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The effect of calcitriol and its analogues on proliferation and hormone expression in cultured cells of thyroid medullary carcinomas

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The study aimed at evaluating the effects of calcitriol and of its analogues on the proliferation of TT and rMTC cells (human and rat line tumour cells originating from thyroid medullary carcinoma) and at examining the effects of the substances on the secretion of the principal hormones of the cells, calcitonin (CT) and calcitonin gene-related peptide (CGRP). Cells of thyroid medullary carcinoma (human TT cells and rat rMTC cells) were cultured for 5 days in the absence or in the presence of calcitriol and of its two analogues (PRI-1906 and PRI-2191) in concentrations of 10^{-9} to 10^{-6} M. Calcitriol and the applied analogues weakly inhibited proliferation of thyroid medullary carcinoma in in vitro *conditions. The evident effect of analogues on hormone secretion points to their effect on the process of CT gene expression.*

key words: calcitonin, CGRP, thyroid medullary carcinoma, calcitriol analogues

INTRODUCTION

Calcitriol and its analogues are examined, amongst other reasons, as potential agents capable of inhibiting the proliferation of tumour cells, including tumours that secrete polypeptide hormones [2]. In the latter case not only inhibited proliferation is taken into account but also effects on hormone secretion, which is linked to the development of side effects that reflect the effects of the hormones on target organs. One of the findings has been the inhibitory effect of calcitriol on the proliferation of hyperplastic parathyroids and on the expression of parathormone [1]. It is also known that some analogues may exert a stronger effect than calcitriol itself [4]. In our previous study we documented the effect of calcitriol on the proliferation and expression of the CT gene in cultured cells of thyroid medullary carcinoma [6].

The present study aimed at evaluating the effects of calcitriol and of its analogues on the proliferation of TT cells and rMTC cells (human and rat tumour cell line originating from thyroid medullary carcinoma) and at examining the effect of the compounds on secretion of the principal hormones of the cells, calcitonin (CT) and calcitonin gene related protein (CGRP). We were particularly interested in the effects on the ratio of secreted CGRP/CT, since the two hormones are produced on the template of the same gene due to alternative splicing.

MATERIAL AND METHODS

Cells of thyroid medullary carcinoma (human TT cells and rat rMTC cells) were cultured for 5 days in the presence or in the absence of calcitriol and its two analogues (PRI-1906 and PRI-2191) at concentrations of 10^{-9} to 10^{-6} M. Proliferation was measured

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by a colourimetric technique, while hormone levels in the medium were measured by radioimmunoassay. The results were subjected to statistical analysis employing Spearman's correlations. Moreover, the cells were immunocytochemically stained for the presence of calcitonin and CGRP.

RESULTS AND DISCUSSION

TT and rMTC produce and store CT and CGRP, as confirmed by immunocytochemical studies (Fig. 1). TT cells secreted, to a moderately significant degree, higher amounts of hormones than those released by rMTC. rMTC cells secreted similar amounts of CT and CGRP, while TT cells secreted more than twice as much CGRP as CT (Fig. 2, 3).

Calcitriol and its two analogues decreased proliferation of rMTC cells in a manner which depended on the concentration of the drug (calcitriol: r = = –0.53; PRI-1906: r = –0.47; PRI-2191: r = –0.59). A similar, although weaker, correlation was also demonstrated for TT cells using calcitriol and PRI-1906 (calcitriol: r = –0.33; PRI-1906: r = –0.50), while PRI-2191 analogue demonstrated no such effect (Fig. 4, 5).

Calcitriol augmented slightly the secretion of CT by TT cells (r = 0.44) but had no effect on the secretion of the hormone by rMTC cells. PRI-2191 analogue clearly decreased CT secretion in rMTC cells (r = –0.64) while PRI-1906 analogue stimulated secretion of the hormone by TT cells (r = 0.80).

Both analogues exerted a strong inhibitory effect, correlated with the dose, on CGRP secretion, both in TT cells (PRI-1906: r = –0.71; PRI-2191: r = = –0.61) and in rMTC cells. Calcitriol itself demonstrated no such effect.

Examination of the ratio of secreted CGRP/CT showed that clearly lowered values of the ratio for both analogues and for both cell lines (for TT PRI-

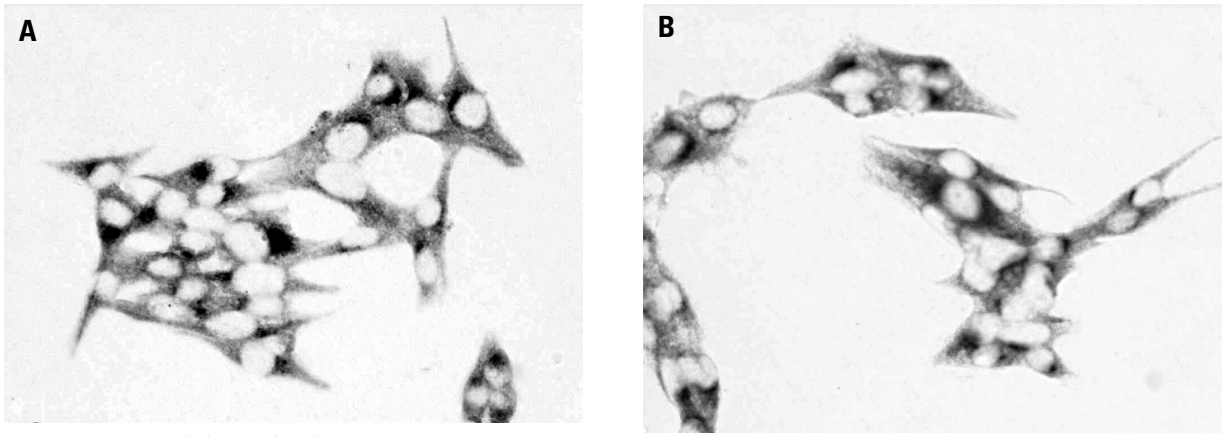

Figure 1. Calcitonin (**A**) and CGRP (**B**) immunocytochemically localised in cultured TT cells in control conditions.

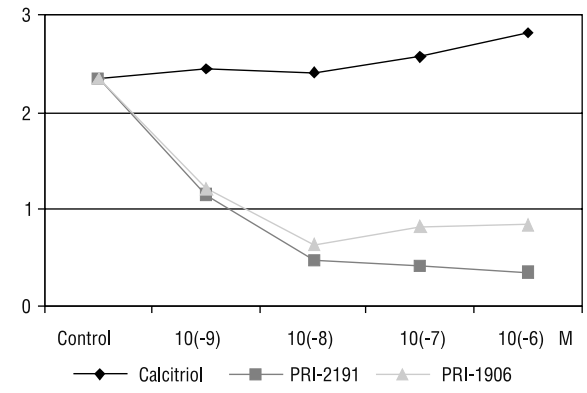

Figure 2. Effect of calcitriol and its analogues on the ratio of CGRP/CT levels in the medium following 5-day culture of TT cells.

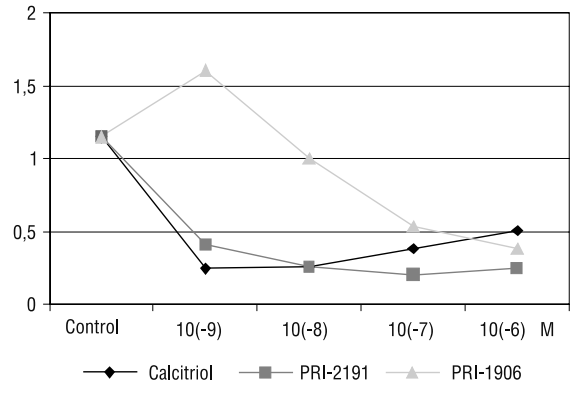

Figure 3. Effect of calcitriol and of its analogues on the ratio of CGRP/CT levels in the medium following 5-day culture of rMTC cells.

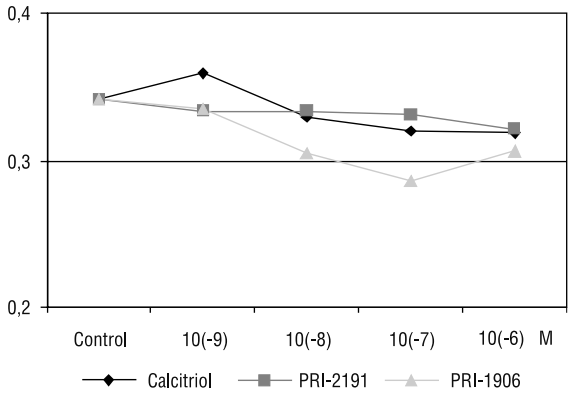

Figure 4. Effect of calcitriol and of its analogues on proliferation of TT cells following 5-day culture.

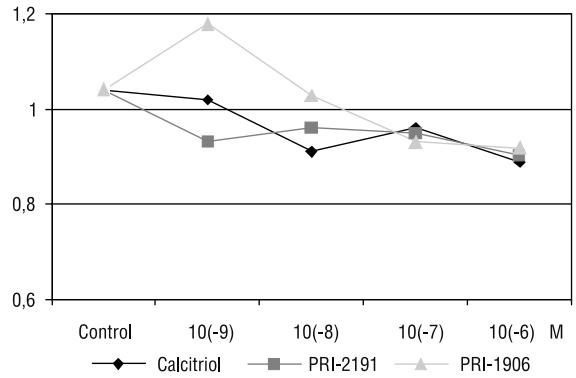

Figure 5. Effect of calcitriol and of its analogues on proliferation of rMTC cells following 5-day culture.

-1906: r = –0.89; PRI-2191: r = –0.63; for rMTC PRI-1906: r = –0.75; PRI-2191: r = –0.77). However, calcitriol itself failed to exert such an effect for TTc (Fig. 2, 3).

Calcitriol and the applied analogues weakly inhibited proliferation of thyroid medullary carcinoma in *in vitro* conditions and it seems improbable that the effect might be of clinical use. Nevertheless, the effects of analogues on hormone expression indicate their effect on the process of CT gene expression. Their effect on secretion is relatively unlikely, since the two hormones are secreted together from the same secretory granules. The analogues most probably influence the alternate splicing, which yields either CT-mRNA or CGRP-mRNA from the same primary transcript, which is in consequence reflected by the levels of the two hormones in the medium [5]. It is also possible that the analogues affect post-translational processes such as those of proprotein convertases, but in the cells studied by us the processes have not yet been clarified [3].

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