Journal of Photoscience (2002), Vol. 9(2), pp. 433-435

Sphingosine-1-phosphate Promotes the Survival of Mel-Ab Cells via ERK and Akt activation

Dong-Seok Kim, Eui-Soo Hwang, Sook-Young Kim, Jai-Eun LEE,
and Kyoung-Chan Park*
Department of Dermatology, Seoul National University, Seoul, 110-744, Korea

In the present study, we investigated the actions of sphingosine-1-phosphate (SPP) in Mel-Ab melanocytes. We observed the cytoprotective effect of SPP on UVB-induced cell death. Following exposure of cells to UVB, a significant protective effect was seen in cultures pretreated with SPP. Since SPP is well known as a mitogenic agent, it is possible that the mitogenic effect of SPP may contribute to cell survival. Surprisingly, we found that SPP inhibited DNA-synthesis significantly. We were next interested in the regulation of the extracellular signal-regulated protein kinase (ERK) and Akt pathways by SPP. We clearly observed that SPP potently stimulated the phosphorylation of both ERK and Akt against UVB-induced cell death. Based on these results, we conclude that SPP may show its cytoprotective effect through ERK and Akt activation.

Key words : Sphingosine-1-phosphate, UVB, melanocytes, ERK, Akt

INTRODUCTION

Sphingolipids have been emerged as bioactive lipid modulators that mediate a variety of cell functions. Sphingosine-1-phosphate (SPP) stimulates proliferation in Rat-1 fibroblasts, airway smooth muscle cells, and arterial smooth muscle cells. On the other hand, SPP has been reported to prevent apoptosis induced by ceramide.

*To whom correspondence should be addressed.
E-mail : gcpark@snu.ac.kr

UV radiation is the most important environmental factor responsible for skin pigmentation and cancer. Thus, UV radiation evokes a number of physiological, pathological, and noxious responses in melanocytes, located at the basal layer of the epidermis. However, in melanocytes, little is known about the role of SPP on the cell death induced by UV. Therefore, we investigated the effects of SPP on Mel-Ab cells. Especially, proliferation and cell survival of Mel-Ab cells were analyzed in terms of changes of the ERK signaling and Akt signaling pathways.

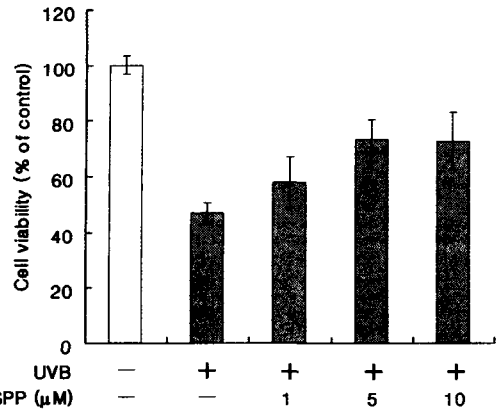

Figure 1. The cells were pretreated with the indicated concentrations of SPP.

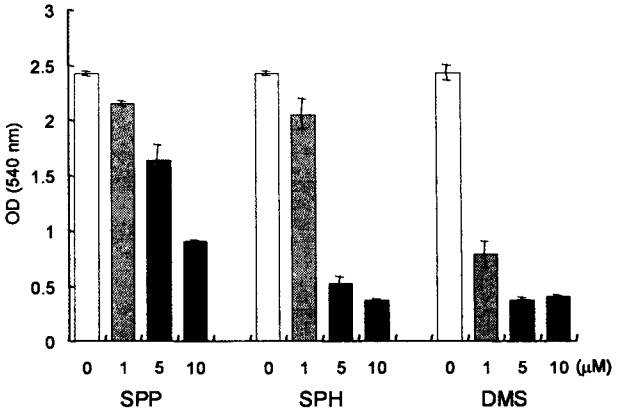

Figure 2. The cells were treated with 1-10 μM SPP, sphingosine (SPH) or N,N-dimethylsphingosine (DMS) for 72 h. The proliferation was determined by the MTT test.

MATERIALS AND METHODS

Cell culture. Mel-Ab cells were maintained at 37℃ in 5% CO_2 as previously described [1].

MTT assay. Cell proliferation was determined by the MTT assay.

UVB irradiation. UVB was irradiated as previously described [2].

Western blot analysis. Western blot analysis was performed as previously described [2].

Cell viability assay. Cell viability was determined by crystal violet assay [2].

RESULTS

Cytoprotective effect of SPP against UVB-induced Cell death. We first tested the protective effect of SPP at concentrations ranging from 1-10 μM. The protective effect of SPP against cell death was dose-dependent and showed a maximal cytoprotection at 5 μM SPP (Fig. 1).

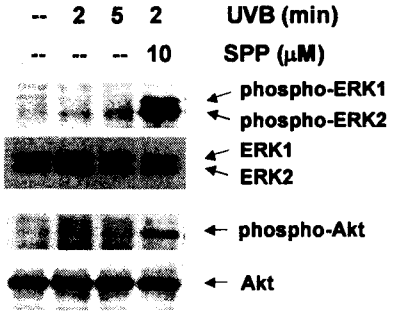

Figure 3. The cells were stimulated with 100 mJ/cm^2 UVB in the absence or presence of 10 μM SPP. Samples were collected for Western Blot at 2 or 5 min after UVB irradiation.

Effects of Sphingolipids on the proliferation of Mel-Ab cells. Since SPP is well known as a mitogenic agent, it is possible that the mitogenic effect of SPP may contribute to cell survival. Surprisingly, we found that SPP, sphingosine and DMS inhibited cell proliferation significantly in the presence of serum. By dosing with various concentrations, SPP treatment was found to have a dose-dependent inhibitory effect (Fig. 2).

Effects of SPP on the ERK and Akt Pathways after UVB Irradiation in Mel-Ab cells. We were interested in the regulation of the ERK and the Akt pathway by SPP against UVB-induced cell death. Therefore, we investigated which signaling pathway is involved in the UVB-induced cell death and the cytoprotective effect of SPP in Mel-Ab cells. We clearly observed that SPP potently stimulated the phosphorylation of both ERK and Akt, which are involved in cell proliferation and survival signaling cascade (Fig. 3).

DISCUSSION

Despite our increasing knowledge about sphingolipids, their effects on proliferation and survival of melanocytes have received little attention. It is well known that SPP stimulates growth of quiescent Swiss 3T3 fibroblasts and may be an important mediator of the mitogenic action of growth factors. Surprisingly, we demonstrated here that SPP inhibits the proliferation of Mel-Ab melanocytes, although SPP induces a strong and sustained activation of ERK. The antiproliferative effect of SPP is not due to the cell cytotoxic effect. It has been proposed that the kinetics of ERK activity is important in determining cellular response and that prolonged activation of the ERK pathway may induce cell growth arrest. Hence, it is likely that SPP may inhibit the proliferation via the sustained activation of ERK. Although mechanisms responsible for the antiproliferative effect are not understood, it is also imaginable that there are different cellular targets for SPP depending on different cell types.

Despite the strong antiproliferative activity, SPP interestingly showed cytoprotective effect against UVB. Recently, it has been reported that Akt plays an important role in apoptosis inhibition. Insulin or other growth factors activates Akt via a pathway that includes PI 3-kinase. Many types of neuronal cells are known to depend on the PI 3-kinase/Akt kinase pathway as a crucial anti-apoptotic signal. Since melanocytes are derived from the neural crest, it is feasible that the Akt kinase pathway is deeply involved in cell death of melanocytes. Thus, our results indicate that Akt activation by SPP may lead to the survival of cells against UVB.

Based on these results, we conclude that SPP may show its cytoprotective effect through ERK and Akt activation.

REFERENCES

1. Dooley T.P., R.C. Gadwood, K. Kilgore, and L.M. Thomasco (1994) Development of an in vitro primary screen for skin depigmentation and antimelanoma agents. *Skin Pharmacol.*, 7,88-200.

2. Kim D.S., S.Y. Kim et al (2001) Ceramide inhibits cell proliferation through Akt/PKB inactivation and decreases melanin synthesis in Mel-Ab cells. *Pigment Cell Res.* 4,10-115