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Hypericin photo-induced apoptosis involves the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and activation of caspase-8

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Abstract Hypericin (HYP) is a photosensitizing pigment from *Hypericum perforatum* that displays cytotoxic effects in neoplastic cell lines. Therefore, HYP is presently under consideration as a new anticancer drug in photodynamic therapy. Here, we investigated the mechanism of action of HYP photo-induced apoptosis of Jurkat cells compared to the cytostatic drug paclitaxel (PXL). Both photoactivated HYP and PXL similarly increased the activity of caspase-8 and caspase-3, and drug-induced apoptosis of Jurkat cells was completely blocked by inhibitors of caspase-8 (Z-IETD-FMK) and caspase-3 (Z-DEVD-FMK). The involvement of death receptors was analyzed using neutralizing monoclonal antibodies against Fas (SM1/23), FasL (NOK-2) and TNF-R1 (MAB225), and a polyclonal rabbit anti-human TNF-related apoptosis-inducing ligand (TRAIL) antiserum. TRAIL antibody blocked TRAIL-induced and HYP photo-induced, but not PXL-induced apoptosis of Jurkat cells. In contrast, PXL-induced, but not HYP-induced apoptosis was blocked by the SM1/23 and NOK-2 antibodies. Anti-TNF-R1 antibody had no effect. These findings suggest that HYP photo-induced apoptosis of Jurkat cells is mediated in part by the TRAIL/TRAIL-receptor system and subsequent activation of upstream caspases.

Key words: Apoptosis; Caspase; Death receptor; Hypericin; Photosensitization

1. Introduction

Hypericin (HYP) is a photosensitizing plant pigment from *Hypericum perforatum* [1,2]. The absorption spectrum of HYP shows peaks in the UV range (330 nm) and in the visible light (VIS) range at 550 and 588 nm [2]. Upon photoactivation, HYP generates highly reactive singlet oxygen molecules [2,3]. The photodynamic action of HYP includes photohemolysis of red blood cells [1], lipid peroxidation, increase of superoxide dismutase activity, and decrease of cellular glutathione levels [4]. Several studies have described cytotoxic effects of HYP in neoplastic cell lines [4–7]. Therefore, HYP is presently under consideration as a new anticancer drug in photodynamic therapy (PDT) [8,9]. During the last years, several pharmacological actions of HYP have been described that may contribute to its antiproliferative activity. These include inhibition of mitochondrial succinoxidase [3], protein kinase C [10,11], tyrosine kinase [12,13], MAP kinases [14], epidermal growth factor receptor [13], and transcription factor NF-κB [15]. Most evidence suggests that photoactivated HYP kills tumor cells by induction of apoptosis [7,14,16–19], although necrosis occurs at higher drug concentrations [18]. At present, the mechanisms of HYP-induced apoptosis are not fully understood. Although HYP is a strong inhibitor of protein kinase C [10] it was shown that protein kinase C inhibition does not correlate with cytotoxicity induced by HYP [16]. HYP-induced apoptosis of glioma cells is not dependent on the level of bcl-2 expression [16]. However, in Jurkat cells overexpression of bcl-2 delayed later stages of HYP photo-induced apoptosis [19]. Recently it was shown that HYP-induced apoptosis is associated with mitochondrial membrane depolarization in glioma cells, HeLa cells and Jurkat cells [17–19], release of cytochrome *c* from mitochondria of HeLa cells [18] and activation of caspase-3 in HeLa and Jurkat cells [14,18,19].

Here, we show that HYP photo-induced apoptosis of Jurkat cells is associated with the activation of caspase-8 and can be blocked by a inhibitor of caspase-8, Z-IETD-FMK. Since caspase-8 is an initiator-caspase upstream of cell death receptors [20], we reasoned that receptors of the tumor necrosis factor (TNF) family might be involved in HYP-induced apoptosis.

Using neutralizing antibodies against Fas, Fas-ligand (FasL), TNF-receptor-1 (TNF-R1) and TNF-related apoptosis-inducing ligand (TRAIL) we could demonstrate that HYP-induced apoptosis can be specifically inhibited by a polyclonal anti-TRAIL antibody. We have shown that this antibody specifically blocks TRAIL-mediated killing of Jurkat cells. For comparison, paclitaxel (PXL)-induced apoptosis was studied. PXL and other drugs that disturb microtubule dynamics kill cells in a Fas/FasL-dependent manner [21].

2. Materials and methods

2.1. Chemicals and antibodies

HYP was obtained from Roth (Karlsruhe, Germany). PXL was obtained from Tocris (Tocris Cookson, MO, USA). Stock solutions were prepared in DMSO and stored in the dark at −20°C. Further dilutions were made with medium to obtain final concentrations as indicated. Cell-permeable non-toxic inhibitors of caspase-8 (Z-IETD-FMK) and caspase-3 (Z-DEVD-FMK) were obtained from R&D

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Abbreviations: HYP, hypericin; PXL, paclitaxel; TRAIL, TNF-related apoptosis-inducing ligand; VIS, visible light

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systems (Wiesbaden, Germany) and were used at a concentration of 50 μM. For blocking experiments, the neutralizing monoclonal antibodies (mAb) NOK-2 (anti-FasL) (Pharmingen, San Diego, CA, USA), SM1/23 (anti-Fas) (Alexis, Grünberg, Germany) and MAB225 (anti-TNF-R1) (R&D systems) were used at a concentration of 10 μg/ml. The polyclonal anti-human TRAIL antiserum 210-732-R100 (Alexis) was used at a 1:50 dilution. Recombinant human soluble TRAIL (rhsTRAIL) (Alexis) was used at a concentration of 100 ng/ml, in the presence of 2 μg/ml enhancer antibody (Alexis).

2.2. Cell culture and drug treatment

Jurkat cells (human acute T cell leukemia) (ATCC, Manassas, VA, USA) were cultured in c-RPMI (RPMI supplemented with 10% FCS, 1% L-glutamine and 1% penicillin/streptomycin) (Gibco, Eggenstein, Germany) at 37°C and 5% CO_2. Before treatment with HYP and VIS, the cells were washed and resuspended in PBS. HYP was prepared and added to cell cultures (250–1000 ng/ml final) under strictly subdued conditions. After 2 h incubation in the dark the cells were irradiated on ice with VIS (520–750 nm, 5.0 J/cm^2), delivered from the incoherent light source PDT 1200 SOA (Waldmann, VS-Schwenningen, Germany) [22]. Following irradiation, the cells were washed and further cultured in c-RPMI in 96 well flat-bottom microtiter plates (Greiner, Nürtingen, Germany). For PXL treatment, the cells were washed and suspended in c-RPMI before PXL was added (1 μM final). The cell numbers were 1×10^6/ml (DNA preparation, caspase assay), 1×10^5/ml (proliferative assay) or 1×10^4/ml (apoptosis assay).

2.3. Proliferative assay

Following drug treatment, the cells were incubated for 24 h in the dark. Cells were then pulsed with 1 μCi [^{3}H]TdR/well for 18 h and [^{3}H]TdR incorporation was determined by liquid scintillation spectroscopy using a Top-Count (Canberra Packard, Frankfurt, Germany) as described [22]. Data are expressed as mean ± S.D. of three independent experiments.

2.4. Photometric determination of apoptosis

Cells were treated with drugs or additives for 24 h as described above. Cells were then evaluated for apoptosis using a cell death detection ELISA (Cell Death Detection ELISA^{plus}, Boehringer, Mannheim, Germany). The principle of this test is the detection of mono- and oligonucleosomes in the cytoplasmic fractions of cell lysates using biotinylated anti-histone and peroxidase-coupled anti-DNA antibodies. The amount of nucleosomes is photometrically quantified at 405 nm by the peroxidase activity retained in the immunocomplexes. Data are expressed as mean ± S.D. of three independent experiments.

2.5. DNA gel electrophoresis

Apoptotic DNA fragments were isolated from 10^6 cells treated with HYP (500 ng/ml)+VIS (5 J/cm^2) as described [23]. Briefly, cells were washed and pelleted by centrifugation. Cell pellets were treated for 10 s with lysis buffer (1% NP40; 20 mM EDTA; 50 mM Tris–HCl, all from Sigma, St. Louis, MO, USA). The cells were then mixed with 1% SDS (Sigma), treated for 2 h with RNase (5 μg/μl) (Boehringer, Mannheim, Germany) at 56°C and digested with proteinase K (2.5 μg/μl) (Sigma) for 2 h at 37°C. After addition of 10 M ammonium acetate (Merck, Darmstadt, Germany) the DNA was precipitated with 100% ethanol at −20°C, resuspended in gel loading buffer (10 mM Tris–HCl, 1 mM EDTA, Sigma) and separated with KiloBase™ DNA marker (Pharmacia, Freiburg, Germany) on a 1% agarose gel.

2.6. Terminal dUTP nick end labeling (TUNEL) assay

To assess apoptosis of HYP (500 ng/ml)+VIS (5 J/cm^2)-treated Jurkat cells at the single cell level, DNA strand breaks were identified using the TUNEL technique. The in situ cell death detection kit from Boehringer Mannheim (Mannheim, Germany) was used according to the manufacturer's instructions. Permeabilized, propidium iodide-positive cells were analyzed for dUTP-FITC staining using the Cell Quest™ research software on a FACScan™ flow-cytometer (both Becton Dickinson).

2.7. Caspase assay

To determine the enzymatic activity of caspase-3 and caspase-8, colorimetric assays were performed. Jurkat cells (1×10^5/ml) were treated for 24 h with HYP (500 ng/ml)+VIS (5.0 J/cm^2) or 1 μM PXL. Cells were lysed in ice-cold hypotonic buffer containing 20 mM Tris–HCl (pH 7.2), 1 mM EDTA and 10 μg protease inhibitors (Boehringer) to prevent non-specific cleavage of proteins. Homogenates were clarified by centrifugation for 10 min at $16\,000\times g$. Cell lysates were then tested for protease activity by the addition of caspase-specific peptides conjugated to the color reporter *p*-nitroanilide (R&D systems). The cleavage of the peptide by the caspase releases the chromophore, which is quantitated spectrophotometrically at 405 nm. The level of caspase enzymatic activity is directly proportional to the color reaction. Data are indicated as percent of untreated control and represent mean+S.D. of three independent experiments.

2.8. Blocking of TRAIL-mediated cell killing

To confirm the blocking activity of the anti-TRAIL antibody, Jurkat cells were incubated for 24 h with 100 ng/ml TRAIL+enhancer antibody in the presence or absence of the anti-TRAIL antibody (1:500, 1:100 and 1:50). Subsequently, cell proliferation was measured by [^{3}H]TdR uptake as described above.

3. Results

3.1. Photoactivated HYP inhibits proliferation of Jurkat cells by induction of apoptosis

Treatment of Jurkat cells with HYP (250–1000 ng/ml) and subsequent irradiation with VIS (520–750 nm, 5.0 J/cm^2) showed a dose-dependent inhibition of cell proliferation, whereas HYP alone had not effect (Fig. 1A). We next evaluated cell lysates of HYP+VIS-treated Jurkat cells for the content of apoptotic oligonucleosomes by a colorimetric ELISA. Fig. 1B shows a dose-dependent increase of DNA-fragmentation after treatment with HYP+VIS. Apoptosis was further confirmed by DNA-gel electrophoresis, showing an increased generation of apoptotic DNA fragments in HYP+VIS-treated cells, but not in cells treated with HYP alone (Fig. 1C). TUNEL-staining revealed that treatment with HYP (500 ng/ml)+VIS (5 J/cm^2) resulted in an apoptosis rate of 80% as compared with 10% in untreated cells (Fig. 1D).

3.2. Photoactivated HYP induces activation of caspase-8 and caspase-3

To assess the involvement of caspases, Jurkat cells were treated with HYP (500 ng/ml)+VIS (5 J/cm^2) and 1 μM PXL. The cells were then incubated for 24 h and cell lysates were evaluated for the enzymatic activity of caspase-8 and caspase-3, respectively. Caspase-8 and caspase-3 activity were increased by treatment with HYP+VIS to a similar extent as compared with PXL (Fig. 2).

3.3. HYP photo-induced apoptosis is blocked by caspase-inhibitors and anti-TRAIL antibody

Both HYP+VIS and PXL-induced apoptosis could be blocked by inhibitors of caspase-8 (Z-IETD-FMK) and caspase-3 (Z-DEVD-FMK) (Fig. 3). Since caspase-8 is an initiator-caspase upstream of death receptors of the TNF family [20], we asked if HYP-induced apoptosis is mediated by death receptors containing a cytoplasmic death domain.

As shown in Fig. 3, polyclonal anti-TRAIL Ab selectively inhibited HYP-induced apoptosis. In contrast, anti-FasL mAb (NOK-2) and anti-Fas mAb (SM1/23) blocked PXL-induced apoptosis, but had no effect on HYP+VIS-induced apoptosis. Anti-TNF-R1 mAb had no effect on HYP+VIS and PXL-induced apoptosis (Fig. 3). The inhibitory activity of the polyclonal anti-TRAIL Ab was confirmed in a separate experiment that shows a dose-dependent inhibition of TRAIL-mediated killing of Jurkat cells by the antibody (Fig. 4).

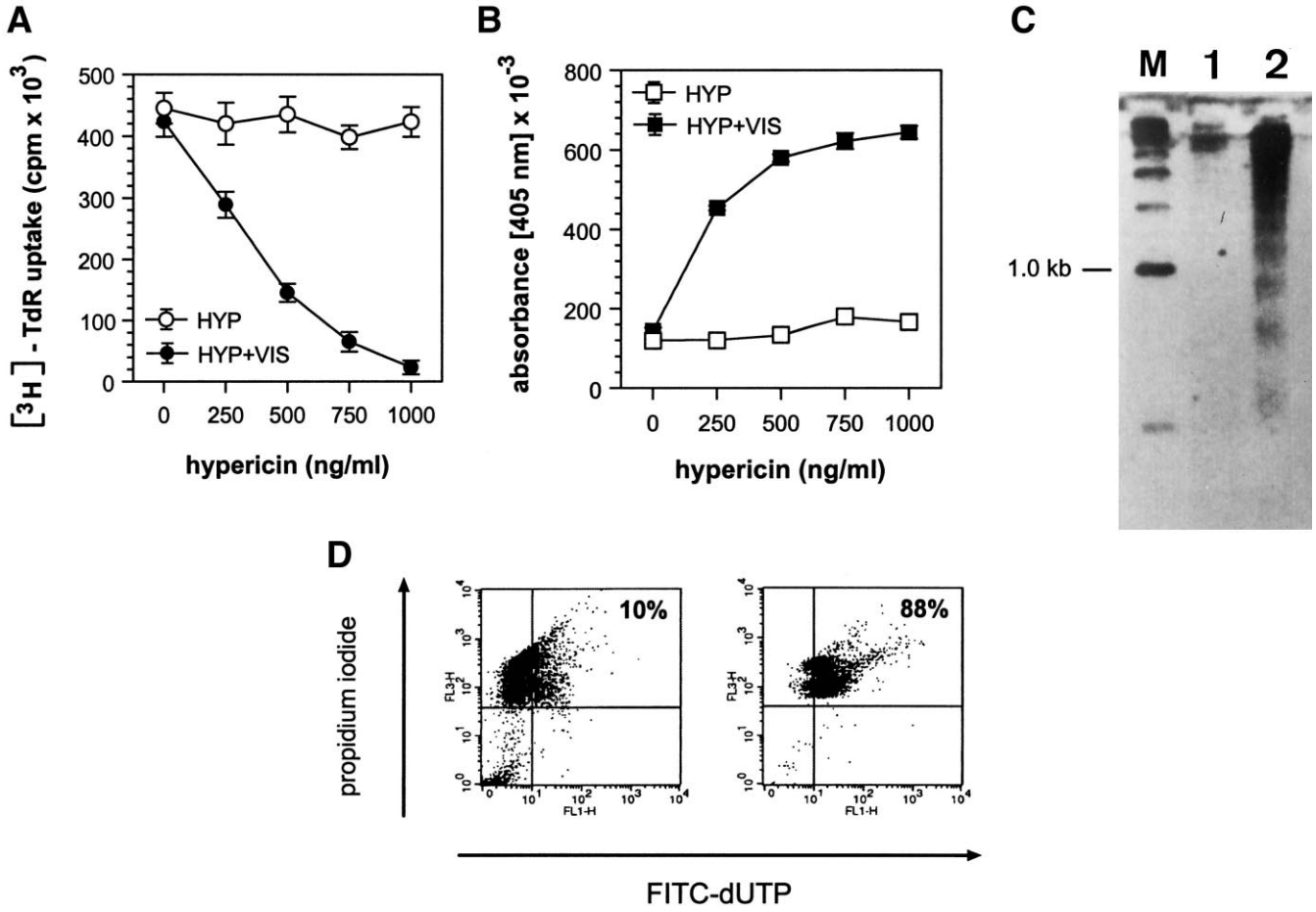

Fig. 1. Effect of photoactivated HYP on cell proliferation and apoptosis of Jurkat cells. A: Proliferative assay. Cell proliferation was assessed 24 h after treatment with HYP+VIS by [^{3}H]TdR incorporation. Data are expressed as mean ± S.D. of three independent experiments. B: Dose-dependent increase of apoptotic oligonucleosomes 24 h after treatment with HYP+VIS. Cell lysates were evaluated for the content of oligonucleosomes by a cell death detection ELISA. Data represent mean ± S.D. of three independent experiments. C: Evaluation of HYP-induced apoptosis by DNA-electrophoresis. DNA was extracted from Jurkat cells 24 h after treatment with HYP (500 ng/ml)+VIS (5 J/cm^2) by NP40 lysis. DNA was separated on a 1% agarose gel. M, KiloBase™ DNA marker; lane 1, cells treated with HYP alone; lane 2, cells treated with HYP+VIS. D: Evaluation of HYP-induced apoptosis at the single cell level. Untreated cells (left) and cells treated with HYP (500 ng/ml)+VIS (5 J/cm^2) (right) were incubated for 24 h. Subsequently, the cells were permeabilized, stained with propidium iodide and FITC-dUTP and analyzed on a FACScan. The percentage of TUNEL-positive cells is indicated.

4. Discussion

Here we show that photoactivated HYP dose-dependently inhibits proliferation of Jurkat cells by induction of apoptosis. Triggering of apoptosis by anticancer drugs may enter the cell death pathway at different sites. The 'extrinsic' cell death pathway involves the TNF-family of death receptors [20,24–26] and subsequent activation of the initiator caspase-8 [26,27]. Alternatively, the apoptosis program can be activated by the 'intrinsic' cell death pathway directly targeting mitochondria. This pathway results in the release of pro-apoptotic factors from mitochondria, i.e. cytochrome *c* and apoptosis-inducing factor (AIF), and caspase activation downstream of mitochondria, i.e. caspase-9, that is part of the mitochondria-associated apoptosome complex [27–32]. Vantieghem et al. demonstrated that HYP-induced apoptosis of HeLa cells can be inhibited by a specific inhibitor of caspase-3 (Z-DEVD-FMK) and by the pan-caspase inhibitor (Z-VAD-FMK), whereas HeLa cells over-expressing the serpin-like inhibitor CrmA, which preferentially inhibits caspase-1 and cas-

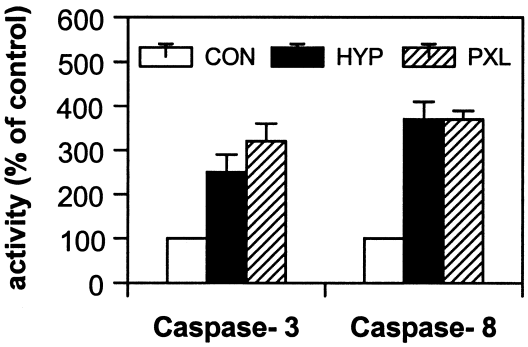

Fig. 2. Involvement of caspases in HYP photo-induced apoptosis of Jurkat cells. Cells were treated with HYP (500 ng/ml)+VIS (5 J/cm^2) or PXL (1 μM) for 24 h. Cell lysates were tested for protease activity by the addition of caspase-specific peptides. The cleavage of the peptide by the caspase releases a chromophore, which is quantitated at 405 nm. Data are indicated as percent of untreated control and represent mean+S.D. of three independent experiments.

pase-8, did not protect the cells from apoptosis [18]. The authors conclude that HYP-induced apoptosis is not mediated by the 'extrinsic' caspase-8-dependent pathway but rather by the 'intrinsic' cell death pathway that involves the release of cytochrome *c* from the mitochondria and subsequent activation of caspase-3 [18]. However, Chaloupka et al. have shown that HYP-induced mitochondrial damage of Jurkat cells does not act through the mitochondrial permeability transition (MPT) pore, but rather through non-specific photo-induced alterations of mitochondrial membranes and a rapid change of the intracellular pH [19]. This is consistent with the earlier finding that the disruption of mitochondrial function is linked to the photooxidative properties of HYP [3]. These findings suggest that the effect of photoactivated HYP on mitochondria may be non-specific or indirect in some cell types (i.e. Jurkat cells), while it can be mediated by the classical 'intrinsic' cell death pathway in other cells (i.e. HeLa cells).

Our finding that HYP-induced apoptosis of Jurkat cells is associated with the activation of caspase-8 prompted us to investigate the involvement of receptors of the TNF family in HYP-induced apoptosis. Using neutralizing antibodies against Fas, FasL, TNF-R1 and TRAIL we have shown that HYP-induced apoptosis can be specifically inhibited by a polyclonal anti-TRAIL antibody. The specificity of the inhibitory activity of this antibody was demonstrated by blocking TRAIL-mediated killing of Jurkat cells. The mechanism by which photoactivated HYP acts on the TRAIL/TRAIL-receptors of Jurkat cells is not clear at the present. It may include increased shedding of TRAIL, stabilization of death-inducing TRAIL-receptors or downregulation of decoy receptors. The signal transduction pathways for the two pro-apoptotic TRAIL receptors (TRAIL-R1/DR4 and TRAIL-R2/DR5) seem to use FADD (Fas-associated death domain), an adapter protein that bridges the receptor with procaspase-8 [33,34]. TRAIL-induced apoptosis involves activation of caspase-8 and caspase-3, is followed by mitochondrial membrane potential dissipation and cytochrome *c* release, and cannot be

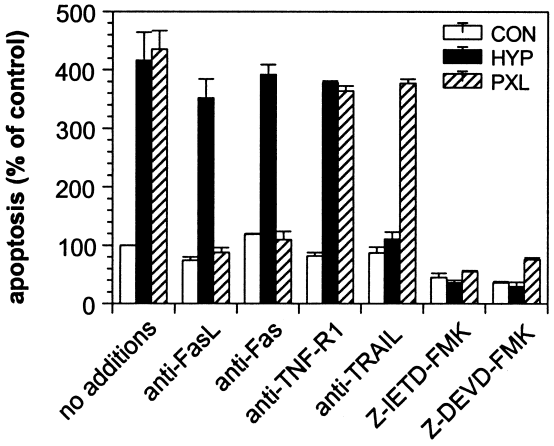

Fig. 3. Involvement of caspases and death receptors in HYP-induced apoptosis. Cell lysates were evaluated for the content of oligonucleosomes by a cell death detection ELISA after treatment with HYP (500 ng/ml)+VIS (5 J/cm^2) or 1 μM PXL, in the presence or absence of the specific inhibitors of caspase-8 (Z-IETD-FMK) and caspase-3 (Z-DEVD-FMK) (50 μM), the neutralizing mAb NOK-2 (anti-FasL), SM1/23 (anti-Fas) and MAB225 (anti-TNF-R1) (10 μg/ml), or the polyclonal rabbit anti-human TRAIL antiserum 210-732-R100 (1:50). Data are indicated as percent of untreated control and represent mean+S.D. of three independent experiments.

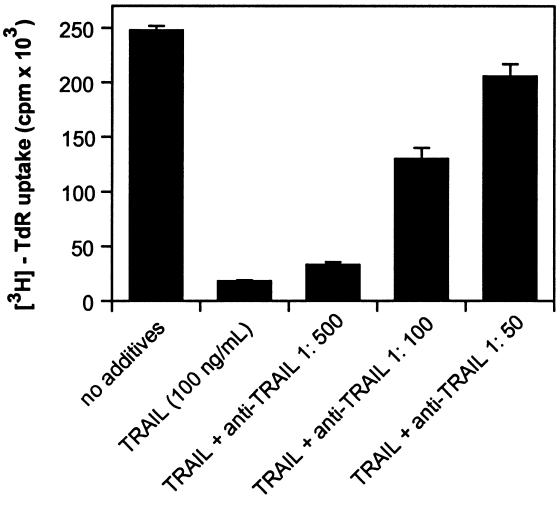

Fig. 4. Inhibitory activity of the polyclonal anti-TRAIL Ab. To confirm the blocking activity of the anti-TRAIL antibody, Jurkat cells were left untreated or were incubated for 24 h with 100 ng/ml TRAIL+enhancer antibody in the presence or absence of the anti-TRAIL antibody (1:500, 1:100 and 1:50). Subsequently, cell proliferation was measured by [^{3}H]TdR uptake.

blocked by bcl-2 overexpression [33]. Thus, the release of cytochrome *c* from mitochondria that occurs during HYP photo-induced apoptosis may also be explained by the apoptosis pathway that involves the TRAIL/TRAIL-receptor system. Tumor cells overexpressing bcl-2 are often resistant to apoptosis induced by chemotherapeutic drugs, but TRAIL may still kill chemoresistant tumors since it bypasses the 'intrinsic' cell death pathway that is controlled by bcl-2 [35]. TRAIL-mediated killing of tumor cells is associated with low toxicity in vivo [36–38]. The present study reports for the first time TRAIL-mediated cell death triggered by HYP in a single cell line. At the present it is not clear if this is also the case in other cell lines. It would also be interesting to study the effects of HYP on cells that are resistant to TRAIL. Taken together, the present study adds to the multi-facetted action of HYP by demonstrating that HYP photo-induced apoptosis of Jurkat cells is mediated at least in part by the TRAIL/TRAIL-receptor system and activation of caspase-8.

References

[1] Pace, N. and Mackinney, G. (1941) J. Chem. Soc. 63, 2570–2574.
[2] Duran, N. and Song, P.S. (1986) Photochem. Photobiol. 43, 677–680.
[3] Thomas, C., Randall, S., MacGill, S., Miller, G.C. and Pardini, R.S. (1992) Photochem. Photobiol. 55, 47–53.
[4] Hadjur, C., Richard, M.J., Parat, M.O., Jardon, P. and Favier, A. (1996) Photochem. Photobiol. 64, 375–381.
[5] Andreoni, A., Colasanti, A., Colasanti, P., Mastrocinque, M., Riccio, P. and Roberti, G. (1994) Photochem. Photobiol. 59, 529–533.
[6] Vandenbogaerde, A.L., Delaey, E.M., Vantieghem, A.M., Himpens, B.E., Merlevede, W.J. and de Witte, P.A. (1998) Photochem. Photobiol. 67, 119–125.
[7] Fox, F.E., Niu, Z., Tobia, A. and Rook, A.H. (1998) J. Invest. Dermatol. 111, 327–332.
[8] Koren, H., Schenk, G.M., Jindra, R.H., Alth, G., Ebermann, R., Kubin, A., Konderhold, G. and Kreitner, M. (1996) J. Photochem. Photobiol. B 36, 113–119.
[9] Alecu, M., Ursaciuc, C., Halalau, F., Coman, G., Merlevede, W.,

Waelkens, E. and de Witte, P. (2000) Anticancer Res. 18, 4651–4654.
[10] Utsumi, T., Okuma, M., Utsumi, T., Kanno, T., Yasuda, T., Kobuchi, H., Horton, H.H. and Utsumi, K. (1995) Arch. Biochem. Biophys. 316, 493–497.
[11] Agostinis, P., Donella-Deana, A., Cuveele, J., Vandenbogaerde, A., Sarno, S., Merlevede, W. and de Witte, P. (1996) Biochem. Biophys. Res. Commun. 220, 613–617.
[12] de Witte, P., Agostinis, P., van Lint, J., Merlevede, W. and Fandenheede, J.R. (1993) Biochem. Pharmacol. 46, 1929–1936.
[13] Kil, K.S., Yum, Y.N., Seo, S.H. and Lee, K.T. (1996) Arch. Pharmacol. Res. 19, 490–496.
[14] Assefa, Z., Vantieghem, A., Declercq, W., Vandenabeele, P., Vandenheede, J.R., Merlevede, W., de Witte, P. and Agostinis, P. (1999) J. Biol. Chem. 274, 8788–8796.
[15] Bork, P.M., Bacher, S., Schmitz, M.L., Kaspers, U. and Heinrich, M. (1999) Planta Med. 65, 297–300.
[16] Weller, M., Trepel, M., Grimel, C., Schabet, M., Bremen, D., Krajewski, S. and Reed, J. (1997) Neurol. Res. 19, 459–470.
[17] Miccoli, L., Beurdeley-Thomas, A., de Pinieux, G., Sureau, F., Oudard, S., Dutrillaux, B. and Poupon, M.F. (1998) Cancer Res. 58, 5777–5786.
[18] Vantieghem, A., Assefa, Z., Vandenabeele, P., Declercq, W., Courtois, S., Vandenheede, J.R., Merlevede, W., de Witte, P. and Agostinis, P. (1998) FEBS Lett. 440, 19–24.
[19] Chaloupka, R., Petit, P.X., Israel, N. and Sureau, F. (1999) FEBS Lett. 462, 295–301.
[20] Ashkenazi, A. and Dixit, V.M. (1998) Science 281, 1305–1308.
[21] Srivastava, R.K., Sasaki, C.Y., Hardwick, J.M. and Longo, D.L. (1999) J. Exp. Med. 190, 253–263.
[22] Schempp, C.M., Simon-Haarhaus, B., Heine, A., Schöpf, E. and Simon, J.C. (1999) Photodermatol. Photoimmunol. Photomed. 15, 13–17.
[23] Herrmann, M., Lorenz, H.M., Voll, R., Grünke, M., Woith, W. and Kalden, J.R. (1994) Nucleid Acids Res. 22, 5506–5507.
[24] Golstein, P. (1997) Curr. Biol. 7, R750–R753.
[25] Martinez-Lorenzo, M.J., Alava, M.A., Gamen, S., Kim, K.J., Chuntharapai, A., Pineiro, A., Naval, J. and Anel, A. (1998) Eur. J. Immunol. 28, 2714–2725.
[26] Krammer, P.H. (2000) Nature 407, 789–795.
[27] Thornberry, N.A. and Lazebnik, Y. (1998) Science 281, 1312–1316.
[28] Zamzami, N., Marchetti, P., Castedo, M., Zanin, C., Vayssière, J.L., Petit, P.X. and Kroemer, G. (1995) J. Exp. Med. 181, 1661–1672.
[29] Liu, X., Kim, C.N., Yang, J., Jemmerson, R. and Wang, X. (1996) Cell 86, 147–157.
[30] Castedo, M., Hirsch, T., Susin, S.A., Zamzami, N., Marchetti, P., Macho, A. and Kroemer, G. (1996) J. Immunol. 157, 512–521.
[31] Reed, J.C. (1997) Cell 91, 559–562.
[32] Green, D. and Reed, J.C. (1998) Science 281, 1309–1312.
[33] Bodmer, J.L., Holler, N., Reynard, S., Vinciguerra, P., Schneider, P., Juo, P., Blenis, J. and Tschopp, J. (2000) Nat. Cell Biol. 2, 241–243.
[34] Sprick, M.R., Weigand, M.A., Rieser, E., Rauch, C.T., Juo, P., Blenis, J., Krammer, P.H. and Walczak, H. (2000) Immunity 12, 599–609.
[35] Walczak, H., Bouchon, A., Stahl, H. and Krammer, P.H. (2000) Cancer Res. 60, 3051–3057.
[36] Gura, T. (1997) Science 277, 768.
[37] Ashkenazi, A., Pai, R.C., Fong, S., Leung, S., Lawrence, D.A., Marsters, S.A., Blackie, C., Chang, L., McMurtrey, A.E., Hebert, A., DeForge, L., Koumenis, I.L., Lewis, D., Harris, L., Bussiere, J., Koeppen, H., Shahrokh, Z. and Schwall, R.H. (1999) J. Clin. Invest. 104, 155–162.
[38] Walczak, H., Miller, R.E., Ariail, K., Gliniak, B., Griffith, T.S., Kubin, M., Chin, W., Jones, J., Woodward, A., Le, T., Smith, C., Smolak, P., Goodwin, R.G., Rauch, C.T., Schuh, J.C. and Lynch, D.H. (1999) Nat. Med. 5, 157–163.