A C-terminal inhibitor of HSP90 decreases GH-promoter activity and growth hormone secretion in a cellular model of somatotropinoma

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Introduction and Objectives

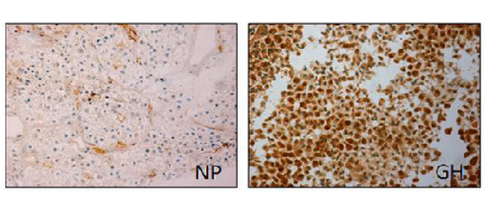
- Heat Shock Protein 90 (HSP90) exerts a pivotal role in the maturation and stabilization of more than 200 client proteins[1]. Many of them are involved in oncogenic signaling and cancer progression[2].
- Strong overexpression of HSP90 was reported in corticotroph adenomas, and treatment with C-terminal HSP90 inhibitors showed anti-tumorigenic and anti-secretory effects in vitro and in vivo[3].
- Aim of the present study was to extend the investigation of the potential tumorigenic role of HSP90 in GH-secreting pituitary adenomas using as a model the somato-lactotroph cell line GH3, by testing the efficacy of N- terminal (**17-AAG**) and C-terminal (**novobiocin, KU174**) HSP90 inhibitors.

Methods

- *Immunohistochemistry*: detection of HSP90 immunostaining in GH-secreting pituitary adenomas
- *Reporter assays*: estimation of GH, CRE and PIT-1 promoters activities
- *Radioimmunoassay*: quantification of GH secretion
- *Western Blot*: evaluation of the expression of pituitary tumor markers (PIT-1, CREB) and HSP90 interactors (Akt, HSF1)

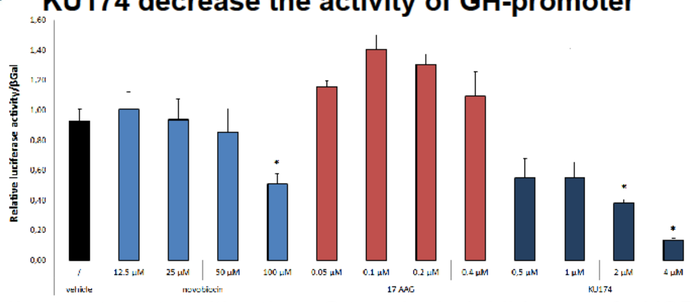
Results

1 HSP90 is overexpressed in biopsy specimens of human GH-secreting pituitary adenomas



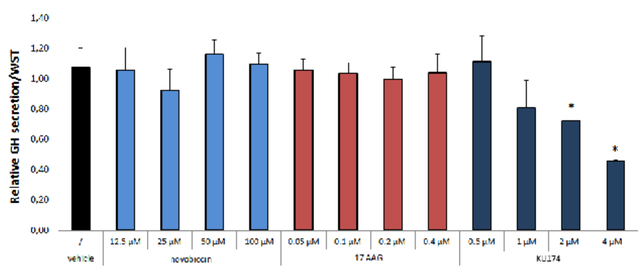
Intense HSP90 immunostaining was detected in 8/25 GH-secreting pituitary tumors (GH) compared to the normal pituitary (NP).

2 C-terminal inhibitors of HSP90 novobiocin and KU174 decrease the activity of GH-promoter



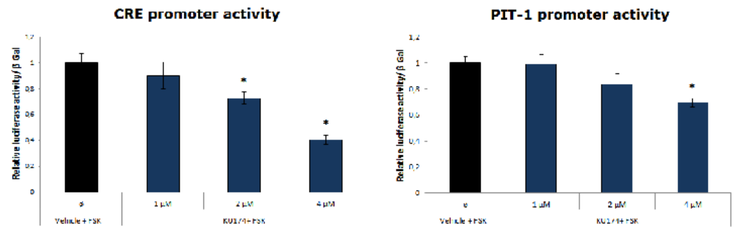
24h treatment with the C-terminal inhibitors of HSP90 novobiocin and KU174 dose-dependently decreased GH-promoter activity, with maximal effect for KU 174 at 4μM concentration (15% compared to control, *P<0.05). Conversely, opposite results were obtained by treatment with the HSP90 N-terminal inhibitor 17 AAG.

3 The C-terminal inhibitor of HSP90 KU174 decreases growth hormone secretion



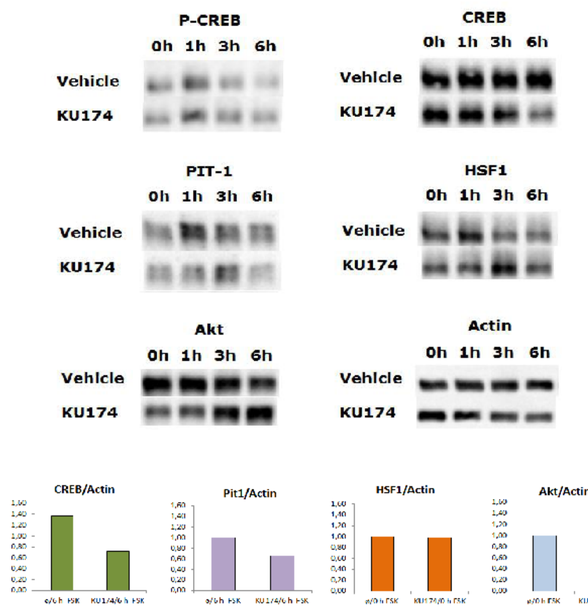
24h treatment with the C-terminal inhibitor of HSP90 KU174 dose-dependently decreased GH secretion, with maximal effect at 4μM (52% compared to control, *P<0.05), whether no effects were reported by novobiocin and 17 AAG treatment.

4 C-terminal inhibition of HSP90 with KU174 decreases the promoter activity of PIT-1 and CRE



24h treatment with the C-terminal inhibitor of HSP90 KU174 dose-dependently decreased CRE and PIT-1 promoter activity after 6 h stimulation with Forskolin 10 μM (FSK), with maximal effect at 4μM (40% and 70% respectively, compared to control *P<0.05).

5 Exposure of GH3 cells to KU174 results in downregulation of Akt, CREB and PIT-1 expression



48h treatment with KU174 decreased Akt expression and the inhibiton of HSP90 activity did not induce an heat shock response, as confirmed by no change in HSF1 expression. During the additional stimulation with FSK it was displayed a decrease in PIT-1 and total CREB expression, the latter without changes in its phosphorilated counterpart (P-CREB).

Discussion and Conclusions

- Considering the intense HSP90 immunostaining reported in a considerable quantity of specimens analysed in this study, HSP90 might be involved in the pathogenetic mechanisms driving the development of GH-secreting pituitary adenomas.
- Treatment of the GH3 cell line with the C-terminal HSP90 inhibitor KU174 showed the downstream effect of reducing GH excessive production both at transcriptional and at secretory levels, suggesting its potential use for the therapeutic management of GH-secreting pituitary adenomas.
- The inhibition of HSP90 by KU174 is both affecting known HSP90 protein interactors (Akt) and the transcriptional activity and the expression of proteins implicated in pituitary adenomas tumorigenesis (CREB, PIT-1), suggesting that HSP90 might also influence the stability of pituitary tumor-related proteins.

References

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ECE 2016 859--EP

Pituitary Basic

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Poster presented at: ECE2016 28 – 31 May 2016 MUNICH, GERMANY 18th EUROPEAN CONGRESS OF ENDOCRINOLOGY

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