Endocrinology & Metabolic Syndrome
ISSN: 2161-1017
Yurekli et al., Endocrinol Metab Syndr 2018, 7:4
DOI: 10.4172/2161-1017.1000e126
Editorial
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AT-101 Decreased 11BHSD2 Expression in AtT20 Cells

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Received date: August 26, 2018; **Accepted date:** August 26, 2018; **Published date:** August 31, 2018

Editorial

Cushing disease results from pituitary adenoma resulting in excess ACTH. Transsphenoidal surgery is the first-line treatment in which recurrence could be seen at a rate of 10-20% during follow-up [1]. The other treatment modalities for the recurrent Cushing disease or for the unsuccessful surgery are radiotherapy and bilateral adrenalectomy. Cushing disease has high mortality and morbidity rates so; there is a need for the effective medical therapy options [1].

Recently, we have showed that AT-101 induced cytotoxicity and apoptosis in mouse pituitary corticotroph tumor AtT20 cells and also AT-101 decreased ACTH secretion significantly.1 AT-101 is an (-/-) enantiomer of gossypol which is a polyphenolic compound found in cottonseed [1]. Tuszysnki and Cossu firstly depicted that gossypol has anticancer effects observed in several cell lines [1]. Apoptotic effect of gossypol comes from the inhibition of Bcl-2 anti-apoptotic proteins and interaction with the mitochondrial caspase pathways [1]. We also showed that AT-101 treatment caused significant changes in mRNA levels of apoptosis-related genes in AtT20 cells [1].

Hypokalemia was observed in subjects using gossypol [1]. It was thought that inhibition of 11 beta hydroxysteroid dehydrogenase 2 (11BHSD2) enzymes was responsible for the hypokalemia [1]. Enzyme of 11BHSD2, which converts active cortisol to inactive cortisone, takes place near to mineralocorticoid receptors in the kidney. When this enzyme is inhibited, mineralocorticoid receptors become activated and hypokalemia develops.

It was shown that cortisol-inactivating enzyme 11BHSD2 is expressed on pituitary tumors [2]. So, our aim was to investigate whether AT-101 decreased the expression of 11BHSD2 in the AtT20 cells.

Cells were grown for 72 h with AT-101 at 37°C. Cell pellets were lysed in M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, Rockford, IL, USA) to prepare cell lysates for Western blot analysis. After centrifugation at 14.000xg for 15 min, protein concentrations were quantitated in duplicate by the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of protein were separated on an SDS polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were blocked with 5% non-fat dry milk prepared in Tris-buffered saline containing 0.1% Tween 20 (TBST) at room temperature for 1 h. The membrane was then incubated with primary antibodies at 4°C overnight. 11BHSD2 antibody (sc-365529) was obtained from Santa Cruz Biotechnology Inc. (Heidelberg, Germany) and prepared according to the manufacturer's instructions. Following several washes in TBST, membranes were incubated with appropriate secondary antibody (1:1000 dilutions, Millipore Upstate USA, Charlottesville, VA) at room temperature for 1 h.

The protein bands recognized by the antibodies were visualized by the Kodak Gel Logic 1500 Imaging System. AtT20 cells were treated with AT-101 at the IC10 and IC50 doses as calculated in the study [1].

As illustrated in Figure 1, 11BHSD2 protein levels were inhibited by IC10 and IC50 values of AT-101 at 72 h investigated by Western blot analysis.

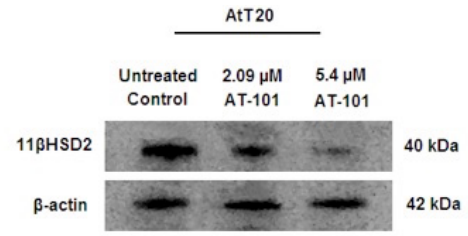

Figure 1: Western blot analysis of 11BHSD2 expression in AtT20 cells treated for 72 h with AT-101.

Korbonits et al. [2] showed that 11BHSD1 protein was expressed in GH and PRL secreting cells. However, ACTH, TSH and gonadotroph secreting cells didn't show 11BHSD1 enzyme activity. But, corticotroph adenomas highly expressed 11BHSD2 enzyme activity. The increased expression of 11BHSD2 activity which converts cortisol to cortisone can change the glucocorticoid feedback control in Cushing disease [2].

Nigawara et al. [3] investigated the effect of 11BHSD2 inhibition on ACTH secretion and apoptosis in AtT20 mouse cortiocotroph tumor cells. They showed that 11BHSD2 inhibition resulted in cellular apoptosis.

Also, glucocorticoid inhibiton of ACTH secretion and POMC expression was increased. It is well known that glucocorticoid hormones are major inducers of apoptosis in some cell types and are associated with growth inhibition of tumor cells. So, 11BHSD2 inhibition may cause apoptosis and inhibition of ACTH secretion by increasing the cortisol levels in the medium.

It is the fact that gossypol inhibits 11BHSD2 enzyme at the level of kidney tubules that leads to hypokalemia as a side effect. Our data point out that AT-101 treatment in AtT20 cells inhibits 11BHSD2 activity.

In conclusion, with the increase of local cortisol concentration at the pituitary level as a result of 11BHSD2 inhibition, AT-101 may cause the effect of anti-proliferation and hormone suppression. Further studies are needed to clarify this effect of gossypol in Cushing disease.

Compliance with Ethical Standards

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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