Fenofibrate has differential effects on cell proliferation and GH secretion in GH_3 cells

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INTRODUCTION

PPARα, a member of the Peroxisome-Proliferator Activated Receptors (PPARs) family, is a partner of the Aryl hydrocarbon receptor Interacting Protein (AIP), which is involved in the pathogenesis of pituitary adenomas (PA). AIP has been reported to repress the transcriptional activity of PPARα **(1)**, whereas in GH_4C_1 cells, Tolon *et al.* (1998) **(2)** have shown that PPARα can stimulate the prolactin promoter by association with Pit-1 and other coactivator proteins. We recently observed that PPARα was expressed in the normal human pituitary, in particular in somatotrophs and lactotrophs an in pituitary adenomas **(3)**. Therefore, we investigated the effects of fenofibrate (FF), a PPARα-agonist, on GH_3 cells.

MATERIAL AND METHODS

Cell culture and treatment: GH_3 cells were obtained from the American Type Culture Collection (ATCC, cultured in Ham's F10 supplemented with 10% FBS, 1% Glutamine/PES, and treated with fenofibrate (FF) (Santa Cruz Biotechnology, USA) at different concentrations (0, 12,5, 25, 50 µM) and with 25 µM of FF at different time points (0,24,48,72 h). Cells were counted with a Burker's chamber. ***ELISA:*** GH concentration was determined in the culture media by rat-specific enzyme-linked immunoassays (A05104 for GH, SPI-BIO, Bertin Pharma, France) and corrected for cell number. ***qRT-PCR analysis:*** Total RNA was extracted by Eurogold TriFast (EuroClone, Pero, Milan); cDNA was obtained from 1µg of RNA and semi-quantitative Real-time RT-PCR (sqRT-PCR) of cDNA was performed in duplex for each sample using commercial Taqman assays (Life Technologies, Monza) for rat GH with Cyclophilin B as housekeeping gene. ***Western Blot analysis:*** Proteins were extracted from GH_3 cells using a RIPA buffer. 60µg of each extract were resolved on 12% SDS-PAGE and elettroblotted to PVDF membranes. Western blot were carried out using primary rabbit polyclonal antibodies (anti-GH and anti--Cyclophilin B ,Thermoscientific, USA). Signals were detected using Pierce ECL WB substrate (Thermo Scientific, USA).

RESULTS (1) Effect of fenofibrate (FF) on GH_3 cell growth

In vitro treatment with fenofibrate induced a dose-dependent decrease on cell growth in GH_3 cells (P<0.0001 for each concentration) in a dose-dependent manner (P=0.0019 at 25 µM *vs* 12.5 µM, P=0.0003 at 50 µM *vs* 25 µM). **Fig. 1A** indicates data obtained at 48h.

Time-dependent data obtained with FF 25µM are shown in **Fig. 1B**. The maximal effect was obtained after 48h and remained significant at 72h (P<0.0001 at 48h and 72h *vs* control cells for both concentrations) (**Fig. 1B**).

Fig. 1A

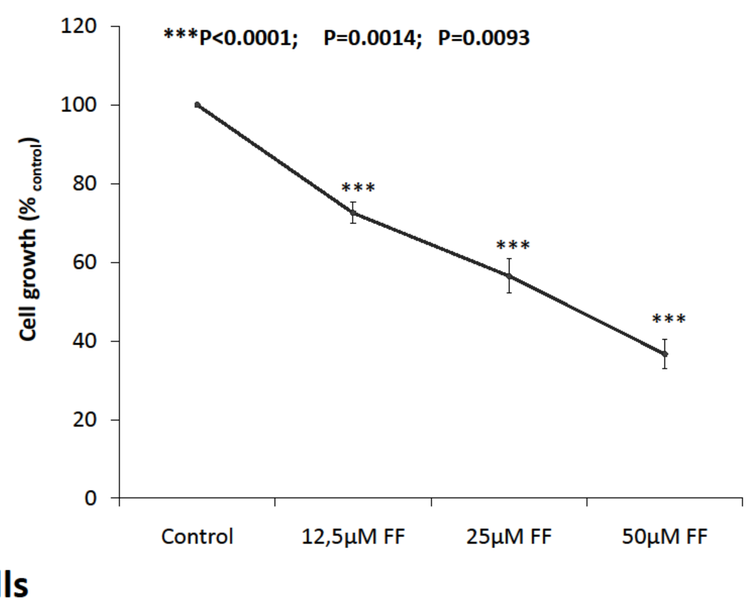
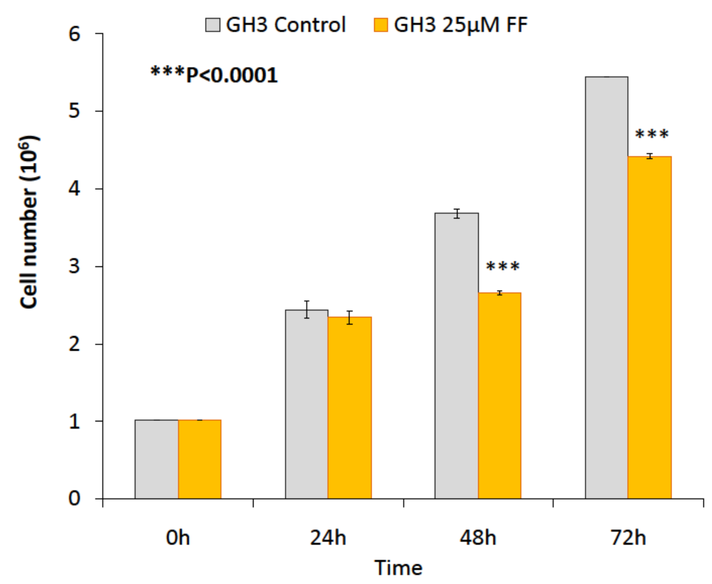


Fig. 1B



RESULTS (2) Endocrinological effect of fenofibrate on GH_3 cells

Time-dependent experiments performed with 25 µM revealed a significant increaase in GH secretion into the medium after 24h and 48h of treatment (P<0.0001 *vs* control for both time points, P=ns at 72h; data not shown). The increase in GH secretion was dose-dependent - about 3-folds at 25 µM and 4-folds at 50 µM *vs* controls – data obtained after 48h of treament (**Fig. 2A**). In contrast, analysis of *GH* mRNA at 48h showed a bimodal response, with a significant increase in gene transcription at 25 µM (P=0.0094) and a return to levels similar to control cells at 50 µM (P=0.002 *vs* 25 µM) (**Fig. 2B**). Compared to control cells, a modest increase in intracellular GH content was also observed at 25 µM, with a modest decrease at 50 µM, respectively (**Fig. 2C**). Taken together, these data indicate that fenofibrate had a predominant effect on hormone release.

Fig. 2A

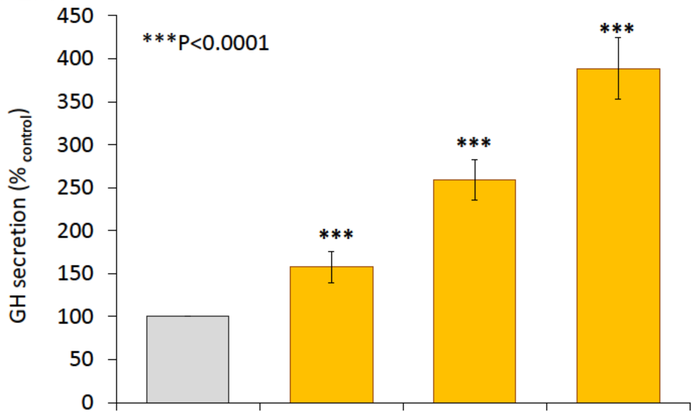


Fig. 2B

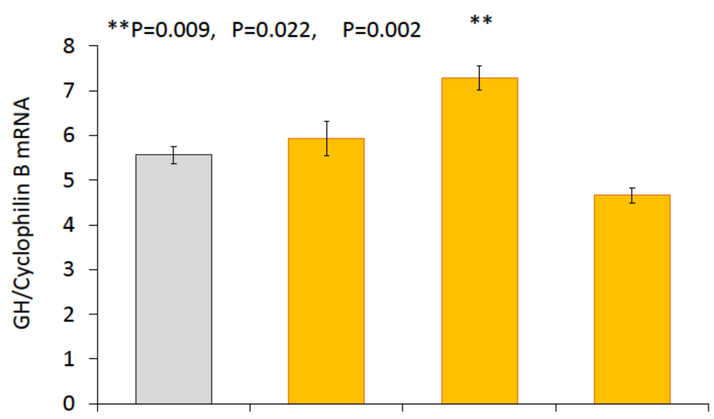
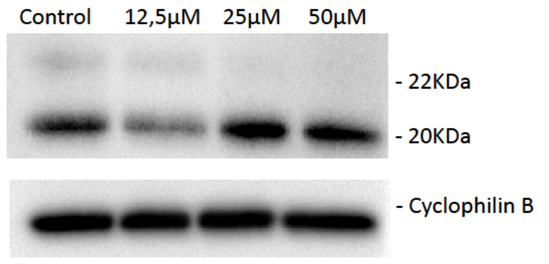


Fig. 2C



1 0.12	0,95 0.12	1,15 0.05	0,8 0	GH 20KDa/Cycl. B GH 22KDa/Cycl. B
1.12	1.07	1.20	0.8	GH/Cycl. B

CONCLUSION

Fenofibrate was able to significantly reduce cell growth in GH_3 cells while increasing GH secretion. Both effects were dose-dependent. The anti-proliferative effects are reminiscent of those reported with rosiglitazone, a PPARγ agonist (4), suggesting common genomic effects on genes sharing similar PPAR-responsive elements (PPRE). The increase in GH secretion may reflect a modest increase in GH synthesis (at low dose) and a predominant increase in GH release (especially at high dose). Whether long-term treatment with fenofibrate would induce sustained GH release is questionable since the anti-proliferative effects may be able to overcome the acute effects, especially at high dose when GH transcription is reduced. Experiments combing fenofibrate treatment with octreotide are ongoing to disclose potential complementary effects of both drugs.

REFERENCES: **(1)** WK Sumanasekera *et al.* Evidence that Peroxisome Proliferator-activated Receptor α is complexed with the 90-kDa Heat Shock Protein and the Hepatitis Virus B X-associated Protein 2. JBC 2003; **(2)** RM Tolón *et al.* Activation of the prolactin gene by Peroxisome Proliferator activated Receptor-α appears to be DNA binding-independent. JBC 1998; **(3)** S Rotondi *et al.* Expression of Peroxisome Proliferator–activated Receptor α in pituitary tumours. ECE 2014; **(4)** Bogazzi *et al.* PPARgamma inhibits GH synthesis and secretion and increases apoptosis of pituitary GH-secreting adenomas. Eur J Endocrinol 2004.

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