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Decrease in circulating autotaxin by oral administration of prednisolone

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ABSTRACT

Background: Autotaxin (ATX), secreted mainly from adipose tissue, functions as a lysophospholipase D (lysoPLD) to hydrolyze lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA). ATX–LPA signaling is implicated in a wide range of physiological and pathophysiological processes including immune response.
Methods: The present study measured serum ATX antigen levels in patients with various autoimmune diseases using a recently developed automated enzyme immunoassay. In addition, serum lysoPLD activity was assessed by measuring choline liberation from the substrate LPC. Moreover, the effect of prednisolone (PSL) on mRNA expression of ATX was evaluated using cultured adipose tissue from mice.
Results: Decreased serum ATX antigen levels were observed after the initiation of treatment with PSL. The decreased levels recovered during tapering of PSL dose in a dose-dependent manner without exacerbation of disease activity. Moreover, decreased ATX mRNA expression in PSL-treated cultured murine adipose tissue suggested that the effect of PSL on serum ATX may have resulted from changes in adipose tissue ATX expression.
Conclusions: Our results suggest that measurement of serum ATX antigen level may be clinically useful for the assessment of steroid treatment effect and drug compliance with steroids. Furthermore, our findings provide many novel insights into the biosynthesis, physiological functions, pathological roles, and clinical significance of circulating ATX.

1. Introduction

Lysophosphatidic acid (LPA; 1- or 2-acyl-*sn*-glycerol-3-phosphate) is a bioactive lipid molecule with a phosphate, a glycerol, and a fatty acid in its structure [1]. The cellular effects of LPA include proliferation, migration, cytokine secretion, and morphological change [2]. These pleiotropic actions allow LPA to participate in a wide variety of biological processes, such as brain development, oncogenesis, wound healing, and vascular development [2,3]. These LPA actions are mediated through the activation of its G protein-coupled receptors. To date, at least 6 subtypes of LPA receptor (LPA_{1-6}) have been identified [4,5] by various laboratories including ours.

Several pathways contribute to LPA production, and it is now clear that production of plasma/serum LPA is regulated by autotaxin (ATX) through its lysophospholipase D (lysoPLD) activity [6,7]. This lipase hydrolyzes lysophospholipids, mainly lysophosphatidylcholine (LPC), to produce LPA.

ATX is a 125-kDa glycoprotein and a potent cell motility-stimulating factor originally isolated from the conditioned medium of A2058 human melanoma cells [8]. ATX belongs to the ectonucleotide pyrophosphatase/phosphodiesterase (ENPP) family and is also known as ENPP2. ATX is widely expressed, with the highest mRNA levels detected in adipose tissue, brain, placenta, ovary, and small intestine [9,10]. ATX is also overexpressed in several human malignancies, including glioblastoma multiforme [11] and prostate cancer [12].

Studies in mice reveal that ATX–LPA has key roles in a wide range of physiological and pathological processes such as vascular and neural development, lymphocyte homing, fibrosis, and cancer [13]. In particular, ATX-deficient mice have been reported to suffer from severe vascular defects and died around E10.5 [6,7,14]. Notably, similar phenotypes at this stage were observed in a fraction of LPA_4-deficient embryos [3]. Therefore, the ATX–LPA–LPA_4 signaling axis may be involved in vascular development.

LPA is present in human serum, plasma, saliva, follicular fluid, and malignant effusions at a physiologically significant level [15,16].

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Moreover, ATX is the only factor known to exhibit lysoPLD activity in serum [17]. In sera from heterozygous ATX-null mice, both lysoPLD activity and LPA concentrations were about half of those observed in sera from wild-type mice, indicating that ATX is responsible for the bulk of LPA production in serum [6,7]. Furthermore, when ATX-depleted human [18], mouse [6], or bovine [19] serum was prepared using immunoprecipitation with an anti-ATX monoclonal antibody, lysoPLD activity was negligible in ATX-depleted serum, indicating that ATX fully accounts for serum lysoPLD activity. In line with these findings, a strong correlation between serum ATX activity and plasma LPA level was observed in humans [20] and mice [21]. Considering the importance of ATX as an enzyme that exerts lysoPLD activity and produces LPA, the measurement of serum ATX concentrations is considered important.

Recently, an ATX immunoenzymetric system has been developed [22], which requires much less time to obtain results compared to ATX activity assays. Using this assay, serum ATX level was strongly correlated with serum ATX activity in humans, with higher levels observed in females than in males [22]. To date, the significance of plasma or serum ATX level in patients has been reported in patients with hypoalbuminemia [22], nephrotic syndrome [23], follicular lymphoma [24], pancreatic cancer [25], liver fibrosis [26], and pregnancy-induced hypertension [27].

Various types of skin diseases are treated in the field of dermatology. In this study, we examined whether serum ATX level could be useful as a biomarker of patients with skin disorders such as autoimmune disease and malignant melanoma. In the process of measurement, we focused particularly on patients with autoimmune diseases including collagen diseases, who often undergo oral steroid therapy. Steroids, such as prednisolone (PSL) and betamethasone (BMS), have potent immunosuppressive effects and are commonly used to treat a range of immunological and inflammatory diseases in many clinical fields including dermatology. We herein report findings concerning the relationship between serum ATX level and steroid therapy.

2. Materials and methods

2.1. Patients

Serum samples were obtained from 55 patients (198 samples) with the following autoimmune diseases, diagnosed based on diagnostic criteria and/or clinical characteristics: systemic sclerosis (SSc) ($n=20$; 14 females and 6 males), dermatomyositis (DM)/polymyositis (PM) ($n=6$; three females and two males), systemic lupus erythematosus (SLE) ($n=18$; 8 females and 10 males), morphea ($n=4$; four females), eosinophilic fasciitis (EF) ($n=5$; 2 female and 3 males), and bullous pemphigoid (BP) ($n=13$; 7 female and 6 males). Additionally, the detailed breakdown of patients with SSc was as follows: diffuse cutaneous SSc (dcSSc) ($n=16$; 11 females and 5 males), limited cutaneous SSc (lcSSc) ($n=4$; 3 females and 1 male), overlapped with SLE ($n=2$, 2 females), and overlapped with DM ($n=1$, 1 female). For comparison, 16 healthy subjects were enrolled ($n=16$ samples; 8 females and 8 males). In addition, 13 patients with malignant melanoma ($n=13$; 8 females and 5 males) were enrolled based on the fact that ATX was originally isolated from human melanoma cells. Serum ATX level is reportedly increased in patients with chronic hepatitis in a manner related to the grade of fibrosis [26]. Therefore, patients with hepatic disorder were excluded from the current study.

All the patients had been treated at the Department of Dermatology, University of Tokyo Hospital, Tokyo, Japan, from 2006 to 2008. This study was carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Research Ethics Committee of the Faculty of Medicine of the University of Tokyo. Informed consent was obtained from the patients for the use of all samples. To obtain the serum samples, whole blood was directly put into glass tubes and left to stand for 15 min at room temperature to allow blood clots to form; then, serum was separated by centrifugation at $1500\times g$ for 5 min and frozen at −80 °C until assayed. As a point of reference, sampling intervals were extended for at least 2 days after the dose change or start of PSL therapy.

2.2. Automated immunoassay for quantitative determination of ATX antigen

Serum ATX antigen concentration was determined using a specific sandwich enzyme immunoassay, as previously described in detail, in which the within-run and between-run CVs were 3.1–4.6% and 2.8–4.6%, respectively [22]. Briefly, the assay reagent was compatible with a commercial automated immunoassay analyzer AIA-system (Tosoh, Tokyo, Japan), which includes automated specimen dispensation, incubation of the reaction cup, a bound/free washing procedure, 4-methylumbelliferyl phosphate substrate dispensation, fluorometric detection, and a result report.

2.3. Measurement of lysoPLD activity

ATX activity was measured as lysoPLD activity, as previously described in detail [17]. Briefly, serum lysoPLD activity was assessed by measuring choline liberation from the substrate LPC. The reactions were performed in 100-μl aliquots; the serum samples (20 μl) were incubated with 2 mmol/l of 1-myristoyl (14:0)-LPC (Avanti Polar Lipids Inc., Alabaster AL) in the presence of 100 mmol/l Tris–HCl (pH 9.0), 500 mmol/l NaCl, 5 mmol/l $MgCl_2$, 5 mmol/l $CaCl_2$, and 0.05% Triton X-100 for 3 h at 37 °C. Liberated choline was detected using an enzymatic photometric method with choline oxidase (Asahi Chemical, Tokyo, Japan), horseradish peroxidase (Toyobo, Osaka, Japan), and TOOS reagent (*N*-ethyl-*N*-(2-hydoroxy-3-sulfoproryl)-3-methylaniline; Dojindo Laboratories, Kumamoto, Japan) as a hydrogen donor. This choline measurement was performed in an absorption spectrometer.

2.4. Collection and culture of murine adipose tissue

Mouse epididymal fat pads were obtained and cultured as described previously with minor modifications [28,29]. Briefly, epididymal adipose tissue was removed from C57BL/6 mice under sterile conditions. The epididymal adipose tissue was washed three times in phosphate-buffered saline containing 0.1% bovine serum albumin (BSA; fraction V, fatty acid-free; Sigma, St. Louis, MO) and once in Dulbecco's Modified Eagle's Medium (DMEM; Sigma) containing 10% fetal bovine serum. A 400-μl aliquot of minced tissue was transferred into 4 ml low-glucose DMEM containing 0.1% BSA in 6-cm culture dishes and incubated at 37 °C with shaking (60 oscillations/min). Some culture dishes also included 100 μg/ml prednisolone (Sigma). After incubation for 24 h, floating adipose tissue was harvested and frozen in liquid nitrogen. All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Tokyo.

2.5. Quantitative real-time PCR

Total RNA was prepared from the primary culture of mouse adipose tissue using Isogen (Nippon Gene, Tokyo, Japan). cDNA was obtained from total RNA with SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA). Using the LightCycler System (Roche Applied Science, Indianapolis, IN), quantitative real-time PCR was performed with 10-fold diluted cDNA reaction mixture. The PCR products were detected via intercalation of the fluorescent dye SYBR Green with the FastStart DNA Master SYBR Green I kit (Roche Applied Science, Indianapolis, IN). The LightCycler PCR parameters were: initial denaturation at 95 °C for 4 min and 50 cycles of denaturation at 95 °C for 15 s, annealing at 65 °C for 5 s with single fluorescence acquisition and elongation at 72 °C for 7 s, all with a temperature