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SHORT COMMUNICATION

Additive Inhibitory Effect of Calcipotriol and Anthralin on Ribonuclease P Activity

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ABSTRACT. The effects of two antipsoriatic compounds, calcipotriol and anthralin, separately or in combination on ribonuclease P (RNase P), were investigated using a cell-free system from the slime mold *Dictyostelium discoideum*. RNase P is an ubiquitous and essential enzyme which endonucleolytically cleaves all tRNA precursors to produce the mature 5′ end. The substrate for RNase P assays was an *in vitro* ^{32}P-labeled transcript of the *Schizosaccharomyces pombe* $tRNA^{Ser}$ gene supS1. Enzyme assays were carried out at 37° in 20 μL 50 mM Tris–HCL 7.6 buffer, containing 10 mM NH_4Cl, 5 mM $MgCl_2$, and 10% isopropanol. Calcipotriol or anthralin alone exerted a dose-dependent inhibitory effect on RNase P activity, with the former being more active than the latter in this respect. Simultaneous exposure of the enzyme to both drugs resulted in an enhancement of RNase P inhibition, which was additive. Considering the lack of structural similarities between the substrate (precursor tRNA) of RNase P and the tested drugs, it seems reasonable to suggest that their effects may be due to binding to allosteric inhibition sites of the enzyme. Although our *in vitro* findings cannot be directly extrapolated to the *in vivo* human condition, they do suggest that the inhibitory effects of calcipotriol and anthralin on tRNA biogenesis may be implicated in the mechanisms of their antipsoriatic action. Moreover, the additive inhibitory effect of these compounds on RNase P activity provides an experimental basis for their possible combined therapeutic application in the management of psoriasis. BIOCHEM PHARMACOL **60**;1:91–94, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. RNase P; ribozymes; vitamin D; dithranol

The efficacy of calcipotriol (Fig. 1, inset), a synthetic analogue of vitamin D_3, in the topical treatment of psoriasis and other keratinization disorders has been established in a large number of clinical trials [1]. This analogue is capable of exerting a marked modulatory effect on epidermal proliferation and differentiation as well as on cutaneous immunology [2]. The therapeutic action of calcipotriol is thought to be mediated by an interaction with the vitamin D_3 receptor of epidermal keratinocytes [3], dendritic cells [4], and activated lymphocytes [5]. This receptor belongs to the superfamily of ligand-induced transcriptional regulators, also comprising the thyroid, steroid, and retinoid receptors [6], and modulates gene transcription by binding to the vitamin D_3 response element [7].

Anthralin (dithranol, 1,8-dihydroxy-anthrone; Fig 1, inset), a derivative of chrysarobine, was introduced in 1916 by Galewsky [8] and Unna [9] and has since become a widely used, safe, and highly effective topical remedy for the treatment of chronic plaque psoriasis. Various investigators have shown that anthralin inhibits key enzymes of metabolic pathways, cellular respiration, and DNA synthesis [10], and exerts distinct immunomodulatory effects [11]; however, the mode of therapeutic action of anthralin in psoriasis still remains to be elucidated.

RNase P§ is an endonuclease responsible for the maturation of 5′ termini of tRNA precursors. In all organisms investigated so far, this enzyme is composed of both RNA and protein [12]. *In vitro*, RNA subunits of RNase P enzymes from bacteria [13] and some archaea [14] are catalytically active in the absence of protein; this phenomenon has never been demonstrated for eukaryotic RNase P enzymes. Although the latter include an RNA subunit of similar size as found in their prokaryotic counterparts, they are composed of multiple protein components which contribute about 70% to the enzyme's molar mass [15]. This high protein/RNA ratio is a common feature of all eukaryotic nuclear RNase P enzymes, including nuclear *Dictyostelium discoideum* RNase P [16], characterized to date [12]. In addition, human orthologues of the yeast RNase P protein subunits have been found [17]. These findings indicate that the structures of RNase P enzymes from different eukaryotes are similar. Thus, *D. discoideum* RNase P is a good model system for other eukaryotic RNase P enzymes, such as

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§ *Abbreviations:* RNase P, ribonuclease P.

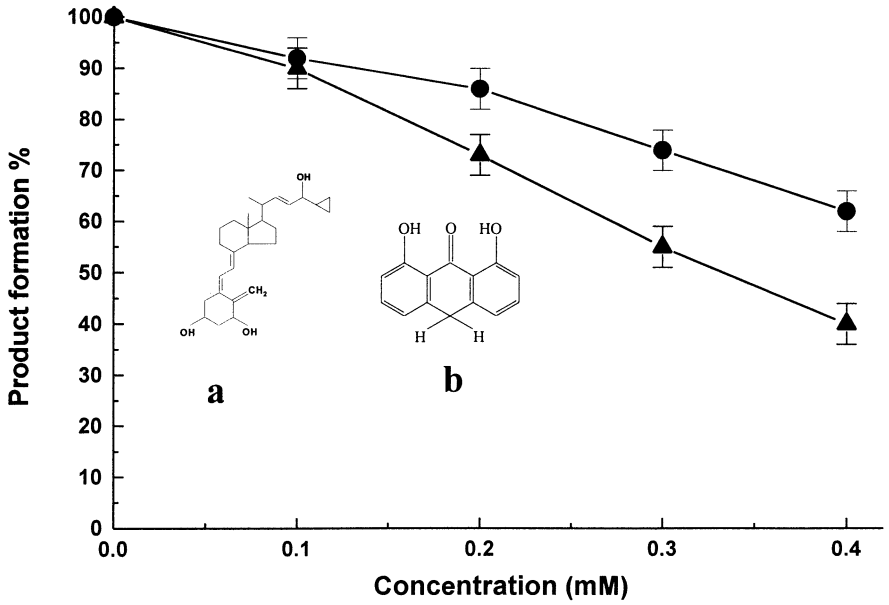

FIG. 1. Dose–response effect of calcipotriol and anthralin on RNase P activity. RNase P activity is shown as a function of increasing concentrations of calcipotriol (▲) and anthralin (●). Results are from 5 experiments for calcipotriol and anthralin ± standard deviations. Inset: Chemical structures of calcipotriol (a) and anthralin (b).

human nuclear RNase P, and could become a promising system for the identification and development of novel inhibitors [18].

MATERIALS AND METHODS

Growth of *D. discoideum* cells (strain AX2 wild-type), cell breakage, and purification of RNase P were essentially carried out as previously described [18]. Enzyme assays were performed at 37° for 20 min in 20 μL buffer D (50 mM Tris–HCl pH 7.6, 10 mM NH_4Cl, 5 mM $MgCl_2$, and 5 mM dithiothreitol) containing 2–5 fmol tRNA substrate, an *in vitro* labeled transcript of the *Schizosaccharomyces pombe* $tRNA^{Ser}$ gene supSI [16], and 1.3 μg protein from the RNase P fraction. When calcipotriol (supplied by Leo Pharmaceutical Products) and anthralin (Sigma) were used, enzyme assays were carried out in the presence of 10% isopropanol. Stock solutions of calcipotriol and anthralin (see Fig. 1, inset) were prepared in 100% isopropanol. The reactions were stopped by addition of 5 μL stop dye (80% formamide, 50 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol). Reaction products were resolved on a denaturing 10% polyacrylamide/8M urea gel and visualized by autoradiography without drying. Activity was quantified by Cerenkov counting of excised gel slices.

RESULTS AND DISCUSSION

In the present study, we investigated the effects of calcipotriol and anthralin, separately or in combination, on RNase P activity using a cell-free system from the slime mold *D. discoideum*. Due to high hydrophobicity of calcipotriol and anthralin, all assays were carried out in the presence of 10% isopropanol. At this concentration, isopropanol reduces tRNA processing 20% on average. Figure 1 clearly shows that either calcipotriol or anthralin alone exerted a dose-dependent inhibitory effect on RNase P activity, with the former compound being more active than the latter in this respect. Interestingly, simultaneous exposure of the enzyme to both drugs resulted in an enhancement of RNase P inhibition (Fig. 2). In a series of further experiments using 0.1 and 0.2 mM calcipotriol and anthralin alone or in combination, it was found that this inhibition was additive (Table 1). This finding indicates an inhibition mode due to the presence of two not mutually exclusive inhibitors.

It is known that there is a poor correlation between the biological activity and the affinity of vitamin D_3 analogues for their receptor [19]. Furthermore, in a recent study on transcriptional activation by these compounds, it was suggested [3] that other molecular events than receptor binding and transcriptional activity may be of importance for the biological potential of vitamin D_3 analogues. Theoretically, the inhibition of *D. discoideum* RNase P could have been caused by a calcipotriol-specific receptor complex co-eluted with RNase P activity during the purification procedure. However, this possibility can be ruled out, since RNase P activity during purification through the Sephacryl S-200 column elutes with the void volume of the column, and the enzyme behaves as a protein with a very high molecular weight (see Ref. 18 for details). Thus, vitamin D_3 receptors, if present in the extract, will elute in later fractions well apart from the RNase P activity due to their low molecular weight (< 50) [6]. Our results point towards the possibility that calcipotriol, apart from its involvement in the regulation of transcription, is also capable of modulating posttranscriptional events by molecular mechanisms

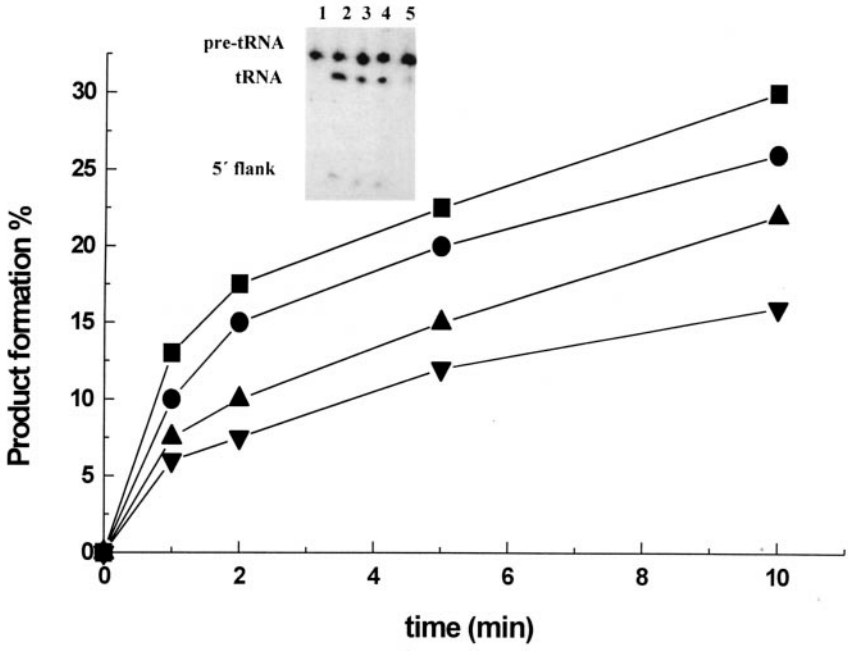

FIG. 2. Kinetics of RNase P cleavage in the absence or presence of calcipotriol or anthralin. Enzyme assays were carried out at 37° in 20 μL buffer D in the presence of 2 fmol tRNA substrate, 1.2 μg protein of RNase P, and 10% isopropanol. (■), without calcipotriol or anthralin; (●), with 0.2 mM anthralin; (▲), with 0.2 mM calcipotriol; and (▼) with 0.2 mM calcipotriol and 0.2 mM anthralin. Inset: Autoradiogram of the cleavage of the supS1 precursor by RNase P in the absence or presence of calcipotriol or anthralin. Lane 1, substrate alone; lane 2, control (RNase P in the absence of calcipotriol or anthralin); lane 3, RNase P in the presence of 0.2 mM calcipotriol; lane 4, RNase P in the presence of 0.2 mM anthralin; lane 5, RNase P in the presence of 0.2 mM calcipotriol and 0.2 mM anthralin.

in which binding of this compound to vitamin D_3 receptors is not involved. Considering the lack of structural similarities between the substrate (precursor tRNA) of RNase P and calcipotriol, it may be suggested that the effects of this compound are perhaps due to its binding to allosteric inhibition sites of the enzyme.

Although anthralin has been used for the topical treatment of psoriasis for more than 80 years, the molecular mechanisms underlying its therapeutic effects are still poorly understood. However, accumulating evidence suggests that the mode of anthralin action might be related to its redox activity, which results in the production of free radical and active oxygen species [10]. The latter are capable of affecting molecules and organelles thought to represent targets of anthralin action, such as DNA, carbohydrates, lipids, enzymes, membranes, and mitochondria [20].

TABLE 1. Separate and joint inhibitory effects of calcipotriol and anthralin on *D. discoideum* RNase P activity

Compound	Concentrations (mM)	Inhibition (%)
Calcipotriol	0.1	10
Calcipotriol	0.2	27
Anthralin	0.1	8
Anthralin	0.2	14
Calcipotriol + anthralin	0.1 + 0.1	23
Calcipotriol + anthralin	0.1 + 0.2	30
Calcipotriol + anthralin	0.2 + 0.1	38
Calcipotriol + anthralin	0.2 + 0.2	47

Binding of anthralin to allosteric inhibition sites of RNase P could explain its inhibitory effect on the activity of this enzyme. Alternatively, the inhibition of the latter could be due to the interaction of anthralin itself or of its free radical products with the RNA component of *D. discoideum* RNase P. It is obvious that our *in vitro* findings cannot be directly extrapolated to the *in vivo* human conditions. Nevertheless, the additive effect of these compounds in the inhibition of RNase P activity could provide an experimental basis for their possible combined therapeutic application in the management of psoriasis. Finally, given that RNase P possesses an RNA subunit structurally related to the RNA subunit of the precursor rRNA-processing enzyme RNase MRP and shares most of its protein subunits with RNase MRP [15], it is likely that calcipotriol and anthralin also inhibit RNase MRP and therefore ribosome biogenesis.

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