



Review

Expression of 11beta-hydroxysteroid-dehydrogenase type 2 in human thymus



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ABSTRACT

11beta-hydroxysteroid-dehydrogenase type 2 (11β-HSD2) is a high affinity dehydrogenase which rapidly inactivates physiologically-active glucocorticoids to protect key tissues. 11β-HSD2 expression has been described in peripheral cells of the innate and the adaptive immune system as well as in murine thymus. In absence of knowledge of 11β-HSD2 expression in human thymus, the study aimed to localize 11β-HSD2 in human thymic tissue.

Thymic tissue was taken of six healthy, non-immunologically impaired male infants below 12 months of age with congenital heart defects who had to undergo correction surgery. 11β-HSD2 protein expression was analyzed by immunohistochemistry and Western blot. Kidney tissue, peripheral blood mononuclear cells (PBMCs) and human umbilical vein endothelial cells (HUVEC) were taken as positive controls.

Significant expression of 11β-HSD2 protein was found at single cell level in thymus parenchyma, at perivascular sites of capillaries and small vessels penetrating the thymus lobuli and within Hassall's bodies.

The present study demonstrates that 11β-HSD2 is expressed in human thymus with predominant perivascular expression and also within Hassall's bodies. To our knowledge, this is the first report confirming 11β-HSD2 expression at the protein level in human thymic tissue underlining a potential role of this enzyme in regulating glucocorticoid function at the thymic level.

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Contents

1. Introduction	36
2. Materials and methods	36
2.1. Tissue and cell samples	36
2.2. Immunohistochemical staining	36
2.3. Western immunoblot analysis	36
2.4. PCR	36
2.5. Concentrations of tissue cortisol and cortisone	37
3. Results	37
3.1. 11β-HSD2 and 11β-HSD1 protein expression	37

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3.2. PCR of 11 β -HSD1 and 11 β -HSD2 gene expression	37
3.3. Concentrations of tissue cortisol and cortisone	37
4. Discussion	37
Disclosure statement	39
Acknowledgement	39
References	40

1. Introduction

1 β -hydroxysteroid-dehydrogenase type 1 (11 β -HSD1) and type 2 (11 β -HSD2) are high affinity dehydrogenases which rapidly inactivate physiological glucocorticoids (cortisole and corticosterone) to inert 11-keto forms (cortisone and 11-dehydrocorticosterone) in order to protect key tissues [1,2]. 11 β -HSD2 is a 405 aminoacid long 44 kDa protein and is highly expressed in kidney and placenta, and also in colon and salivary glands [3]. 11 β -HSD2 may not only play a role in embryonic development [4] and fetal programming [5] but also in human hypertension [6]. Glucocorticoids as a key target of 11 β -HSD1 and 11 β -HSD2 have been shown to have an impact on thymic development and selection processes, but most studies investigating the influence of glucocorticoids on lymphoid organs are derived from mouse tissue [7,8]. 11 β -HSD1 and 11 β -HSD2 expression have been described in peripheral cells of the innate [9,10] and the adaptive immune system [11,12] as well as in murine thymus [13–18]. Increased attention has been paid towards a potential influence of 11 β -HSD1 and 11 β -HSD2 on immune functions [19]. A partly different role of 11 β -HSD1 and 11 β -HSD2 has been described for inflammatory conditions in humans [9,12,19,20]. So far, expression of 11 β -HSD2 has not been localized in human thymic tissue.

Considering the wide-spread functions of 11 β -HSD2 and the essential role of glucocorticoids in thymocyte apoptosis considered by many authors [8,21], the study aimed to localize 11 β -HSD2 in human thymic tissues. Significant expression of 11 β -HSD2 protein was found at single cell level in thymus parenchyma, at perivascular sites of capillaries and small vessels penetrating the thymus lobuli and within Hassall's bodies.

2. Materials and methods

2.1. Tissue and cell samples

Thymic tissue of six healthy, non-immunologically impaired male infants below 12 months of age with congenital heart defects who had to undergo correction surgery was taken and snap frozen in liquid nitrogen immediately after removal by the surgeon at the Department of Surgery, Medical University Innsbruck. The thymic tissue had to be removed for surgical reasons to improve the surgical access to the heart as described previously [22]. Peripheral blood mononuclear cells (PBMCs) were obtained from immunological healthy donors at the Department of Pediatrics, University of Wuerzburg. Human umbilical vein endothelial cells (HUVECs) were provided by the Laboratory of Autoimmunity, Innsbruck. Kidney sections of patients without known renal impairments were provided by the Department of Pathology, Medical University Innsbruck. Kidney tissue was used as positive control. HUVECs were used to represent a positive control for staining of von Willebrand factor (vWF) to characterize endothelial cells. The study was approved by the local ethics committee at the Medical University Innsbruck, Austria and all parents gave their written informed consent.

2.2. Immunohistochemical staining

Acetone-fixed cryostat sections (5 μ m) of the respective frozen tissue samples were stained immunohistochemically using a sheep anti-human 11 β -HSD2 antibody (The Bindingsite, Schwetzingen, Germany; #PC545, final protein concentration 124 μ g/ml) or rabbit F(ab)₂ anti-sheep IgG labeled with horse radish peroxidase (Chemicon, Billerica, USA; #AQ147P, dilution 1:40) and diaminobenzidine (Sigma, St. Louis, USA; #D4168) for visualization. Counterstaining was performed using Mayer's hematoxylin (Dako, Glostrup, Denmark; #S3309, dilution 1:8). Normal sheep serum (Dako; #X0503, final protein concentration: 124 μ g/ml) was applied for negative control purposes respectively. To determine the expression of 11 β -HSD1, rabbit anti-human 11 β -HSD1 antibody (Santa Cruz Biotechnologies, TX, USA; #SC-20175, 1 μ g/mL) was used. After washing steps with phosphate buffered saline (PBS, pH 7.2), slides were incubated with anti-rabbit antibody (Advance HRP Link, Dako, Carpinteria, California; kit #K4069) for 20 min, followed by a washing step with PBS and incubation with antibodies polymerized with horseradish peroxidase (Advance HRP Enzyme, Dako; kit #K4069) for 20 min. Protein expression was visualized with 3,3'-diaminobenzidine (Dako; #K3468) and counterstained with Mayer's hematoxylin. To display vascular endothelial cells mouse IgG₁ anti-human von Willebrandt Factor (Dako; #M0616, final protein concentration 243 μ g/ml) antibody was incubated over night at 4 °C. Protein expression and counterstained was performed as indicated before.

2.3. Western immunoblot analysis

11 β -HSD1 and 11 β -HSD2 protein expression was determined using the rabbit-anti-human 11 β -HSD1 antibody (Santa Cruz Biotechnology, Dallas, TX, USA; #SC-19259, dilution 1:200) and the rabbit-anti-human 11 β -HSD2 antibody (Santa Cruz Biotechnology; #SC-20176, dilution 1:200) [23] in western blotting of PBMCs, HUVECs, thymus, kidney and liver tissue with 50 μ g total protein. Protein extracts were obtained using cell lysis buffer 1 \times (Cell signaling, Danvers, USA; #9803) supplemented with protease inhibitor cocktail (Sigma-Aldrich, Saint Louis, USA; #P2714). Proteins were separated in reducing 8% polyacrylamide gels. Band detection was performed using goat-anti-rabbit Infrared Fluorescence IRDye-800cw (Li-cor, Lincoln, Nebraska; #926-32211). For β -actin expression, the blot was hybridized with mouse-anti- β -actin antibody (Li-cor, Lincoln, Nebraska; #926-42212, dilution 1:5000) and identified using goat-anti-mouse IRDye-680 (Li-cor, Lincoln, Nebraska). Analysis was performed using Li-cor Odyssey (Licor). For detection of protein lengths, size marker 1 (SM 1) (Spectra Multicolor High Range Protein ladder, Fermentas, St. Leon-Rot, Germany; #SM1851) and SM 2 (PageRuler Prestained Protein ladder, Thermo Fisher Scientific, Waltham, USA; #26616) were used.

2.4. PCR

Total RNA was extracted from frozen human thymus, kidney, and PBMCs following the manufacturer's instructions (Macherey-Nagel, Düren, Germany). First strand cDNA synthesis of each

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