Dissecting the in vitro efficacy of octreotide and cabergoline in GH- and GH/PRL-secreting pituitary tumors

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Introduction

Acromegaly is a systemic endocrine disease caused by the prolonged exposure to **high levels of growth hormone (GH) and** its peripheral mediator, **insulin-like growth factor-1 (IGF-1)**. In over 95% of cases, acromegaly is due to the presence of a **GH-secreting pituitary tumor**. About **25% of GH-secreting pituitary tumors co-secrete prolactin (PRL)**.
First-generation somatostatin receptor ligands (fg-SRLs), such as **octreotide (OCT)**, show a **preferential binding affinity for the somatostatin receptor (SST) subtype 2 (SST_2)** and still represent the **first-line medical therapy for acromegaly**.
In the view of a cheap and manageable medical therapy, the dopamine agonist **cabergoline (CAB)**, an ergot derivative showing **preferential binding affinity for the dopamine receptor type 2 (D2R),** is used as **off-label treatment in acromegaly**, particularly in the presence of mild disease.

Aim of the Study

To perform **a head-to-head comparison between OCT and CAB in inhibiting GH (and PRL) secretion in primary cultures** derived **from acromegalic patients** harboring **GH- or GH/PRL-secreting pituitary tumors**. We **stratified our primary cultures based on the preferential response to each drug**, thus identifying peculiar tumor subtypes. Finally, we **correlated the expression profile of both SSTs and D2R with the observed tumor characteristics**

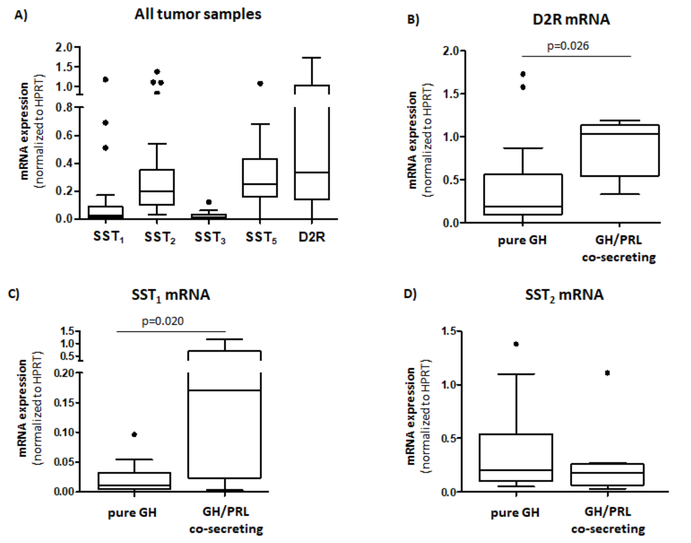
Patients and Methods

Pituitary **tumor samples** were obtained from **23 acromegaly patients** that underwent transsphenoidal surgery at the Erasmus Medical Centre in Rotterdam (The Netherlands).
Inclusion criteria of the study were: i) **availability of enough viable cells** to establish a primary culture, ii) adequate cell number to **test** in the same experiment, at least in triplicate, **the anti-secretory efficacy of 72h OCT and CAB treatment (10 nM concentration), alone and/or in combination (vs control)**.

Directly after obtaining the tissue, **a piece was used for cell culture, and mRNA analysis was carried out** from freshly isolated cells.
Tumor samples were stratified as pure GH-secreting (n=15) and **GH/PRL co-secreting (n=8) lesions**. Based on previous studies from our group, we classified as mixed tumors those samples in which both GH and PRL secretion was detectable in cultured cells, while the pure somatotroph tumors showed GH secretion alone.

Results

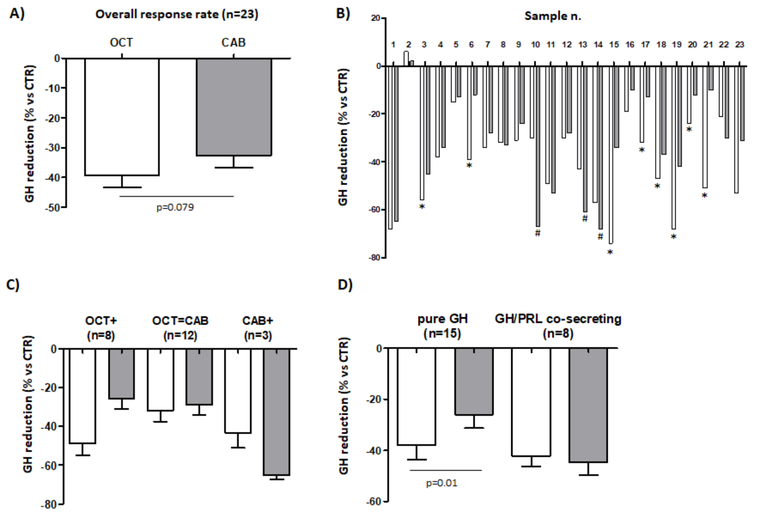
Figure 1. mRNA expression of dopamine type 2 receptor and somatostatin receptor subtypes



The **D2R showed the highest mRNA expression** among all the membrane receptors evaluated (Fig 1A). Among SSTs, **SST_5 and SST_2 were the most predominantly expressed subtypes**.

D2R and SST_1 mRNA levels were higher in GH/PRL-secreting tumors. SST_2, SST_5 and SST_3 expression was almost superimposable between the two groups (Fig 1B-C-D).

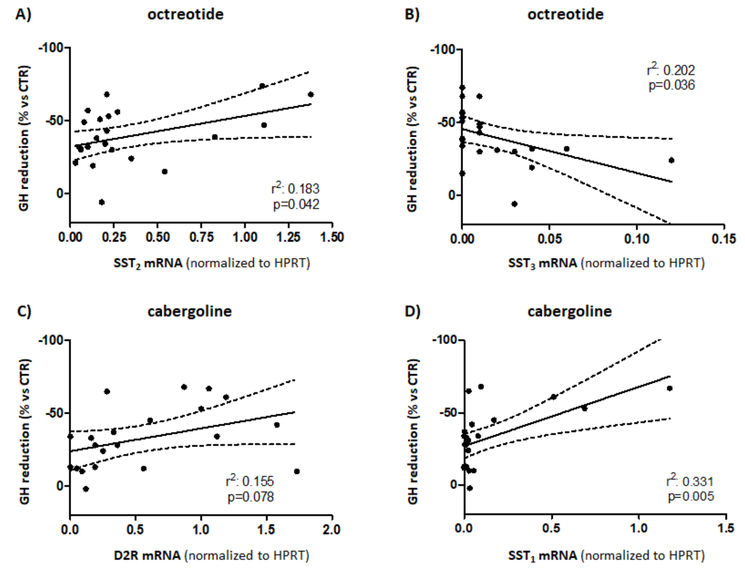
Figure 2. In vitro response to octreotide (OCT) and cabergoline (CAB) treatment



Overall, **OCT treatment was (slightly) more effective** in reducing GH secretion, **compared with CAB** (mean GH decrease -39.5% ± 19.0 vs. -32.5% ± 20.2; p=0.07; Fig 2A).

We identified **8 (35%) cell cultures where OCT was more effective than CAB** in reducing GH secretion (OCT+ group), **12 (52%) in which the efficacy of the two compounds did not significantly differ** (OCT=CAB group), and **3 (13%) where CAB was more effective than OCT (CAB+)** (Fig 2C).

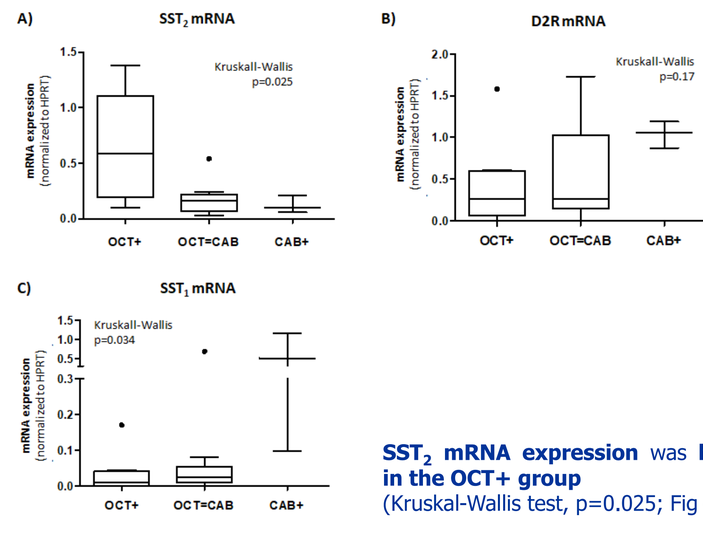
Figure 3. Correlation between drug antisecretory effect and membrane receptor mRNA expression



SST_2 expression was significantly and **positively correlated** with the ability of **OCT to reduce GH secretion in vitro** (r2: 0.183, p=0.042), while **SST_3 expression** was **negatively associated with OCT efficacy** (r2: 0.202, p=0.036) (Fig 3. A-B).

D2R expression showed a **trend for a positive correlation** with the inhibitory **effect of CAB** on GH secretion (r2: 0.155, p=0.078).
SST_1 was **significantly and positively correlated with CAB efficacy** (r2: 0.331, p=0.005) (Fig 3. C-D)

Figure 4. Differential mRNA expression of membrane receptors in tumor samples showing a prefential response to octreotide (OCT) and/or cabergoline (CAB)

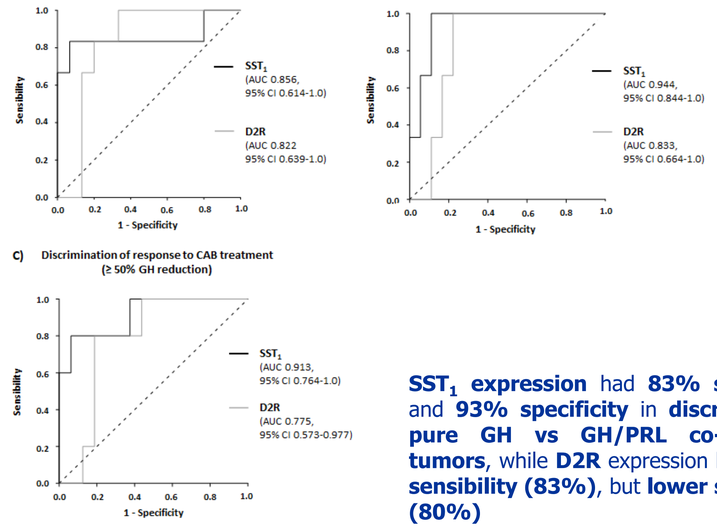


SST_2 mRNA expression was **higher in the OCT+ group**
(Kruskal-Wallis test, p=0.025; Fig 4A).

D2R expression showed a slight **trend** toward **higher levels in CAB+ tumors**, compared to both OCT=CAB and OCT+ groups (Kruskal-Wallis test, p=0.17; Fig 4B).

SST_1 mRNA levels were significantly **higher in the CAB+ group** (median value 0.51/hprt), compared to the OCT=CAB and the OCT+ groups (median 0.023/hprt and 0.01/hprt; Kruskal-Wallis test, p=0.034; Fig 4C).

Figure 5. Receiver-operating characteristic (ROC) curves on the predictive discrimination of SST_1 and D2R mRNA expression

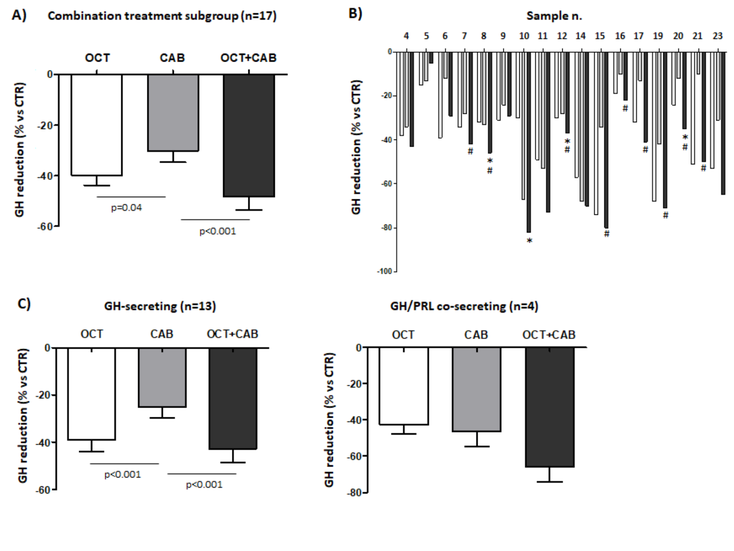


SST_1 expression had **83% sensibility** and **93% specificity** in **discriminating pure GH vs GH/PRL co-secreting tumors**, while **D2R** expression **had equal sensibility (83%)**, but **lower specificity (80%)**

SST_1 had the **highest capability in discriminating** those tumors with a preferential response to CAB (**CAB+ group**) (AUC 0.944, p=0.016; 100% sensibility, 89% specificity) (Fig 5B)

SST_1 discriminated the response to CAB treatment (≥ 50% GH reduction) **with satisfactory values** (p=0.006; 80% sensibility, 94% specificity) (Fig 5C).
D2R showed a lower discrimination power (p=0.069; 80% sensibility, 81% specificity).

Figure 6. In vitro response to octreotide and cabergoline combination treatment (OCT+CAB), compared to single-agent therapy



OCT+CAB treatment resulted in a GH decrease of -48.2% ± 22.0 (mean ± SD), which was **significantly more effective compared to CAB treatment alone** (-31.1% ± 18.7; p<0.001) (Fig 6A).
Combination treatment was not superior to OCT alone (GH decrease -39.8% ± 16.5).

In the **GH/PRL co-secreting tumors**, **OCT+CAB treatment showed a higher efficacy** (-65.8% ± 16.7) **compared to both OCT** (-42.5% ± 10.5) and **CAB** (-46.3% ± 16.9) **alone**

Conclusions

- **The in vitro efficacy of OCT**, **in reducing GH secretion**, is **slightly superior compared to that of CAB**. The **efficacy of the two drugs** is **superimposable in 65% of tested primary cultures**, and in about 10% of them CAB is even superior.
- As expected, **the efficacy of CAB correlates** with the **hormone secretion pattern (GH plus PRL)** and **D2R mRNA expression**.
- We have highlighted **SST_1 as a reliable marker of GH/PRL co-secreting lesions showing a good response to CAB**. **SST_1** showed a **higher specificity compared to D2R** in identifying this tumor type.