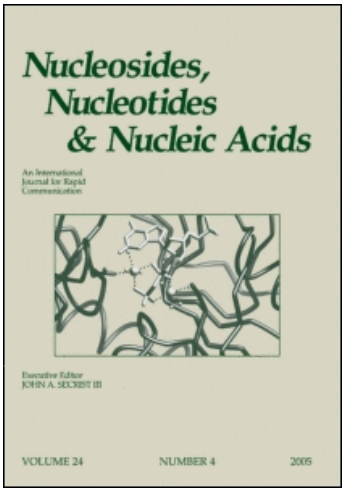

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F. Di Pietrantonio[a]; E. Di Matteo[a]; M. Di Nicola[a]; O. Trubiani[a]; R. Di Primio[b]; E. Serra[a]; G. Spoto[a]
[a] Department of Applied Sciences of Oral and Dental Diseases, University "G. D'Annunzio,", Chieti, Italy [b] Istituto di Morfologia Umana Normale, Università di Ancona, Ancona, Italy

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Cyclase and Phosphodiesterase Activity on Pre-T Lymphoid Human Cells, Treated with Dimethyl Sulfoxide (DMSO)

F. Di Pietrantonio,[1,*] E. Di Matteo,[1] M. Di Nicola,[1] O. Trubiani,[1] R. Di Primio,[2] E. Serra,[1] and G. Spoto[1]

[1]Department of Applied Sciences of Oral and Dental Diseases, University ''G. D'Annunzio,'' Chieti, Italy
[2]Istituto di Morfologia Umana Normale, Università di Ancona, Ancona, Italy

ABSTRACT

Our aim is to estimate the role of the DMSO on pre-T lymphoid human cells, we have searched the cyclase and phosphodiesterase activity. We have studied the *GTPspecific cyclase* (G-Case) and have observed an analogous course to that one of the cAMP-PDE, where, in both cases, the differences ratio is approximately 5. For the cyclase activity values it has been found that cAMP neo formed is undeterminable in these cells, for the controls and the treated samples.

Key Words: Cyclase; Phosphodiesterase; Pre-T lymphoid human cell; cGMP; cAMP; Dimethyl sulfoxide.

INTRODUCTION

Cellular death can take two different forms respectively called necrosis and Apoptosis (PCD). These two forms differ from each other due to morphological and biochemical characteristics and biological incidence and meaning.[1] The necrosis is an

*Correspondence: F. Di Pietrantonio, Department of Applied Sciences of Oral and Dental Diseases, University ''G. D'Annunzio,'' Chieti, Italy.

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event regulated from an active metabolism that, in a complex organism composed from several tissues, is physiologically important to the resorption and/or the remaking of the same tissues. The PCD is a complex event highly, composed of several phases that occur chronologically: phase of induction, phase of execution, phase of degradation. It can be imagined that the regulatory mechanisms regulators in the first phase, the induction one, are particularly rigorous, because generally the started mechanism of apoptosis is irreversible. At the nuclear level, the apoptosis is characterized from the chromatinic condensation, which is followed by the DNA fragmentation. The inhibitors of the endo-nuclease delay but do not prevent the process and this demonstrates that the fragmentation of the nuclear DNA is usually a recurrent event during the apoptosis, even if not essential. Almost all cells can experience PCD independently from the synthesis of new proteins or from the S-phase of cellular cycle in which they are. In the immune system, PCD is a mechanism that is widely used to select the clones that are functionally helpful for differentiation T and B lymphocytes. DMSO typically promotes cellular differentiation due to its ability to arrest cellular growth at the G0–G1 phase. Cyclase and phosphodiesterase activity are very important to metabolize cyclic nucleotides, ubiquitous intracellular second messenger that transmits information to several proteins regulating diverse cellular functions as Ca^{2+} influx, excitability, permeability and gene expression, as well as cell-specific processes such growth cell differentiation and apoptosis. Nevertheless, in other experimental conditions, for example in cellular cultures of pre-T lymphoid human cells (RPMI-8402) DMSO induce apoptosis instead of a terminal differentiation. Using this cell type we studied phosphodiesterase and cyclase activity during apoptosis DMSO-induced.

MATERIALS AND METHODS

cAMP Phosphodiesterase analysis: The enzymatic analysis has been carried out using the method of Spoto et al.[2] The reaction has been started with the addiction of 44 μM of cAMP, in a final volume of 500 μl. The time course of reaction was 60 minutes. The reaction was terminated by transferring the tubes with the reaction mixture in a boiling water bath for 3 min after acidification with HCl 2 M. The sample was then centrifuged and filtered. The clear filtrate obtained was used directly for HPLC assay or stored at − 80°C. *ATP specific cyclase(A-Case) activity analysis*: the enzymatic analysis has been carried out using the method[2] with minor modifications: phosphate 0,1 M buffer, pH 7.0 $MgCl_2$ 33.2 mM with addiction of IBMX 2.25 mM (MixA) to 37°C. The reaction has been started with the addiction of 54 μM of ATP, in a final volume of 500 μl. The mixture has been put to incubate for 60 minutes to 37°C, after the reaction was stopped with 10 μl HCl 2 M and boiled for 5 minutes in bath water. Later the sample has been centrifuged to 12000 rpm and has been filtered. The obtained limpid filtered has been used directly for the analysis in HPLC or has been conserved to − 80°C. *G-Caseactivity analysis*: the enzymatic analysis has been carried out similar to adenylate cyclase activity but with the GTP to the concentration of 54 μM in a final volume of 500 μl. *Phosphodiesterase and cyclase activity in HPLC inverted-phase analysis*: for the analysis is proceeded like described.[2]

Table 1. Phosphodiesterase activity and guanilate cyclase activity in different times.

Samples	cAMP—PDE nmols/mg prot. Differences DMSO-controls	G—Case nmols/mg prot. differences DMSO-controls
8402 0 h	0	
8402 Contr. 12 h DMSO 12 h	5,7	5,424
8402 Contr. 24 h DMSO 24 h	1,737	
8402 Contr. 48 h DMSO 48 h	− 30,131	
8402 Contr. 72 h DMSO 72 h	− 29,728	

RESULTS

The guanilate cyclase activity has shown increasing values, about the controls, to growing of the time of incubation (Table 1). Same course we have obtained with the cells treated with DMSO, but with higher values.

The adenylic activity, instead, has not given values, probably for problems associated to the sensibility limits of our experimental methodologies. About the phosphodiesterase activity, our studies are centralized to adenylic one, that has shown values, in terms of differences, with rational course. The cAMP-PDE has given undeterminable values about the parameter 0 h (that is the reference element, without incubation and treatment with DMSO).

The controls to 12, 24, 48, 72 h have shown respective values: 9.45, 3.29, 30.13, and 29.73 nmoles/mg. Instead, the samples treated with DMSO, to the same intervals of time, have been respective: 15.24, 5.02, 0,0 nmoles/mg.

DISCUSSION

The treated cells with DMSO stimulated the cAMP-PDE activity in the first 24 hr; in fact, its values were higher than respective controls. Instead, about the treated cells with DMSO for 48 and 72 h is had radical change of direction: the controls became numerically higher than respective treated. In the first 12 hours the induced activity cAMP-PDE is a mark of the cellular answer to the action of the DMSO, while from the 48 hours in then, the advance of the apoptotic phenomenon stops the cellular activities and consequently enzymatic activities. From the cyclase activity values it has been found that cAMP neo formed is undeterminable in these cells, for the controls and the treated samples. That would seem in contrast with values obtained in phosphodiesterase activity because the total absence of cAMP would not explain the cAMP-PDE activity. We have searched analogies in the guanylyl methods knowing that the presence of the cGMP-PDE activity exists, for personal communication supplied from the authors. We have studied the G-Case and have observed an analogous course

to that one of the cAMP-PDE, where, in both cases, the differences ratio is approximately 5. In conclusion, the G-Case and the cAMP-PDE are enzymes involved in the apoptotic process.

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