start than girls.

Surprisingly, there was no correlation between cGVHD and low salivary secretion, as the sicca syndrome is one of the major symptoms of cGVHD particularly in adults (Ringdén and Deeg, 1996). The reason for this might be that in our study cohort, the sicca syndrome was not a major feature of cGVHD. This is in line with previous reports from our research group on salivary function in children after HSCT (Dahllöf *et al*, 1997).

In conclusion, we found no difference in long-term salivary function after HSCT in adolescents at 15 years of age receiving conditioning with sTBI, fTBI or Bu. Girls had significantly lower salivary secretion than boys. Furthermore, we found a negative correlation between total systemic exposure of Bu and the stimulated whole salivary secretion.

Acknowledgements

The study was supported by grants from the Swedish Dental Society, Swedish Medical Society, Swedish Childhood Cancer Foundation (014/09; 090/08), Swedish Cancer Foundation (100280) and Karolinska Institutet. The authors declare no conflict of interest.

Author contribution

All authors have substantially contributed to this study by designing it, collecting and analysing the samples, analysing the data and/or writing the manuscript.

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