TCDD-dependent modulation of urokinase-plasminogen activator and plasminogen activator inhibitor-2 in human keratinocytes.

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Treatment of the human keratinocyte cell line, SCC-12F, with TCDD, a potent modulator of keratinocyte growth and differentiation, induces plasminogen activator inhibitor-2 (PAI-2) gene expression[1]. PAI-2 is a rapid inhibitor of urokinase-plasminogen activator (u-PA), a serine protease which catalyzes the conversion of plasminogen to the active enzyme plasmin. Plasmin is a broad spectrum protease involved in degradation of the extracellular matrix and may also play a role in activation of latent growth factors[2]. Controlled extracellular proteolytic activity is essential in a wide variety of physiological events including, cell migration, proliferation and differentiation, wound repair, and inflammation[3]. Inappropriate control of proteolytic activity is associated with certain skin disorders such as psoriasis, pemphigus and tumorigenesis[4]. Thus, plasminogen activators and their inhibitors may play a role in the actions of TCDD on keratinocyte growth and differentiation. The present study further characterizes the actions of TCDD on PAI-2 expression and also looks at u-PA, tissue plasminogen activator (t-PA), and plasminogen activator inhibitor-1 (PAI-1) expression in SCC-12F cells and in cultured human keratinocytes.

SCC-12F cells were plated at 2.5×10^4 cells/cm^2 in DMEM plus 5% fetal calf serum and incubated for 48 hours. After incubation cells were treated with 0.1% DMSO (control) or 10 nM TCDD. Human keratinocytes (obtained from Clonetics Corp., San Diego, CA) were plated at 2×10^4 cells/cm^2 in keratinocyte serum-free media (Gibco/BRL, Gaithersburg, MD) supplemented with bovine pituitary extract and epidermal growth factor. The cultures were incubated for 48 hours. After incubation the cultures were treated with 0.1% DMSO or 10 nM TCDD in

keratinocyte basal medium (Clonetics Corp.) containing 0.1% BSA, 5μg/ml insulin plus or minus 1.8 mM calcium.

Conditioned media from SCC-12F and human keratinocyte cultures was collected at various times after treatment and assayed for the presence or absence of u-PA, t-PA, PAI-1 and PAI-2 by ELISA (American Diagnostica, Greenwich, CT). mRNA was isolated at various times following treatment and used for Northern blot analysis.

For Nuclear runoff analysis, nuclei were isolated by NP-40 lysis from control and TCDD treated cells and incubated in buffer containing ATP, CTP, GTP, and ^{32}P-labelled UTP. After incubation for 30 min radiolabelled mRNA was isolated and hybridized to DNA probes which had been immobilized on nylon membranes. After hybridization, the filters were analyzed by autoradiography and quantified by densitometry.

To determine the effect of TCDD on cell-associated plasminogen activity, cells were plated and treated, as described above, in 96-well dishes. At various times after treatment, cultures were washed with buffer and incubated at 37^{o}C in buffer containing plasminogen plus the chromogenic substrate, S-2251. The difference in absorbance between 405 nm and 650 nm was measured using a microplate reader. Plasminogen activity in all samples was referenced to a human urokinase standard curve.

Treatment of SCC-12F cells with 10 nM TCDD for 24 h resulted in a 2-fold increase in u-PA mRNA and a 20-fold increase in PAI-2 mRNA. The increase in PAI-2 mRNA was due in part to an increase in PAI-2 gene transcription as determined by nuclear runoff analysis. The rate of transcription of u-PA was not altered, indicating that the induction of u-PA mRNA following TCDD treatment may occur posttranscriptionally. t-PA and PAI-1 were not altered by TCDD. Conditioned media from TCDD-treated SCC-12F cultures contained approximately 2-fold higher levels of u-PA protein and 5-fold higher levels of PAI-2 protein, relative to control values. In addition, treatment of SCC-12F cells with TCDD resulted in an increase, relative to control values, in cell-associated plasminogen activity.

Treatment of cultured human keratinocytes with TCDD for 24 h resulted in an approximately 2-fold increase in the mRNAs for PAI-2. No significant alterations in u-PA mRNA were detected. These results do not rule out transient changes in u-PA mRNA or changes that may occur later than 24 h following TCDD treatment. Conditioned media from human keratinocyte cultures treated with TCDD contained significantly higher levels of both PAI-2 and u-PA protein. t-PA protein concentrations in conditioned media from both control and TCDD treated cultures were below the level of detection. PAI-1 protein levels were not altered following treatment with TCDD. In addition, treatment of human keratinocytes with TCDD resulted in an increase in cell-associated plasminogen activity.

These results demonstrate that TCDD induces both u-PA and PAI-2 in SCC-12F cells and in cultured human keratinocytes. Changes in the relative levels of u-PA and its inhibitor, PAI-2, result in an overall increase in cell-associated plasminogen activity. Inappropriate regulation of keratinocyte extracellular proteolysis can affect availability of growth factors, migration, proliferation and differentiation and thus, may be a factor in the biological actions of TCDD on keratinocytes.

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