this metabolite. Further experiments revealed that sphinganine and 1-deoxy-sphinganine levels are regulated in a highly coordinated, counter oscillatory, manner during the PMA induced differentiation of monocytic THP1 cells into macrophages. The inhibition of SPT with myriocin blocked this differentiation process.

These results indicate a regulatory process for the generation of 1-deoxy-sphinganine which seems to be important for THP1 monocyte-macrophage differentiation.

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Daunorubicin-induced neutral sphingomyelinase 2 gene expression in MCF7 cells

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Ceramide is generated by the hydrolysis of sphingomyelin (SM) through sphingomyelinases (SMase) or de novo synthesis from serine and palmitoyl CoA. The Mg^{2+}-dependent neutral SMase (NSMase), namely NSMase2, have emerged as the prime candidate for the stress-induced ceramide production out of 3 NSMases identified at present. In the current study, we analyzed the regulatory mechanism of NSMase2 gene expression of a human breast cancer cell line, MCF7, treated with Daunorubicin (DA). DA-induced increase of NSMase2 mRNA but not NSMase1 and NSMase3 was observed, whereas acid SMase mRNA shoed a mild increase with DA. DA also increased NSMase enzyme activity and cellular ceramide. Promoter analysis revealed that three Sp1 binding sites located between −148 and −42 bp upstream of the first exon were important in the basic promoter activity and that the most distal Sp1 site was mainly responsive to DA, while ASMase and NSMase1 promoter activities did not change by DA. Moreover, DA-treated MCF7 showed higher promoting activity against the reporter vector containing the stretch of consensus Sp1 binding sites than that of control MCF7. Electrophoresis mobility shift assay using the most distal Sp1 site as the probe showed increased band intensity by DA, which was reduced by anti-Sp3 antibody, but not anti-Sp1 antibody. Furthermore, Sp3 protein of MCF7 cells was increased by DA. Taken together, DA-induced Sp3 may play an important role in NSMase2 gene expression of MCF7 cells, leading to DA-induced cell death due to increased cellular ceramide.

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PO 41

Ezetimibe inhibits expression of acid sphingomyelinase in liver and intestine

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Ezetimibe is a drug that inhibits cholesterol absorption in the intestine. Sphingomyelin (SM) has strong interaction with cholesterol. We investigated effects of ezetimibe on sphingomyelinase (SMase) expression in intestine and liver. After feeding rats with ezetimibe (5 mg/kg/day) for 14 days, acid SMase activity in the liver and in the proximal part of the small intestine were reduced by 34% and 25%, respectively. Alkaline SMase (Alk-SMase) was increased only in the proximal part of small intestine. Administration of lower dose of ezetimibe only reduced acid SMase in the liver but not the intestine. In cell culture studies ezetimibe at doses significantly inhibited cholesterol uptake decreased acid SMase activity in Hep G2 and Caco-2 cells dose-dependently. The effects were stronger and more rapid in Hep G2 cells than in Caco-2 cells. Western blot further confirmed the decreased acid SMase protein in both Hep G2 and Caco-2 cells by 100 μM ezetimibe. SM content in HepG2 cells but not Caco-2 cells was increased 24 h after ezetimibe treatment. Following the reduction of acid SMase, there was a slight increase of alk-SMase activity induced by high dose of ezetimibe in both Caco-2 and Hep G2 cell lines. To assess the specificity, the changes of acid and alkaline phosphatases were also determined. Ezetimibe only slightly decreased the activity of acid phosphatase but had no effect on that of alkaline phosphatase. In conclusion, the study demonstrates a novel effect of ezetimibe on acid SMase expression in both liver and intestine.

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PO 42

The influence of sphingomyelin (SM) and platelet-activating factors (PAF) on apoptosis in human leukaemic cell lines HL60, Jurkat, U937, KG1a and BV173 in vitro culture

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Recent studies have pointed out that phospholipids such as SM and PAF play an important role in apoptosis pathways. The SM breakdown product – ceramide (Cer) is deemed to have a second messenger function in programmed cell death. Additionally PAF, formatted during cell shrinkage, stimulates sphingomyelinase to SM hydrolysis, leading to scrambling in the cell membrane. Consequently, it leads to a loss of asymmetry of the cell membrane and cell-surface exposure of phosphatidyloserine (PS).

On the basis of our previous studies with the application of spectroscopy ^{31}P NMR, we have shown a SM and PAF concentration reduction in mononuclear blast cells of acute leukaemia. The aim of the present studies was to estimate apoptosis in blast cells under the influence of SM and PAF in cell culture in vitro. These investigations were carried out on leukaemic cell lines HL60, Jurkat, U937, KG1a and BV173, cultured with lyposomal SM and PAF. The percentage of apoptotic cells was flow-cytometric measured using FITC-labelled Anexin V and propidium iodide. In all studied cell lines we observed