Fenofibrate has differential effects on cell proliferation and GH secretion in GH₃ 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 acitivity of PPARα (1), whereas in GH₃ 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 in pituitary adenomas (3). Therefore, we investigated the effects of fenofibrate (FF), a PPARα-agonist, on GH₃ cells.

MATERIAL AND METHODS

Cell culture and treatment: GH₃ cells were obtained from the American Type Culture Collection (ATCC, cultured in Ham’s F10 supplemented with 10% FBS, 1% Glutamine/PE, 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 (AOS104 for GH, SPI-BIO, Berlin Pharma, France) and corrected for cell number. qRT-PCR analysis: Total RNA was extracted by Eurogentec TriFast (EuroClone, Pero, Milan); cDNA was obtained from 1µg of RNA and semi-quantitative Real-time RT-PCR (qRT-PCR) of cDNA was performed in duplex for each sample using commercial Tagman assays (Life Technologies, Monza) for rat GH with Cyclophilin B as housekeeping gene. Western Blot analysis: Proteins were extracted from GH₃ cells using a RIPA buffer. 60µg of each extract were resolved on 12% SDS-PAGE and electrophorized to PVDF membranes. Western blot were carried out using primary rabbit polyclonal antibodies (anti-GH and anti-Cyclophilin B, Thermofischer, USA). Signals were detected using Pierce ECL WB substrate (Thermo Scientific, USA).

RESULTS (1) Effect of fenofibrate (FF) on GH₃ cell growth

In vitro treatment with fenofibrate induced a dose-dependent decrease on cell growth in GH₃ 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).

RESULTS (2) Endocrinological effect of fenofibrate on GH₃ cells

Time-dependent experiments performed with 25 µM revealed a significant increase 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 treatment (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.

CONCLUSION

Fenofibrate was able to significantly reduce cell growth in GH₃ 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 combining fenofibrate treatment with octreotide are ongoing to disclose potential complementary effects of both drugs.

REFERENCES:

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