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Targeted anti-apoptosis activity for ovarian protection against chemotherapy-induced ovarian gonadotoxicity

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Abstract Chemotherapy damages the reproductive system by enhancing apoptosis, and evidence suggests that targeted anti-apoptotic therapy may preserve fertility in patients receiving chemotherapy. To investigate the protective effect of sphingosine-1-phosphate (S1P) on chemotherapeutic agent-induced ovarian gonadotoxicity, busulfan-treated female mice were pre-treated with low (0.5 mM) and high (2.0 mM) doses of S1P or vehicle 1 h before busulfan injection. In the S1P groups, each mouse was injected with low-dose S1P in one ovary and high-dose S1P in the contralateral ovary. Four weeks later, the ovaries were removed for histological and biochemical examinations. Caspase 3 immunoreactivity was greater in mice treated with busulfan compared with mice pre-treated with S1P, in which more primordial follicles were observed ($P < 0.05$). The mRNA level of anti-Müllerian hormone was higher in mice pre-treated with S1P than those that received busulfan only, indicating a better ovarian function in mice pre-treated with S1P. No difference was observed in the levels of growth differentiation factor-9 among all groups. In conclusion, S1P protects primordial follicles from chemotherapy-induced gonadotoxicity, and may partially preserve ovarian function.

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Introduction

Cytotoxic chemotherapy is widely used to treat cancers, autoimmune diseases or haematological diseases, resulting in the prolongation of the life expectancy of recipient patients. Some agents, however, including cyclophosphamide, ifosfamide, chlorambucil, melphalan, procarbazine, and butanediol dimethanesulfonate (busulfan), have high gonadotoxicity (Oktem and Urman, 2010; Park et al., 2013). Our previous study also demonstrated that busulfan destroyed ovarian tissue and depleted the follicle pool (Tan et al., 2010). Chemotherapy-induced gonadotoxicity may cause premature ovarian failure in women of reproductive age (Larsen et al., 2003a), which is a common sequela of the depletion of a limited ovarian reserve; a menopausal state and infertility ensue. Therefore, a strategy to maintain and restore ovarian function in patients undergoing chemotherapy who desire future fertility is urgently needed.

Apoptosis is thought to be one of the mechanisms underlying ovarian follicle loss (Hancke et al., 2007; Morita et al., 2000; Perez et al., 1997). The phospholipids ceramide and sphingosine-1-phosphate (S1P) play an important role in this process. Ceramide is involved in many cell activities, including growth arrest and apoptosis; these result from an increase in endogenous ceramide levels caused by various stress factors, such as cytokines, drugs, and chemotherapeutic agents (Perry, 1999). In contrast with the action of ceramide, S1P regulates cell growth, cell differentiation, inflammation and vasculogenesis, and counterbalances the ceramide-activated apoptotic pathways (Cuvillier et al., 1996; Payne et al., 2002; Spiegel and Kolesnick, 2002). Previous studies have shown the ability of S1P to prevent apoptosis-related ovarian damage caused by chemotherapy or radiotherapy (Hancke et al., 2007; Morita et al., 2000; Perez et al., 1997; Spiegel and Kolesnick, 2002), indicating a potential role for S1P therapy in fertility preservation.

The identification of women with prematurely decreased ovarian reserves, although challenging, is clinically important and urgent. Ovarian reserve and function can be evaluated by clinical parameters, such as pregnancy rate and resumption of menstruation, hormone markers (including FSH, oestradiol and inhibin-B), and ultrasonographic markers (antral follicle count and measurement of ovarian volume) (Beck-Fruchter et al., 2008; Domingues et al., 2010). Unfortunately, most tests in current clinical use have low predictive accuracy and are best regarded as screening tools (Broekmans et al., 2006; Domingues et al., 2010). Anti-Müllerian hormone (AMH), expressed in granulosa cells of growing follicles, is considered as the best hormonal marker of ovarian reserves because of its cycle independence and strong correlation with the follicle pool (Hansen et al., 2011; Kevenaar et al., 2006; La Marca et al., 2006; Van Disseldorp et al., 2010). The concentration of AMH falls rapidly during the administration of chemotherapy (Anderson et al., 2006), and reduced AMH concentrations have been reported in cancer survivors who have undergone chemotherapy (Lie Fong et al., 2009). A recent study showed that long-term ovarian function after chemotherapy can be predicted by the serum AMH concentration measured before the treatment (Anderson and Cameron, 2011). Therefore, AMH can be used for clinical evaluation of ovarian reserve and function.

Growth differentiation factor 9 (GDF-9) is an oocyte-specific protein that is secreted by growing oocytes. It plays an important role in stimulating the proliferation of granulosa cells and folliculogenesis (Dong et al., 1996; Fitzpatrick et al., 1998; Hreinsson et al., 2002; Kedem et al., 2011; McGrath et al., 1995). The expression of the GDF-9 mRNA and protein was first detected in the oocytes within the primary follicles and at more advanced stages in mice and humans in in-vitro and in-vivo studies (Hayashi et al., 1999; McGrath et al., 1995; Sadeu et al., 2008). In mice lacking GDF-9, an early blockage of folliculogenesis at the one-layer primary follicle stage was reported (Dong et al., 1996; McNatty et al., 2005), indicating the role of GDF-9 in folliculogenesis. These findings suggest that GDF-9 could be considered as another marker that reflects the number and function of primary follicles, and may be used to assess oocyte quality and fertility.

Follicular counting in ovarian tissues from biopsies is a direct and more accurate method of estimating ovarian reserve (Lass, 2004). Although it is considered inappropriate for clinical evaluation in women because of the high variation in follicular distribution and the invasiveness and surgical risk of the procedure, histological examination of ovarian tissue in animal models can provide additional information and promote the understanding of the mechanisms involved more easily and clearly (Hancke et al., 2007; Tan et al., 2010). Caspase-3, which is a cysteine protease that is required for the activation of DNAse and promotion of DNA cleavage in internucleosomal spaces, acts as an effector of apoptosis (Shi, 2002). It may be used as an apoptosis marker in granulosa cells and oocytes in humans (Glamoclija et al., 2005; Hurst et al., 2006; Poljicanin et al., 2013) and mice (Fenwick and Hurst, 2002), as it is involved in almost all apoptotic pathways.

In this study, the effect of busulfan on gonadal function was investigated using histological examinations (follicle counting and caspase-3 immunohistochemical staining) and molecular markers (AMH and GDF-9) in mice. Moreover, the protective effect of the anti-apoptotic S1P on busulfan-induced ovarian gonadotoxicity was examined, and an attempt was made to verify the role of apoptosis in fertility damage.

Materials and methods

Animals

Twenty-four sexually mature, virgin, female FVB/NJNarl mice (8 weeks of age) were obtained from the National Laboratory Animal Centre in Taipei, Taiwan, and housed at the Animal Centre of the Taipei Medical University under a 12-h light–dark cycle with free access to food and water. All procedures were in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, and approved by the Animal Experimental Committee at the Taipei Medical University and Taipei Medical University Hospital in Taipei, Taiwan (reference number LAC-2013-0048, 27 January 2014).

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