Preventable effect of L-threonate, an ascorbate metabolite, on androgen-driven balding via repression of dihydrotestosterone-induced dickkopf-1 expression in human hair dermal papilla cells

Mi Hee Kwack¹, Ji Sup Ahn², Moon Kyu Kim¹, Jung Chul Kim¹ & Young Kwan Sung¹,*
¹Department of Immunology, School of Medicine, Kyungpook National University, Daegu 700-422, ²Dr. Ahn Medical Hair Clinic, Seoul 135-996, Korea

In a previous study, we recently claimed that dihydrotestosterone (DHT)-inducible dickkopf-1 (DKK-1) expression is one of the key factors involved in androgen-potentiated balding. We also demonstrated that L-ascorbic acid 2-phosphate (Asc 2-P) represses DHT-induced DKK-1 expression in cultured dermal papilla cells (DPCs). Here, we investigated whether or not L-threonate could attenuate DHT-induced DKK-1 expression. We observed via RT-PCR analysis and enzyme-linked immunosorbent assay that DHT-induced DKK-1 expression was attenuated in the presence of L-threonate. We also found that DHT-induced activation of DKK-1 promoter activity was significantly repressed by L-threonate. Moreover, a co-culture system featuring outer root sheath (ORS) keratinocytes and DPCs showed that DHT inhibited the growth of ORS cells, which was then significantly reversed by L-threonate. Collectively, these results indicate that L-threonate inhibited DKK-1 expression in DPCs and therefore is a good treatment for the prevention of androgen-driven balding. [BMB reports 2010; 43 (10): 688-692]

INTRODUCTION

The dermal papilla (DP) and dermal sheath of a mammalian hair follicle are derived from the mesenchyme. Hair follicles also contain epithelial cells in the outer root sheath (ORS), inner root sheath, matrix, and hair shaft that are derived from the epithelium (1). Reciprocal interactions between the epithelium and mesenchyme are essential for postnatal hair growth (2). The DP is known to play a key role in the regulation of hair growth and is encapsulated by the overlying follicular keratinocytes during hair growth period. Factors from the DP are believed to stimulate proliferation and differentiation of follicular keratinocytes into the hair shaft (3).

Male-pattern baldness (MPB) is the most common type of hair loss in men. Although its molecular pathogenic mechanism is not clear, dihydrotestosterone (DHT)-dependence has been well demonstrated in MPB (4, 5). Further, the treatment effects of finasteride, a selective inhibitor of type II 5α-reductase (5α-R II) that converts testosterone to DHT, support DHT-dependence in MPB (6). Circulating androgens such as DHT enter the follicle via capillaries in the DP, bind to androgen receptor (AR) within dermal papilla cells (DPCs), and then activate or repress target genes (7). Recent studies suggest that DHT-driven release of autocrine and paracrine factors from DPCs may be the key to androgen-potentiated balding (8-10).

We recently found that dickkopf 1 (DKK-1) is one of the most upregulated genes in balding DPCs (11). DKK-1 encodes a potent and specific endogenously-secreted Wnt antagonist that binds and inhibits low-density lipoprotein (LDL) receptor-related protein co-receptors that are involved in canonical Wnt signaling during hair induction and growth (12-14). Based on the finding that DHT-inducible DKK-1 expression in balding DPCs causes apoptosis in follicular keratinocytes, we claimed that DKK-1 is one of the key factors involved in androgen-potentiated balding (11).

L-ascorbic acid 2-phosphate (Asc 2-P) liberates L-ascorbic acid (AsA) via alkaline phosphatase present on the plasma membrane of various kinds of cells (15). This is followed by the incorporation of AsA into the cells. Very recently, we demonstrated that Asc 2-P represses DHT-induced DKK-1 expression in cultured DPCs of human hair follicles (16). In this study, we first investigated whether or not L-threonate, a metabolite of Asc 2-P, could attenuate DHT-induced DKK-1 expression in cultured DPCs by RT-PCR and ELISA. We next examined whether or not L-threonate could reverse the growth inhibitory role of DHT-inducible DKK-1 in follicular ORS kera-
tinocytes using an in vitro co-culture system.

RESULTS AND DISCUSSION

L-threonate represses DHT-induced DKK-1 expression
Consistent with our previous report (11), we observed that 100 nM DHT induced DKK-1 mRNA expression by RT-PCR analysis (Fig. 1A, compare lanes 1 and 2). When L-threonate was added together with DHT, DHT-induced DKK-1 mRNA expression in DPCs was significantly attenuated (Fig. 1A, compare lanes 2, and 3 and 4). We next measured the concentration of DKK-1 in conditioned medium using ELISA. The mean concentration of DKK-1 was 11.49 ng/ml in the presence of 100 nM DHT and 5.25 ng/ml in the absence of DHT, demonstrating upregulation of DKK-1 in response to DHT (Fig. 1B, compare lanes 1 and 2). When 0.25 and 1 mM L-threonate was added together with DHT, the mean amount of DKK-1 was reduced to 5.03 and 5.62 ng/ml, respectively, demonstrating that DHT-induced DKK-1 secretion was repressed by L-threonate in DPCs (Fig. 1B, compare lanes 2, and 3 and 4).

L-threonate represses DHT-induced activation of DKK-1 promoter activity
A pGL3-DKK-1 promoter plasmid that expresses a luciferase reporter gene at different levels in response to various levels of DKK-1 promoter activity was constructed and used to further confirm the repression of DHT-induced DKK-1 expression by L-threonate. We found that DKK-1 promoter activity was increased by DHT treatment (Fig. 2, compare lanes 1 and 2). When L-threonate was added together with DHT, DHT-induced activation of luciferase activity in DPCs was significantly repressed (Fig. 2, compare lanes 2, and 3 and 4).

L-threonate attenuates DHT-induced growth inhibition of co-cultured keratinocytes
A co-culture system employing DPCs and keratinocytes has been used previously to analyze epithelial-mesenchymal inter-