4.2. SUMMARY

Mechanism of 1α,25-dihydroxyvitamin D_3 induced apoptosis in human keratinocytes

In the present study the mechanism of 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) induced apoptosis was investigated in human keratinocytes. This was done with different methods at distinct cellular levels. It could be demonstrated that 1α,25-$(OH)_2D_3$ mediated its effect on cell proliferation and apoptosis in human keratinocytes via upregulation of tumor necrosis factor (TNF) α gene expression. After treatment of cells with 1α,25-$(OH)_2D_3$ increased TNFα mRNA is detected, which is then translated into protein. TNFα protein is secreted and it affects the cell through an autocrine mechanism via the 55 kDa receptor. When cells are preincubated with neutralizing antibodies against the 55 kDa TNFα-receptor, the 1α,25-$(OH)_2D_3$ induced inhibition of cell proliferation and apoptosis are partially blocked. TNFα leads to sphingomyelin hydrolysis in HaCaT cells, whereby ceramide is generated, which as a second messenger transduces the signal into the cell. The reduction of the basal ceramide concentration with fumonisin B1, a specific inhibitor of an enzyme in the ceramide biosynthesis, the sphinganin-*N*-acyltransferase, antagonizes 1α,25-$(OH)_2D_3$-induced apoptosis. Exogenously applied cellpermeable ceramide analogues such as C_2-Cer=O (*N*-acetylsphingosine), C_2-Cer=S (*N*-thioacetylsphingosine) and FS-5 (4-dodecanoylamino-decan-5-ol) lead also to inhibition of cell growth and apoptosis in HaCaT cells, whereas the analogues C_2-Cer=O and C_2-Cer=S are strong agonists and FS-5 a weak agonist of the inhibition of proliferation and apoptosis. The weak effect of FS-5 could be most probably explained by the lack of the double bond between carbon atom 4 and 5 of the sphingosine backbone.

In addition to 1α,25-$(OH)_2D_3$ its analogues EB 1213, GS 1500, Tacalcitol and Calcipotriol were also tested in HaCaT cells. All four analogues caused sphingomyelin hydrolysis, whereas with EB 1213 the hydrolysis was detectable even after 6 h of treatment. Furthermore, the analogues were shown to be antiproliferativ and proapoptotic. Nevertheless, only EB 1213 could upregulate the mRNA for TNFα, which could be explained by the fact that the analogues have different promotor selectivity.