Caspases-3 and -7 are activated in goniothalamin-induced apoptosis in human Jurkat T-cells

Salmaan H. Inayat-Hussain[a,1,*], Annuar B. Osman[b], Laily B. Din[c], Abdul M. Ali[d], Roger T. Snowden[e], Marion MacFarlane[e], Kelvin Cain[e]

[a] *Department of Biomedical Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia*
[b] *Department of Microbiology, Universiti Kebangsaan Malaysia, Bangi, 43600 Selangor, Malaysia*
[c] *Department of Chemistry, Universiti Kebangsaan Malaysia, Bangi, 43600 Selangor, Malaysia*
[d] *Department of Biotechnology, Universiti Putra Malaysia, Serdang, 43400 Selangor, Malaysia*
[e] *MRC Toxicology Unit, Hodgkin Building, University of Leicester, Lancaster Road, Leicester LE1 9HN, UK*

Received 14 June 1999; received in revised form 28 June 1999

Abstract Goniothalamin, a plant styrylpyrone derivative isolated from *Goniothalamus andersonii*, induced apoptosis in Jurkat T-cells as assessed by the externalisation of phosphatidylserine. Immunoblotting showed processing of caspases-3 and -7 with the appearance of their catalytically active large subunits of 17 and 19 kDa, respectively. Activation of these caspases was further evidenced by detection of poly(ADP-ribose) polymerase cleavage (PARP). Pre-treatment with the caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp fluoromethyl ketone (Z-VAD.FMK) blocked apoptosis and the resultant cleavage of these caspases and PARP. Our results demonstrate that activation of at least two effector caspases is a key feature of goniothalamin-induced apoptosis in Jurkat T-cells.

Key words: Apoptosis; Caspase-3; Caspase-7; Goniothalamin; Jurkat T-cell

1. Introduction

A wealth of information is currently available on the pharmacological activities of many natural products, in particular their potential for use in cancer chemoprevention [1]. The activities of these compounds include anti-proliferative, anti-inflammatory, anti-mutagenic and anti-oxidative mechanisms in addition to effects on drug metabolism, cell differentiation and induction of apoptosis [2]. Dysregulation of apoptosis has now been implicated in the onset or progression of cancer [3]. Consequently, apoptosis represents an innate cellular defense against carcinogen-induced cellular damage by removing and inhibiting survival and growth of altered cells at different stages of carcinogenesis [4].

Overwhelming evidence suggests the involvement of caspases, a family of cysteine proteases which cleave after aspartic acid, in mediating the biochemical events that culminate in apoptosis (reviewed in [5,6]). Currently, 14 different caspases have been identified in humans [7,8]. Caspases exist as inactive pro-forms and require processing to active subunits either by autoprocessing or via activation by other caspases. It has been proposed that 'initiator' caspases with long pro-domains, such as caspases-8 and -9, either directly or indirectly activate effector 'caspases', such as caspases-3, -6 and -7 [5]. A number of proteins have been shown to be cleaved in cells undergoing apoptosis by the activated caspases [9]. Among these, poly(ADP-ribose) polymerase (PARP, 113–116 kDa), a nuclear enzyme which is activated during DNA damage, is known to be cleaved by caspases, more specifically, caspases-3 and -7. Other cellular proteins that are cleaved by caspases include lamins, U1, 70 kDa, DNA-PK, actin and retinoblastoma (reviewed in [9]). Many potent selective reversible and irreversible peptide-based inhibitors have been developed, which have advanced our understanding of the involvement of proteases during apoptosis [10]. Benzyloxycarbonyl-Val-Ala-Asp fluoromethyl ketone (Z-VAD.FMK), a general caspase inhibitor, has been shown to block apoptosis in many cell types including rat hepatocytes, thymocytes and human leukemic T-cells [11–16].

In this study, the apoptotic potential of goniothalamin, a biologically active plant styrylpyrone derivative isolated from *Goniothalamus andersonii*, was investigated in the leukemic T-cell line Jurkat. This plant extract has recently been demonstrated to have anti-proliferative activities in a number of transformed cell lines including MCF-7 and HeLa cells [17,18]. We now demonstrate for the first time that goniothalamin induces apoptosis in Jurkat T-cells and that at least two caspases, the effector caspases-3 and -7, are activated as evidenced by cleavage of their intracellular substrate PARP.

2. Materials and methods

2.1. Jurkat T-cell culture and reagents

The leukemic T-cell line Jurkat (Clone E6-1) was obtained from ECACC. The cells were cultured in RPMI 1640 supplemented with 10% foetal bovine serum and 1% Glutamax. Media and serum were obtained from Life Technologies (Paisley, UK). Annexin V/FITC was purchased from Bender Medsystems (Vienna, Austria). Z-VAD.FMK was from Enzyme Systems (Dublin, CA, USA).

2.2. Extraction of goniothalamin

The dried powdered bark of *G. andersonii* (500 g) was extracted in a Soxhlet apparatus with light petroleum ether for 72 h as described previously [19]. Evaporation of the light petroleum ether afforded a mixture of solid and thick brown oil (10 g). Column chromatography on silica gel using light petroleum and ethylacetate mixtures as the eluent yielded a white solid (1.5 g). Recrystallisation from light petroleum yielded white crystals (1 g), which were identified through spectroscopic data (IR, UV, MS and NMR) as goniothalamin (molecular weight 200).

*Corresponding author. Fax: (1) (303) 315-0274.
E-mail: salmaan.inayat-hussain@uchsc.edu

[1] Current address: Molecular Toxicology and Environmental Health Program, Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Health Sciences Center, Campus Box C238, 4200 East Ninth Avenue, Denver, CO 80262, USA

PII: S0014-5793(99)00984-9

2.3. Quantification of apoptosis by annexin V/propidium iodide staining

Apoptosis in Jurkat T-cells was induced with goniothalamin (10 μg/ml, equivalent to 50 μM) for 5 h. In some experiments, the cells were pre-treated for 30 min with Z-VAD.FMK (20 μM) prior to exposure to the apoptotic stimulus. Apoptosis was quantified by phosphatidylserine (PS) exposure as described previously [20]. Briefly, 10^6 cells were centrifuged for 4 min at $200 \times g$ and resuspended in 1 ml annexin buffer. Annexin V (1.5 μl) was added to the suspension and incubated for 8 min at room temperature prior to addition of propidium iodide (PI) (2.5 μg/ml). Following a further incubation for 2 min at 4°C, flow cytometric analysis was carried out using a FACScan (Becton Dickinson).

2.4. Western blotting

Jurkat cells (0.5×10^6 cells) were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously [21]. Cellular proteins were resolved on 12% (caspases-3 and -7) or 7% (PARP) SDS-polyacrylamide gels under denaturing conditions and blotted onto a nitrocellulose membrane (Hybond-C extra, Amersham, Bucks, UK). Pro-caspase-3 and its catalytically active large subunit were detected with a rabbit polyclonal antibody raised against the p17 subunit of caspase-3 (obtained from Dr D. Nicholson, Merck Frosst, Que., Canada). Pro-caspase-7 and its catalytically large subunit were detected with a rabbit polyclonal antibody raised against the p19 subunit of caspase-7 [21]. Intact PARP (116 kDa) and its 85 kDa signature fragment were detected with a mouse monoclonal antibody (C-2-10) (obtained from Dr G. Poirier, Laval University, Que., Canada). Proteins were detected by enhanced chemiluminescence (Amersham).

3. Results

3.1. Goniothalamin-induced apoptosis in Jurkat cells

The effects of goniothalamin, a plant extract from *G. andersonii*, were determined in Jurkat T-cells. Cells were incubated with various concentrations of goniothalamin (1–30 μg/ml) for 5 h. As shown in Fig. 1, this plant extract induced apoptosis as assessed by annexin V staining in a concentration-dependent manner. The morphology of the annexin V-labelled cells was confirmed as apoptotic by examination using fluorescence microscopy (data not shown). However, at the highest concentration of goniothalamin (30 μg/ml), the percentage of apoptotic cells was reduced to 26.7% concomitant with an increase in cells including PI (51%). This is in contrast to lower concentrations of goniothalamin where the percentage of cells including PI was significantly lower (Fig. 1).

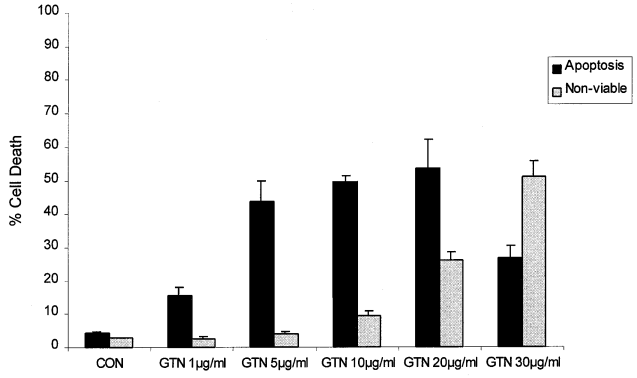

Fig. 1. Concentration-dependent induction of apoptosis by goniothalamin in Jurkat T-cells. Cells (10^6 cells/ml) were incubated either alone (CON) or in the presence of various concentrations of goniothalamin (GTN) for 5 h. The percentage of cell death was determined by the annexin V-FITC/PI method. Apoptosis refers to cells which showed high annexin and low PI while non-viable refers to cells with both high annexin and high PI staining. The data represent the means (±S.E.M.) of at least three separate experiments.

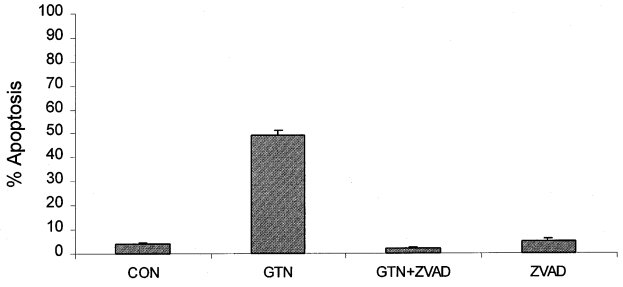

Fig. 2. Inhibition of goniothalamin-induced apoptosis by Z-VAD.FMK. Cells were pre-incubated for 30 min with 20 μM Z-VAD.FMK (ZVAD) before adding 10 μg/ml goniothalamin (GTN) and the % of apoptotic cells was determined by the annexin V assay. The data represent the means (±S.E.M.) of at least three separate experiments.

When analysed by fluorescence microscopy, the PI including cells comprised 'secondary necrotic' or late apoptotic cells (data not shown). Based on these observations, we concluded that the optimal concentration of goniothalamin which induced maximum apoptosis, with minimal necrosis, was 10 μg/ml (53.45%) and this concentration was therefore used in our future studies.

3.2. Z-VAD.FMK blocked goniothalamin-induced apoptosis

In order to understand the role of caspases in goniothalamin-induced apoptosis, we pre-treated cells with Z-VAD.FMK, a general broad-spectrum caspase inhibitor, prior to goniothalamin exposure. This peptide-based covalent inhibitor has been shown to inhibit apoptosis in many mammalian cell systems including Jurkat cells [5,15]. Apoptosis induced by goniothalamin was inhibited back to control levels by pre-treatment of cells with Z-VAD.FMK (20 μM) (Fig. 2). The cytogram in Fig. 3 shows a bivariate PI/annexin V analysis of the Jurkat cells. Viable cells were negative for both PI and annexin V as shown in the lower left quadrant, apoptotic cells were annexin V positive and PI negative as shown on the upper left quadrant, while non-viable cells were positive for both PI and annexin V (upper right quadrant). After a 5 h exposure to goniothalamin (10 μg/ml), the number of apoptotic cells increased from 6.7% in the control culture to 54.5%. Interestingly, the percentage of non-viable cells remained similar to that in control cells confirming that at this concentration, goniothalamin specifically induced apoptosis. Pre-treatment by Z-VAD.FMK completely abrogated apoptosis in Jurkat cells with the cytogram profile being similar to that observed in control cells (Fig. 3).

3.3. Caspases were activated in goniothalamin-induced apoptosis

The involvement of caspases-3 and -7 in goniothalamin-treated Jurkat cells was investigated by immunoblotting. As shown in Fig. 4A, caspase-3 antibody detected the 32 kDa pro-form of caspase-3 in control Jurkat cells (lane 1). In goniothalamin-treated cells, 45–55% of the cells were apoptotic and a marked decrease in the levels of intact caspase-3 was observed (lane 2). This was accompanied by the appearance of a band corresponding to 17 kDa that represents the larger subunit of the catalytically active caspase-3 enzyme [22]. The loss of intact caspase-3 was reversed in cells pre-treated with 20 μM Z-VAD.FMK (lane 3). However, an additional inter-

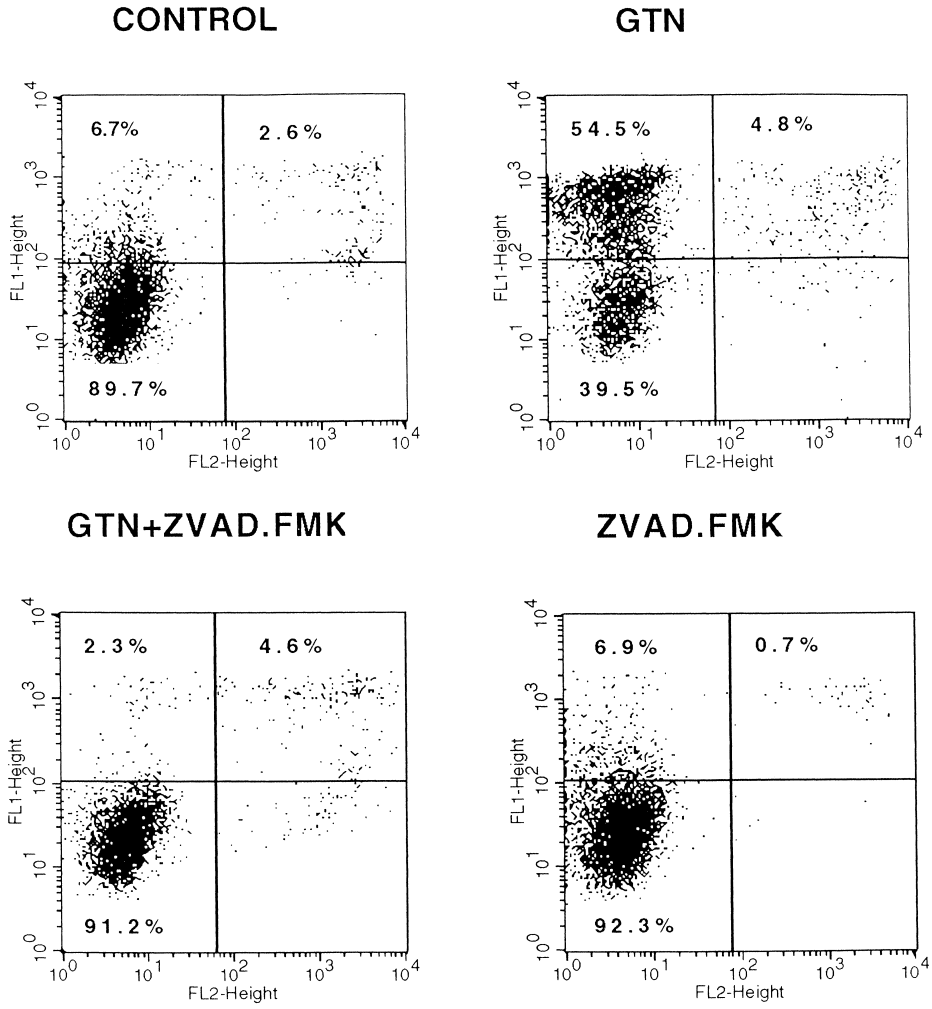

Fig. 3. Flow cytometric profile of Z-VAD.FMK inhibition of goniothalamin-induced apoptosis. Cells treated as in Fig. 2 were analysed for PS exposure by measuring annexin V binding by flow cytometry. The *x*-axis (FL-2 height) represents the PI staining while the *y*-axis (FL-1 height) represents the annexin V labelling. The cytogram represents data typical of three separate experiments.

mediate band of ~20 kDa appeared in these cells. This 20 kDa band was also previously observed when etoposide-mediated cleavage of caspase-3 was inhibited by Z-VAD.FMK in Jurkat T-cells [23].

In control Jurkat cells, caspase-7 was detected as a 35 kDa protein (Fig. 4B, lane 1). After goniothalamin treatment, this 35 kDa band was reduced in intensity and was accompanied by the appearance of a 19 kDa fragment (lane 2). The 19 kDa band corresponds to the larger subunit of the catalytically active caspase-7 enzyme [24]. Pre-treatment of the cells with Z-VAD.FMK completely inhibited processing of caspase-7 to its active subunit (lane 3). Pre-treatment of cells with Z-VAD.FMK alone did not have any effect on the levels of either caspase-3 or -7 (Fig. 4A and B, lane 4).

The goniothalamin-induced processing of caspases-3 and -7 would indicate that these enzymes were activated in Jurkat cells exposed to this agent. We therefore investigated whether PARP, a preferred substrate for both caspases-3 and -7, was cleaved in goniothalamin-treated Jurkat cells. Control cells contained intact PARP protein of 116 kDa (Fig. 4C, lane 1). Following exposure to goniothalamin for 5 h, PARP was cleaved extensively to its 85 kDa cleavage product (lane 2). Pre-treatment of cells with Z-VAD.FMK completely abrogated cleavage of PARP in goniothalamin-treated cells (lane 3). Thus, PARP cleavage, which is indicative of a caspase-3/-7-like proteolytic activity, was both activated and inhibited in parallel with the processing/inhibition of these effector caspases.

4. Discussion

The continuing magnitude and severity of the cancer problem makes it imperative that a preventive approach to this disease is developed. As advances in the molecular and cellular biology of carcinogenesis continue, specific targets for preventive intervention are being identified and effective new chemopreventive agents are being discovered, synthesised and tested. In this regard, modulation of the apoptotic response represents a novel mechanism-based approach in the identification or development of new therapeutic regimens for the treatment of cancer [25,26].

In this study, we have investigated the potential role and mechanisms of goniothalamin-induced apoptosis in the leukemia T-cell line Jurkat. Goniothalamin is a natural plant extract isolated from a widely found local Malaysian plant called *G. andersonii*. Although goniothalamin has been shown

to cause growth arrest, the potential apoptogenic properties of goniothalamin have not previously been demonstrated [17,18]. When Jurkat cells were treated with increasing concentrations of goniothalamin for 5 h, flow cytometric analysis of PS exposure revealed a concentration-dependent increase in apoptosis (Fig. 1). Morphological analysis by fluorescence microscopy (data not shown) confirmed that the cells were apoptotic and further verified the assessment of apoptosis by quantitation of PS exposure. At lower concentrations of goniothalamin, i.e. below 20 μg/ml, the morphological effects observed were mainly apoptotic with only a small percentage of cells exhibiting either late apoptotic or 'secondary necrotic' effects. These results demonstrate that goniothalamin is a potent apoptotic inducer in Jurkat T-cells.

This is in contrast to a previous study where goniothalamin was shown to cause primarily necrotic cell death in MCF-7 breast carcinoma cells [17]. However, it should be noted that although maximal cytotoxicity for this cell type was observed 36 h after treatment with goniothalamin, morphological analysis was carried out at 60 h. Therefore, the possibility of secondary necrosis occurring in these cells cannot be ruled out.

Caspases, especially members of the caspase-3 subfamily, are crucial mediators of apoptosis in many mammalian cells and previous studies have demonstrated that peptide-based caspase inhibitors, such as Z-VAD.FMK, are capable of abrogating apoptosis induced by different agents in a variety of systems [6,13,16,27–29]. To further characterise the role of caspases in our system, we have examined the effects of Z-VAD.FMK, a general caspase inhibitor, on apoptosis induced by goniothalamin. Pre-treatment of cells with Z-VAD.FMK completely blocked apoptosis as assessed by the externalisation of PS. This suggests that the apoptotic signalling events in goniothalamin-treated cells are extremely sensitive to Z-VAD.FMK. We further confirmed that the effector caspases-3 and -7 were cleaved in goniothalamin-treated cells, suggesting a role for caspase activation during the execution of apoptosis. In addition, Z-VAD.FMK blocked the cleavage of caspases-3 and -7 to their active subunits (p17 and p19, respectively). Interestingly, the inhibition of caspase-3 processing by Z-VAD.FMK produced a band migrating at approximately 20 kDa. It has been shown previously that processing of caspase-3 occurs via the generation of a p20 intermediate that is subsequently processed to a p17 fragment [30]. Binding of the tri-peptide inhibitor Z-VAD.FMK to the p20 subunit of caspase-3 has also been shown to attenuate its proteolytic activity [31].

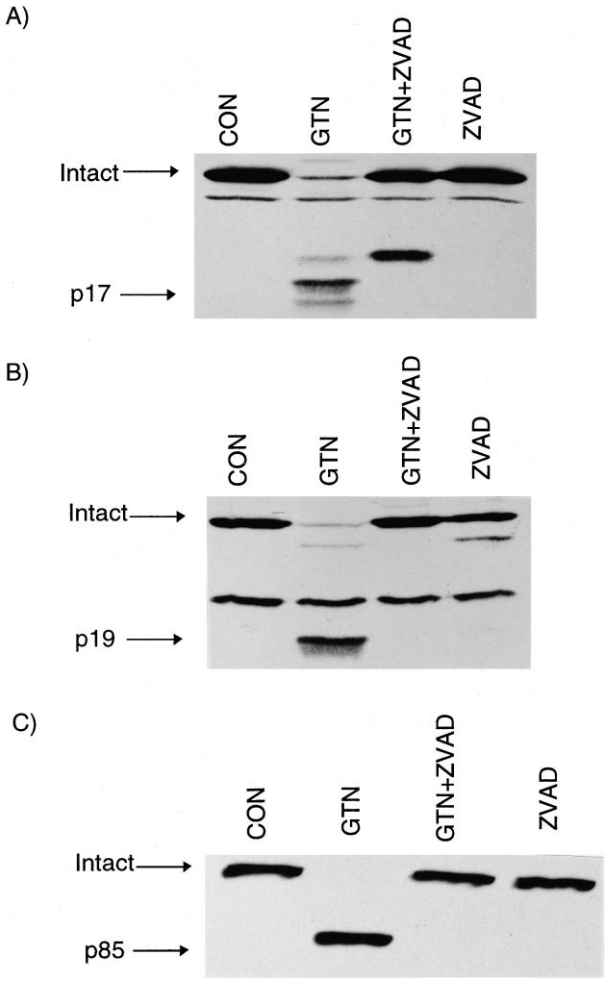

Fig. 4. Goniothalamin-induced processing of caspases and PARP in Jurkat cells. Cells were treated as in Fig. 2 and immunoblotted for (A) caspase-3, (B) caspase-7 and (C) PARP. The intact proteins are 32, 35 and 116 kDa, respectively.

Further evidence supporting the role of caspases-3 and -7 in goniothalamin-induced apoptosis was obtained from the immunoblotting of PARP (Fig. 4C). PARP, a nuclear DNA repair enzyme, has been shown to undergo proteolytic cleavage to its signature 85 kDa product during apoptosis in many cell types and has therefore been proposed as a biochemical marker of apoptosis [32,33]. Although most of the caspases are capable of cleaving PARP in vitro, caspases-3 and -7 are believed to be primarily responsible for cleavage of this protein in intact cells [5]. In this study, intact PARP was completely degraded to its 85 kDa signature fragment in goniothalamin-treated cells. In this respect, Jurkat T-cells were extremely sensitive to this treatment and almost no intact PARP was observed in treated cells. However, goniothalamin-induced PARP cleavage in Jurkat cells was completely abrogated by Z-VAD.FMK. These data indicate that prevention of caspase-3 and -7 activation by Z-VAD.FMK leads to inhibition of PARP cleavage and therefore survival of these cells.

To our knowledge, this is the first report which demonstrates the pharmacological chemopreventive activity of goniothalamin in inducing apoptosis in human tumour cell lines. Although the mechanisms by which goniothalamin impinges on the cell death signal are currently unknown, one cannot rule out the potential involvement of reactive oxygen intermediates, genotoxic damage or upregulation of Fas ligand in this model [34–36]. Further studies will also be required to determine if goniothalamin can selectively induce apoptosis in cancerous cells while leaving normal cells unharmed. In this respect, a recent study has shown that goniothalamin reduces rat mammary tumours induced by 7,12-dimethyl benzanthracene [18]. It will therefore be important to determine whether the tumour regression observed is a result of increased apoptosis.

In conclusion, we have demonstrated that goniothalamin is a potent apoptosis inducer in Jurkat T-cells. Induction of apoptosis results in the activation of the effectors caspases-3 and -7 during execution of the death program. The specific

mechanism(s) by which goniothalamin initiates the apoptosis signalling pathway is currently being investigated.

Acknowledgements: Salmaan H. Inayat-Hussain is the recipient of a Wellcome Trust Research Travel Award and would like to thank the Wellcome Trust for this support. We thank Dr Don Nicholson for caspase-3 antibody, Dr G. Poirier for PARP antibody and Dr X.-M. Sun for supplying us the caspase-7 antibody. We also thank Prof. Gerald M. Cohen for his continued support and helpful discussions.

References

[1] Jacobs, M.M. (1995) in: Diet and Cancer: Markers, Prevention and Treatment, Adv. Exp. Med. Biol., Vol. 354, Plenum Press, New York.
[2] Fujiki, H., Komori, A. and Suganuma, M. (1997) in: Comprehensive Toxicology (McQueen, A.C. and Gandolfi, A.J., Eds.), Vol. 12, pp. 453–471, Pergamon Press, Oxford.
[3] Hetts, S.W. (1998) JAMA 279, 300–307.
[4] Goldsworthy, T.L., Fransson-Steen, R.L. and Moser, G.J. (1995) CIIT Act. 15, 1–10.
[5] Cohen, G.M. (1997) Biochem. J. 326, 1–16.
[6] Porter, A.G. and Janicke, R.U. (1999) Cell Death Differ. 3, 255–267.
[7] Nicholson, D.W. and Thornberry, N.A. (1997) Trends Biochem. Sci. 22, 299–306.
[8] Thornberry, N.A. and Lazebnik, Y. (1998) Science 281, 1312–1316.
[9] Kumar, S. and Lavin, M.F. (1996) Cell Death Differ. 3, 255–267.
[10] Kumar, S. and Harvey, N.L. (1995) FEBS Lett. 375, 169–173.
[11] Cain, K., Inayat-Hussain, S.H., Couet, C. and Cohen, G.M. (1996) Biochem. J. 314, 27–32.
[12] Inayat-Hussain, S.H., Couet, C., Cohen, G.M. and Cain, K. (1997) Hepatology 25, 1516–1526.
[13] Fearnhead, H.O., Dinsdale, D. and Cohen, G.M. (1995) FEBS Lett. 375, 283–288.
[14] Zhu, H., Fearnhead, H.O. and Cohen, G.M. (1995) FEBS Lett. 374, 303–308.
[15] Slee, E.A., Zhu, H., Chow, S.C., MacFarlane, M., Nicholson, D.W. and Cohen, G.M. (1995) Biochem. J. 315, 21–24.
[16] Chow, S.C., Weis, M., Kass, G.E.N., Holstrom, T.H., Eriksson, J.E. and Orrenius, S. (1995) FEBS Lett. 364, 134–138.
[17] Ali, A.M., Mackeen, M.M., Hamid, M., Aun, Q.B., Zauyah, H., Azimathol, H.L.P. and Kawazu, K. (1997) Planta Med. 63, 81–83.
[18] Azimahtol, H. and Stanslas, J. (1998) In Vivo 12, 403–410.
[19] Jewers, K., Davis, J.R., Dougan, J., Machanda, A.H., Blunden, G., Kyi, A. and Wetchapinan, S. (1972) Phytochemistry 11, 2025–2030.
[20] Zhuang, J., Ren, Y., Snowden, R.T., Zhu, H., Gogvadze, V., Savill, J.S. and Cohen, G.M. (1998) J. Biol. Chem. 273, 15628–15632.
[21] MacFarlane, M., Cain, K., Sun, X.-M., Alnemri, E.S. and Cohen, G.M. (1997) J. Cell. Biol. 137, 469–479.
[22] Nicholson, D.W., Ali, A., Thornberry, N.A., Vaillancourt, J.P., Ding, C.K., Gallant, M., Gareau, Y., Griffin, P.R., Labelle, M., Lazebnik, Y.A., Munday, N.A., Raju, S.M., Smulson, M.E., Yamin, T.-T., Yu, V.L. and Miller, D.K. (1995) Nature 376, 37–43.
[23] Sun, X.-M., MacFarlane, M., Zhuang, Z., Wolf, B.B., Green, D.R. and Cohen, G.M. (1999) J. Biol. Chem. 274, 5053–5060.
[24] Fernandes-Alnemri, T., Armstrong, R.C., Krebs, J., Srinivasula, S.M., Wang, L., Bullrich, F., Fritz, L.C., Trapani, J.A., Tomaselli, K.J., Litwack, G. and Alnemri, E.S. (1996) Proc. Natl. Acad. Sci. USA 93, 7464–7469.
[25] Kerr, J.F.R., Winterford, C.M. and Harmon, B.V. (1994) Cancer 73, 2013–2026.
[26] Haq, R. and Zanke, B. (1998) Cancer Metastasis Rev. 17, 233–239.
[27] Lazebnik, Y.A., Kaufmann, S.H., Desnoyers, S., Poirer, G.G. and Earnshaw, W.C. (1994) Nature 371, 346–347.
[28] Enari, M., Hug, H. and Nagata, S. (1995) Nature 375, 78–81.
[29] Los, M., Van De Craen, M., Penning, L.C., Schenk, H., Westendorp, M., Baeuerle, P.A., Droge, W., Krammer, P.H., Fiers, W. and Schulze-Osthoff, K. (1995) Nature 375, 81–83.
[30] Schlegel, J., Peters, I., Orrenius, S., Miller, D.K., Thornberry, N.A., Yamin, T.T. and Nicholson, D.W. (1996) J. Biol. Chem. 271, 1841–1844.
[31] Martin, S.J., Amarante, M.-G.P., Shi, L., Chuang, T.H., Casiano, C.A., O'Brien, G.A., Fitzgerald, P., Tan, E.M., Bokoch, G.M., Greenberg, A.H. and Green, D.R. (1996) EMBO J. 15, 2402–2416.
[32] Kaufmann, S.H. (1989) Cancer Res. 49, 5870–5878.
[33] Kaufmann, S.H., Desnoyers, S., Ottaviano, Y., Davidson, N.E. and Poirier, G.G. (1993) Cancer Res. 53, 3976–3985.
[34] Clement, M.V. and Pervaiz, S. (1999) Free Radic. Res. 30, 247–252.
[35] Schwartzman, R.A. and Cidlowski, J.A. (1993) Endocr. Rev. 14, 133–151.
[36] Bae, M.A., Kang, S.K., Rue, S.W., Seu, J.H. and Kim, Y.H. (1998) Biochem. Biophys. Res. Commun. 246, 276–281.