# Biphenyl Azepanyl Methanone rescues glucocorticoid-induced growth arrest in human hair follicle dermal papilla cells.

HC-95

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## Introduction:

Stress related hormones such as glucocorticoids have negative effects both on skin and hair. Sustained exposure to cortisol leads to the atrophy of skin tissue mainly through extracellular matrix degradation, and increased levels of cortisol have been associated with hair growth disorders [1, 2]. In skin, cortisone is converted intracellularly into its active form cortisol (hydrocortisone) by 11beta-hydroxysteroid dehydrogenase-1 (11βHSD1) [3] (Fig. 1) and the expression of this enzyme is increased by glucocorticoid treatments through a positive feedback loop [4]. The expression of 11βHSD1, which is upregulated in aged skin and upon UV-irradiation, is not only restricted to the skin tissue [5, 6]. Indeed, it was recently shown that this enzyme was also expressed in human hair follicles and more specifically, 11βHSD1 was detected both in matrix keratinocytes and in hair follicle dermal papilla cells (HFDPCs) [7].
It was shown that blockage of the cortisol pathway in skin was able to rescue age associated changes [8]. Along those lines, we recently developed Biphenyl Azepanyl Methanone (BAM), a specific inhibitor of the 11βHSD1 enzyme, that we have shown to be able to protect skin against cortisol-induced collagen degradation [9].
In the present study, our goal was first to confirm the inhibitory effect of cortisol on HFDPC proliferation and then to investigate, if this effect could be rescued by BAM.


**Figure 1:** Conversion from cortisone (inactive) to hydrocortisone (active) and back is catalyzed by 11betaHSD1 and 11betaHSD2, respectively, in skin. Drawing after Slominski et al. 2014 [3].

## Materials & Methods:

### Cell culture
Human hair follicle dermal papilla cells (HFDPCs) [10] were used at the 8th passage and grown in DMEM supplemented with 2 mM L-glutamine, 50 U/ml Penicillin and 50 µg/ml Streptomycin, and 10% fetal calf serum (FCS). Assay medium was the same but with only 1% FCS. Hydrocortisone (Cortisol) was from Sigma (#H0135), Cortisone was from Sigma (#C2755).

### 11βHSD1 inhibitors
-BAM (biphenyl azepanyl methanone, batch 1992/02486 (DSM Nutritional Products, Kaiseraugst, Switzerland), CAS 1910069-14-5
-10j, (Merckmillipore/Sigma-Aldrich #385581), CAS 1009373-58-3

### Cytotoxicity measurement
Cytotoxicity of 11βHSD1 inhibitors was determined using the MTT assay [11]. In brief, cells were incubated in assay medium together with inhibitors for 72 hrs followed by MTT assay and measurement of $OD_{540}$. Inhibitors were subsequently used in non-cytotoxic concentrations.

### Measurement of cell proliferation
HFDPCs were seeded in 96-well plates in growth medium for 24 hours. For the assay, medium was replaced with assay medium containing the test substances. Cell were further incubated for 72 hours, and proliferation was assessed by means of BrdU-incorporation using a BrdU-ELISA kit (Roche, #11647229001) according to the manufacturer's instructions.

## Results & Discussion:

### Glucocorticoids inhibited BrdU-incorporation into HFDPCs

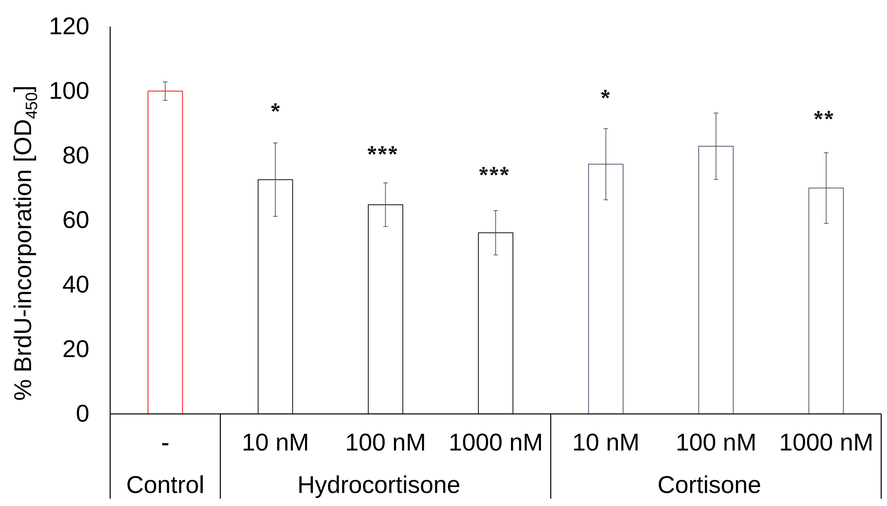


**Figure 2:** Effect of hydrocortisone and cortisone on the proliferation of HFDPCs. HFDPCs were treated for 72 hrs with the indicated concentrations of both hydrocortisone (black bars) or cortisone (grey bars). Red bar = control cells normalized to 100% proliferation. *p<0.05, **p<0.01, ***p<0.001, all vs control by unpaired Student's t-test (n = 3). Error bars represent standard error of the mean.

This result in Fig. 2 indicated and confirmed the growth/proliferation inhibitory effect of glucocorticoids on HFDPCs [7, 12]. We thus investigated, if our 11βHSD1-inhibitor BAM was able to rescue this proliferation inhibition phenotype.

### BAM rescued glucocorticoid-induced proliferation inhibition

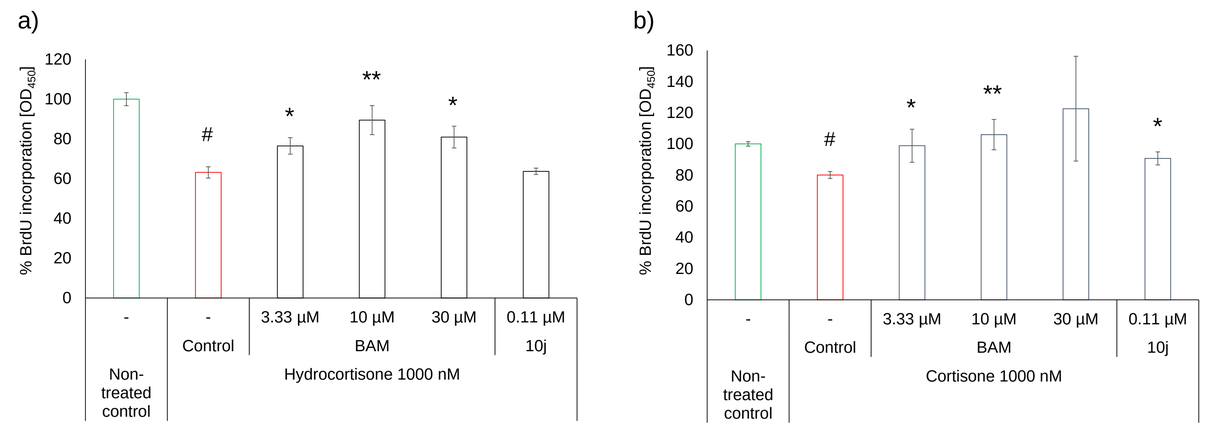


**Figure 3:** Effect of 11βHSD1 inhibitors on glucocorticoid reduced proliferation in HFDPCs. HFDPCs were treated with glucocorticoids and inhibitors for 72 hrs. a) HFDPCs were treated with 1000 nM hydrocortisone (red bar) and inhibitors (black bars) and compared to untreated cells (green bar). b) HFDPCs were treated with 1000 nM cortisone (red bar) and inhibitors (grey bars) and compared to untreated cells (green bar). #p<0.001 vs non-treated control, *p<0.05, **p<0.01, both vs control, by unpaired Student's t-test (n = 3). Error bars represent standard error of the mean.

The results in Fig. 3 indicated that BAM was indeed able to rescue the HFDPC growth inhibition phenotype induced by both hydrocortisone and cortisone.

## Conclusions:

In this study, we provide evidence that a specific inhibitor of 11βHSD1 (BAM) was able to rescue a glucocorticoid induced proliferation phenotype in HFDPCs. In addition to previous studies, we also showed that HFDPCs displayed reduced proliferation when exposed to cortisone which again could be rescued by BAM. Our results suggest, that BAM could be an effective treatment option for glucocorticoid dependent hair growth disorders in vivo.

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