# Apoptosis induction by inhibitors of Ser/Thr phosphatases 1 and 2A is associated with transglutaminase activation in two different human epithelial tumour lines

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**Abstract Two epithelial tumour lines, HeLa and KB, were treated with okadaic acid and calyculin A, specific inhibitors of Ser/Thr phosphatases (PP), esp. PP1 and PP2A. Morphological criteria, analysis of DNA fragmentation and studies of membrane integrity revealed that both agents concentration- and time-dependently induced apoptosis at nanomolar concentrations which in these cells was associated with the stimulation of a transglutaminase activity. Since a non-functional derivative of okadaic acid did not affect cell viability apoptosis was apparently related to the inhibition of PP1 and PP2A. Membrane damage marker activity was delayed by at least 24 h when compared to nuclear alterations.**

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*Key words:* Ser/Thr phosphatase inhibitor; Okadaic acid; Calyculin A; Apoptosis; Transglutaminase; HeLa cell; KB cell; MCF-7 cell

## 1. Introduction

Apoptosis is an essential process in growth, development and tissue homeostasis of multicellular organisms. Characteristic features are viability loss, DNA fragmentation and membrane blebbing accompanied by sustained membrane integrity. As an active process apoptosis requires an intact metabolism and in some cells RNA and protein synthesis [1–4].

Certain cell lines respond with apoptosis when Ser/Thr phosphatases are continuously inhibited for more than 12–24 h by agents like okadaic acid, cantharidic acid and calyculin A [5,6]. These changes can be followed by viability assays, nuclear and cytosolic morphological appearance, DNA fragmentation analysis and flow cytometry. A possible target of inhibitors of Ser/Thr phosphatases, esp. of PP1 and PP2A, as inducers of apoptosis seems to be the control of the cell cycle which requires the coordinated phosphorylation and dephosphorylation of regulatory proteins. Inhibition of Ser/Thr phosphatases by okadaic acid has been reported to result in cell cycle deviation with subsequent progression into apoptosis [7]. In addition, in a histiocytic lymphoma line resistant to tumour necrosis factor (TNF) induced apoptosis okadaic acid and calyculin A as specific inhibitors of Ser/Thr phosphatases synergized with TNF. In combination with these agents TNF triggered apoptosis in the initially resistant cells [8].

A characteristic feature of apoptosis even in cultured cells is the presence of gross structural changes but in contrast to necrosis still intact membrane function which seems to be necessary for the encapsulation of cellular components and debris in definite structures, so-called apoptotic bodies. This structural behaviour underlies the observation that apoptotic cell death does not affect neighbouring cells and therefore is not accompanied by inflammatory responses [1,9,10]. An important factor involved is presumed to be the activation or induction of a tissue transglutaminase that modulates membrane texture [11,12]. Such an increase in transglutaminase activity has not always been reported for agents that induce apoptosis [13,14]. For example in neuroblastoma cells apoptosis induction by cisplatin and retinoic acid but not by okadaic acid was accompanied by an increased transglutaminase activity [13].

Studies in cultured cells are complicated by the fact that secondary necrosis with leaky membranes follows initial apoptosis. In asynchronously growing cultures this might result in some association of apoptotic markers (e.g. DNA fragmentation, apoptotic body formation) with cytosolic enzyme release, e.g. of LDH, in the medium albeit kinetic differences in the appearance of apoptosis and necrosis markers would be expected.

We studied the effects of okadaic acid and calyculin A on three different human epithelial tumour lines (HeLa, KB, MCF-7). Both agents have been isolated from marine organisms [15,16] and produce diarrhetic shellfish poisoning. These toxins specifically inhibit Ser/Thr phosphatases, predominantly types 1 and 2A, at nanomolar concentrations [17–19] whereas PP2B and PP2C are not inhibited at submicromolar concentrations. While okadaic acid inhibits PP2A with an $IC_{50}$ of about 0.5–1 nM, PP1 is about 10–30-fold less sensitive ($IC_{50}$ 15–50 nM) [17–19]. Calyculin A inhibits both enzymes with $IC_{50}$ values of 1–2 nM [17–19].

Here, we report for the first time that apoptosis induction in two epithelial human tumour lines by okadaic acid and calyculin A, inhibitors of Ser/Thr phosphatases, is concentration-dependently correlated with an increased transglutaminase activity. Our conclusion is supported by the observation that maximum membrane damage as measured by lactate dehydrogenase release was delayed by 24 h when compared to maximum DNA fragmentation.

## 2. Materials and methods

### 2.1. Materials

Cell culture reagents were from Seromed (Berlin, Germany), Boehringer-Mannheim (Mannheim, Germany) and Life Technologies (Eggenstein, Germany). Okadaic acid and calyculin A were purchased from RBI (Biotrend, Köln, Germany) and okadaic acid tetraacetate from Calbiochem (Bad Soden, Germany). [$^{14}$C]Putrescine-dihydrochloride (80–120 mCi/mmol) was from NEN Du Pont (Dreieich,

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Germany) and unlabelled putrescine from Sigma (Deisenhofen, Germany).

*2.2. Cell culture*

Cervix carcinoma HeLa [20], pharynx carcinoma KB [21] and mammary epithelial carcinoma MCF-7 [22] cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 7.5% foetal bovine serum, penicillin (100 000 U/l), streptomycin (100 mg/l) and amphotericin B (250 μg/l). Treatment of cells was performed in serum-free insulin–transferrin–selenite (ITS) supplemented DMEM without phenol red.

*2.3. MTT viability assay*

Cells ($20\times10^4$/well) were plated in 96-well plates and cultured for 48 h in complete medium. Then medium was changed to ITS with the individual treatments. After 24 h 10 μl of thiazolyl blue (MTT 5 mg/ml, Sigma, Deisenhofen, Germany) were added for 1 h. Cells and the converted dye were lysed with 20% SDS in 0.02 N HCl for 24 h at 37°C. Mitochondrial conversion to the formazan product was quantified in an ELISA reader (Molecular Devices, USA) at 550 nm. Values of treated cells were presented as percent of the respective controls.

*2.4. Lactate dehydrogenase activity*

Lactate dehydrogenase (LDH) in medium (20 μl) was measured at 365 nm in an Eppendorf photometer by following the oxidation of NADH to NAD accompanying the conversion of pyruvate into lactate [23]. LDH activity was calculated from the mean ΔE/min in the linear range according to the Lambert-Beer law.

*2.5. Quantitative DNA fragmentation assay*

The assay procedure was modified from reported methods [24–26]. Cells ($0.7–0.9\times10^6$) were plated in 6-cm dishes. After a 24 h treatment the supernatant (medium) was removed and centrifuged for 3 min at $120\times g$ (4°C). The pellet was resuspended with the adherent cells which were scraped off the plates. The cell pellet was lysed for 30 min at room temperature with lysis buffer (5 mM Tris-HCl (pH 8), 20 mM EDTA, 0.5% Triton X-100). Low molecular weight DNA was separated from the genomic DNA by centrifugation at $14\,000\times g$ for 20 min. The supernatant was transferred into another vial containing perchloric acid (PCA, 11.6 N) resulting in a final concentration of 1.5 N PCA. The pellet was dissolved in 1.5 N PCA. Protein was precipitated by heating to 80°C for 15 min followed by centrifugation ($1500\times g$, 10 min). DNA in 125 μl of cleared supernatant or pellet mixture was quantified by its deoxyglucose content [27,28] at 600 nm. Herring sperm DNA (0.4 mg/ml in 5 mM NaOH) was used for standards (0–200 μg/ml). The DNA fragmentation rate was calculated from the ratio of DNA in the supernatant to total DNA.

*2.6. Transglutaminase assay*

Quantification of transglutaminase activity followed the methods of [12] and [29]. Cells ($2–3\times10^6$) were cultured in 10-cm dishes for 24 h under the individual conditions. Then medium was discarded and cells lysed in 150 μl of lysis buffer (150 mM Tris-HCl (pH 7.2), 1 mM EDTA, 0.5% Triton X-100). Transglutaminase activity of each sample was measured after two reaction periods (10 and 70 min) with dimethylcaseine as substrate and labelled putrescine. Conditions per reaction (100 μl) were 3 mg/ml dimethylcaseine, 5 mM DTT, 0.2 μCi [$^{14}$C]putrescine (0.5 μM), 5 mM $CaCl_2$ and 100–150 μg extract protein at pH 7.5. Negative controls contained EDTA instead of $CaCl_2$. Reactions at 37°C were stopped after 10 and 70 min by the addition of 1 ml of ammonium sulfate (35%). After 15 min at room temperature the precipitated protein was pelleted by centrifugation at $14\,000\times g$ for 5 min. The supernatant was discarded and the pellet washed with 0.5 ml of ammonium sulfate. Following a second centrifugation the pellet was dissolved in 300 μl of water. Incorporation of activity into the substrate was quantified by liquid scintillation counting after addition of 4 ml of scintillation cocktail (Instant Scint, Packard, Frankfurt, Germany) to 280 μl of dissolved pellet. Activity was calculated from the difference between the 70 and 10 min values. As a positive control a purified transglutaminase (Sigma, Deisenhofen, Germany) was used at concentrations from 1.25 to 320 mU/ml. Protein was measured in 10 μl of the extract by the biscinchonic acid assay (Pierce, Rockford, USA) with bovine serum albumin as standard.

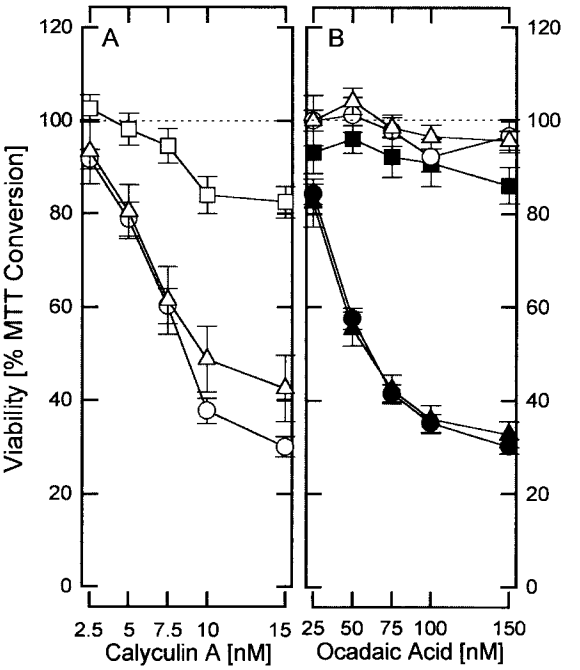


Fig. 1. Viability of HeLa (circles), KB (triangles) and MCF-7 (squares) cells following a 24 h treatment with calyculin A (A) and okadaic acid (B). Open symbols in (B) are viabilities of cells treated with okadaic acid tetraacetate, the non-functional derivative of okadaic acid. Means ± SEM of 6–8 experiments.

## 3. Results and discussion

HeLa and KB cells concentration-dependently respond with a decreased viability to a 24 h treatment with okadaic acid and calyculin A (Fig. 1A,B). In contrast, MCF-7 cells were nearly unresponsive to both treatments (Fig. 1). In our experiments both responsive lines displayed a maximal 60–70% decrease in mitochondrial conversion of the tetrazolium dye MTT while in MCF-7 cells even high concentrations of calyculin A (>10 nM) reduced viability only by about 20%. Okadaic acid toxicity was seen above 25 nM, while calyculin A induced comparable changes at 8–10-fold lower concentrations (>2.5 nM). Okadaic acid tetraacetate, a derivative not inhibiting PP1 and PP2A [18] did not reduce viability in both responsive lines (Fig. 1B).

Within the same concentration range both agents induced morphological changes resembling apoptosis induction. More and more cells developed characteristic membrane blebbings and became rounded and condensed (Fig. 2a–d). Therefore, treatment for more than 12 h with okadaic acid and calyculin A can (HeLa, KB) but also may not (MCF-7) induce apoptosis at the tested concentrations and the according periods of treatment.

Qualitative analysis of DNA fragmentation revealed in KB and HeLa cells the DNA ladder phenomenon resulting from internucleosomal DNA cleavage [31] usually observed in apoptotic cells, but no comparable alterations in MCF-7 cells (data not shown). To study the concentration-dependencies in more detail a quantitative DNA fragmentation analysis of cells treated with the phosphatase inhibitors was performed by measuring the appearance of low molecular weight DNA (Fig. 3A,B). After 24 h HeLa and KB cells responded with an increase in fragmented DNA up to 20% (okadaic acid) and

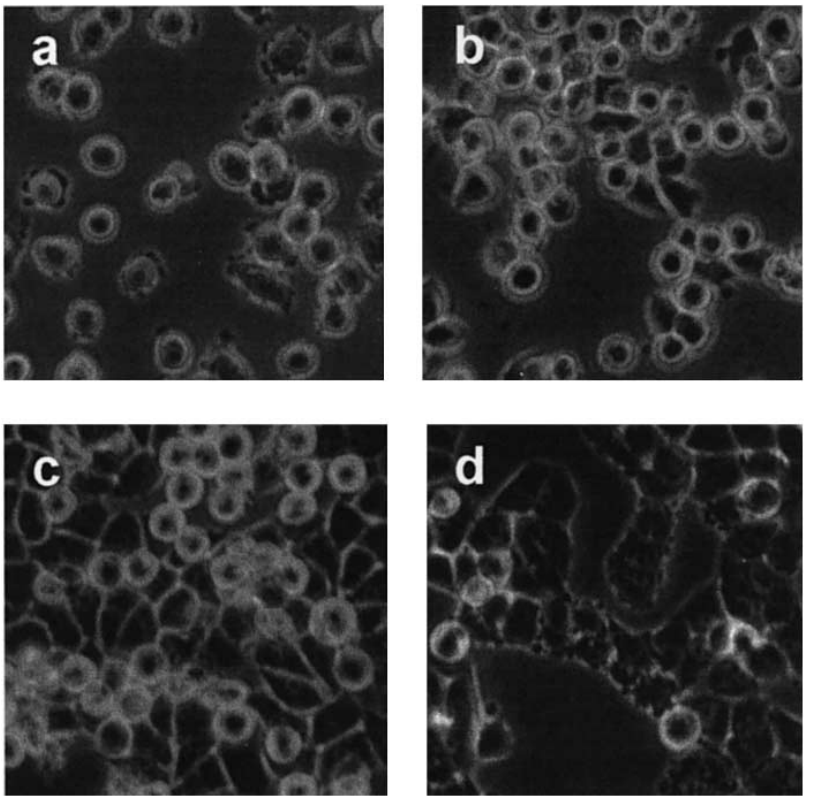


Fig. 2. Phase contrast microscopy (200-fold magnification) of KB (a,b) or HeLa (c,d) cells treated with 50 nM okadaic acid (a,c) or 7.5 nM calyculin A (b,d).

12–14% (calyculin A) while MCF-7 cells revealed no comparable rise in DNA cleavage under the same conditions. Interestingly, in both responsive tumour lines the fragmentation rate levelled off at 15 nM calyculin A. Higher concentrations of this agent did not result in maximal fragmentation rates comparable to that seen with okadaic acid. These observations differed from those with $GH_3$ cells, a rat pituitary line. In these cells both inhibitors induced the same extent of DNA fragmentation with a comparable concentration ratio of 10:1 between okadaic acid and calyculin A [5]. This observation supports the assumption that apoptosis induction by inhibitors of Ser/Thr phosphatases is a phenomenon seen with many but not all cells and in addition that cell-specific differences exist even between individual responsive lines. The missing sensitivity of MCF-7 cells for okadaic acid cytotoxicity was further confirmed by staining DNA with a fluorescent dye, which showed the typical nuclear fragmentation and condensation in KB and HeLa cells treated with 150 nM okadaic acid, but no changes in MCF-7 cells. These findings are supported by observations of Oberhammer et al. [30] with four different epithelial cell lines. The hormone-sensitive MCF-7 cells were the only line which did not respond with apoptosis to serum withdrawal or to TGF-β or etoposide treatment. The observed lack of sensitivity of the MCF-7 cells might be the result of high endogenous levels of antiapoptotic factors, e.g. Bcl-2 [32], or decreased intracellular accumulation of the drug as the result of increased drug extrusion, e.g. by the P-glycoprotein mediating multidrug resistance [33].

DNA fragmentation analysis of cells treated with okadaic acid tetraacetate (150 nM) revealed no signs of cellular damage by this non-functional derivative with fragmentation rates of $1.4 \pm 0.3\%$ (KB) and $2.0 \pm 0.8\%$ (HeLa) vs. $1.7 \pm 0.5\%$ (KB) and $2.0 \pm 0.4\%$ (HeLa) in untreated cells while 150 nM okadaic acid induced rates of $18.6 \pm 1.3\%$ (KB) and $23.5 \pm 0.9\%$ (HeLa). Taken together with the data from the viability assays (Fig. 1B) these results substantiate the hypothesis that prolonged inhibition of PP1 and/or PP2A triggers apoptosis.

A rise in tissue transglutaminase activity has been described as a prominent feature of some cells undergoing chemically induced apoptotic cell death [12,29,34–37]. This activity is considered to be important for the organised involution of apoptotic cells which does not negatively affect and thus renders protection for the surrounding tissue. Okadaic acid induced apoptosis was described for many different cells [5,6,32,38] albeit no concomitant activation of transglutaminase activity was observed [13,14]. Other apoptotic signals, e.g. retinoic acid, resulted in apoptosis in the same cell lines which was associated with an enhanced transglutaminase activity [13,14]. To address a relation between apoptosis and transglutaminase activation in our systems we studied transglutaminase activity in the okadaic acid and calyculin A responsive KB and HeLa cells. In contrast to previous reports an increased transglutaminase activity was observed in extracts from okadaic acid and calyculin A treated HeLa and KB cells (Fig. 4). Both lines displayed a concentration-dependent increase which correlated with the concentration-dependency for DNA fragmentation. While the concentration response to the phosphatase inhibitors was comparable, basal transglutaminase activity was different. HeLa cells displayed a 2-fold higher basal activity ($\approx 300$ mU/mg protein) with a treatment associated activation by 2 (calyculin A) to 2.5-fold (okadaic acid). Basal activity in KB cells was lower (165 mU/

mg protein) but comparably activated by 2.3- and 4-fold by calyculin A and okadaic acid, respectively. Again the effects of calyculin A were observed at about 10-fold lower concentrations. Calyculin A which produced a lower maximal DNA fragmentation also activated transglutaminase to a lower extent in both lines. Thus, two different biochemical markers of apoptosis induction, transglutaminase activation and DNA fragmentation, gave comparable results when calyculin A and okadaic acid, two structurally and functionally different inhibitors of Ser/Thr PP1 and PP2A, were studied.

As a functional assay substantiating that the initial cytotoxic effects of okadaic acid and calyculin A are apoptotic and not necrotic we compared the time course of DNA fragmentation with that of lactate dehydrogenase release as a marker of membrane integrity. Both cell lines were treated for up to 96 h with 50 nM okadaic acid, a concentration that resulted only in a slight increase in DNA fragmentation (Fig. 3B) and cell death (Fig. 1B) but marked morphological alterations (Fig. 2a,c) after 24 h. With this concentration a marked more than 30% DNA fragmentation was observed after 48 h in both lines (Fig. 5A,B). LDH release as a marker of membrane damage reached its maximal values 24–48 h later. This delayed release of a cytosolic enzyme can be considered to be the consequence of the co-ordinated packing and salvage of intracellular components characteristic for apoptosis which was suggested to result from transglutaminase activation [39].

It has been reported that cells undergo apoptosis from a defined point of the cell cycle depending on the individual substance inducing apoptosis [13,39–41]. The rise in LDH release at 48 h might be explained by the fact that in our asynchronously growing cultures the cells develop apoptosis also in an asynchronous way. Therefore, at the peak of DNA fragmentation some cells already could have passed apoptosis and would have proceeded to secondary necrosis, if they were

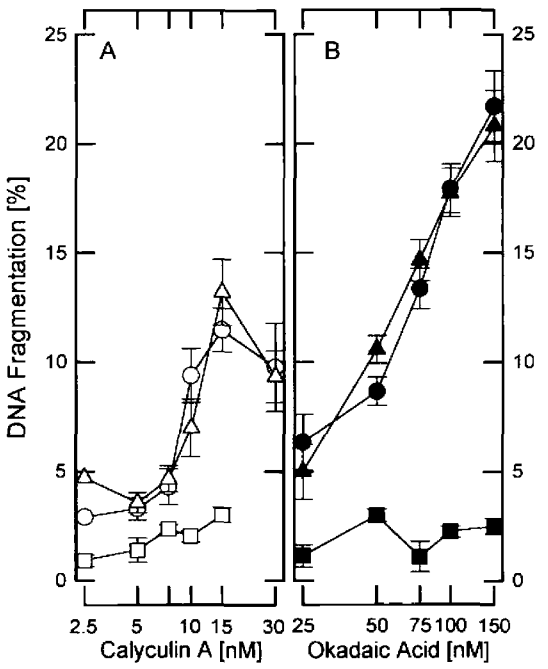


Fig. 3. DNA fragmentation in HeLa (circles) or KB (triangles) cells after a 24 h incubation with the indicated concentrations of calyculin A (A) and okadaic acid (B). Means ± SEM of 4–5 experiments.

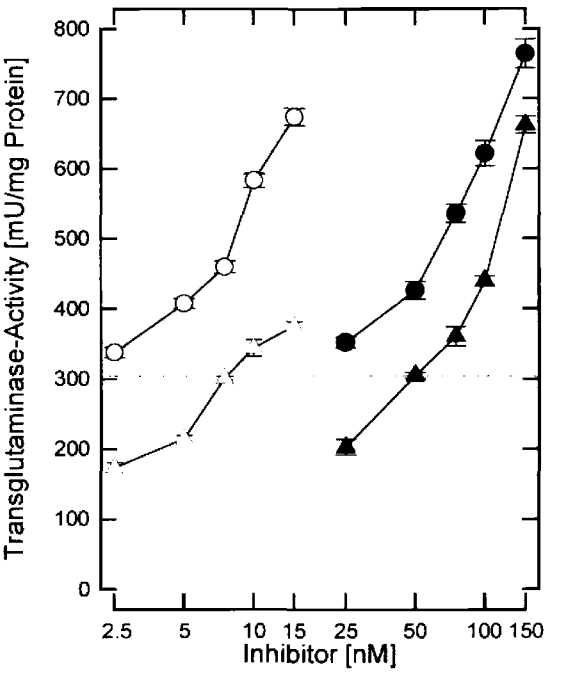


Fig. 4. Activation of a tissue transglutaminase activity in HeLa (circles) and KB (triangles) cells following a 24 h incubation with calyculin A (open symbols) or okadaic acid (filled symbols). Means ± SEM of 5 measurements. Basal activities are given by the lines and were 165 mU/mg protein (KB cells, solid line) and 300 mU/mg protein (HeLa cells, broken line).

at the susceptible point of the cell cycle when the inhibitor was added. Even after dissolution into apoptotic bodies the membrane integrity is initially preserved. In cell culture secondary necrosis, during which the apoptotic bodies go through a swelling process, follows initial apoptosis since no subsequent phagocytosis occurs [1]. This might be the reason that after 48 h LDH activity increases in the medium (Fig. 5A,B) despite the fact that transglutaminase was activated.

Calyculin A was more potent than okadaic acid but concentrations above 15 nM resulted in less prominent apoptotic features (lower fragmentation rate, cellular swelling instead of condensation). Therefore, we would suggest that these concentrations induce necrotic instead of apoptotic features. Such concentration-dependent differences in cytotoxicity either as apoptosis at lower or necrosis at higher concentrations have been reported for many physical and chemical cytotoxic stimuli [4]. Whether the observations of necrotic characteristics with HeLa and KB cells are the consequences of a small apoptotic concentration window for calyculin A or a fast developing secondary necrosis following an initial apoptosis cannot definitely be answered.

In summary, okadaic acid and calyculin A, two structurally different inhibitors of Ser/Thr PP1 and PP2A, induced apoptosis in HeLa and KB cells, two human epithelial cancer lines. Under the same conditions no comparable toxicity was observed with MCF-7 breast tumour cells. Okadaic acid tetraacetate, a derivative of okadaic acid that does not inhibit phosphatases did not induce apoptosis. When comparing the concentration-response to okadaic acid and calyculin A consistently a discrepancy by 8–10-fold was observed between both drugs. This is most likely caused by the differences in their affinities towards PP1 and PP2A. Calyculin A inhibits

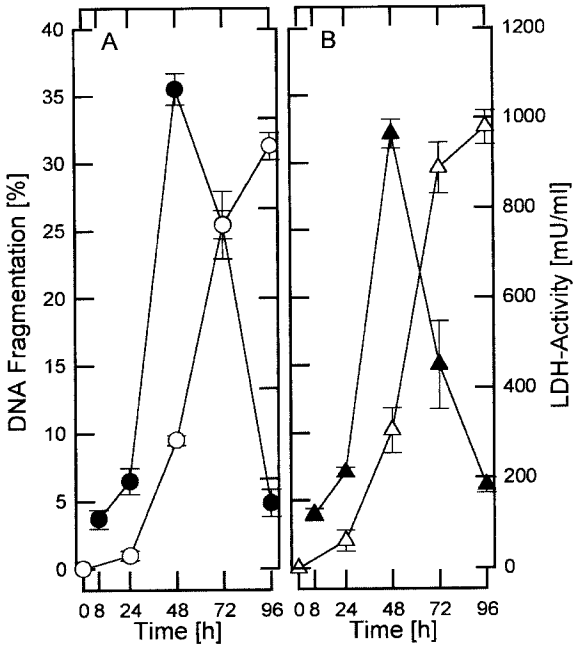


Fig. 5. Kinetics of DNA fragmentation (filled symbols) and LDH release (open symbols) in HeLa (A) and KB (B) cells treated with 50 nM okadaic acid. Means ± SEM of 3 experiments.

PP1 and PP2A at a similar concentration range while okadaic acid inhibits PP1 at about 10–30-fold higher concentrations than PP2A which is in accordance with the concentrations (> 50 nM) necessary for triggering apoptosis after 24 h. Apoptosis induction seems to be related to the preferential inhibition of PP1 or the simultaneous inhibition of PP1 and PP2A. For the first time it could be shown that in two lines responding with apoptosis to okadaic acid this response was associated with the rise of a tissue transglutaminase activity. An explanation for the discrepant reports on the involvement of transglutaminase activation accompanying apoptosis by okadaic acid might be the fast progression of okadaic acid induced apoptosis when compared to e.g. retinoic acid. In addition, cell-specific differences between the tested tumour lines are likely, e.g. their individual content of transglutaminase which differed even in the two investigated responsive lines. Thus, inhibitors of Ser/Thr PP1 and PP2A can be included in the group of drugs inducing apoptosis with concomitant activation of a transglutaminase.

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## References

[1] Cotter, T.G., Lennon, S.V., Glynn, J.G. and Martin, S.J. (1990) Anticancer Res. 10, 1153–1159.
[2] Binder, C. and Hiddemann, W. (1994) Ann. Hematol. 69, 45–55.
[3] Cohen, J.J. (1993) Immunol. Today 14, 126–130.
[4] Martin, S.J., Green, D.R. and Cotter, T.G. (1994) Trends Biochem. Sci. 19, 26–30.
[5] F. Tergau, J. Weichert, I. Quentin, R. Opitz, C.A. von Zezschwitz, J. Marwitz, V. Ritz, and H.J. Steinfelder, Naunyn Schmiedeberg's Arch. Pharmacol., 1997 in press.
[6] Morana, S.J., Wolf, C.M., Li, J., Reynolds, J.E., Brown, M.K. and Eastman, A. (1996) J. Biol. Chem. 271, 18263–18271.
[7] Wertz, I.E. and Hanley, M.R. (1996) Trends Biochem. Sci. 21, 359–364.
[8] Wright, S.C., Zheng, H., Zhong, J., Torti, F.M. and Larrick, J.W. (1993) J. Cell Biochem. 53, 222–233.
[9] Arends, M.J. and Wyllie, A.H. (1991) Int. Rev. Exp. Pathol. 32, 223–254.
[10] Bortner, C.D., Oldenburg, N.B. and Cidlowski, J.A. (1995) Trends Cell Biol. 5, 1–26.
[11] Fesus, L., Thomazy, V. and Falus, A. (1987) FEBS Lett. 224, 104–108.
[12] Piacentini, M., Fesus, L., Farrace, M.G., Ghibelli, L., Piredda, L. and Melino, G. (1991) Eur. J. Cell Biol. 54, 246–254.
[13] Piacentini, M., Fesus, L. and Melino, G. (1993) FEBS Lett. 320, 150–154.
[14] Ishii, I. and Ui, M. (1994) J. Biochem. Tokyo 115, 1197–1202.
[15] Tachibana, K., Scheuer, P.J., Tsukitani, Y., Kikuchi, H., van Engen, D., Clardy, J., Gopichand, Y. and Schmitz, F.J. (1981) J. Am. Chem. Soc. 103, 2469–2471.
[16] Kato, Y., Fusetani, N., Matsunaga, S. and Hashimoto, K. (1988) Drugs Exp. Clin. Res. 14, 723–728.
[17] Ishihara, H., Martin, B.L., Brautigan, D.L., Karaki, H., Ozaki, H., Kato, Y., Fusetani, N., Watabe, S., Hashimoto, K. and Uemura, D. (1989) Biochem. Biophys. Res. Commun. 159, 871–877.
[18] Cohen, P., Klumpp, S. and Schelling, D.L. (1989) FEBS Lett. 250, 596–600.
[19] Honkanen, R.E., Codispoti, B.A., Tse, K. and Boynton, A.L. (1994) Toxicon 32, 339–350.
[20] Gey, C.O., Coffman, W.D. and Kubicek, M.T. (1952) Cancer Res. 12, 264–265.
[21] Eagle, H. (1955) Proc. Soc. Exp. Biol. Med. 89, 362–364.
[22] Soule, H.D., Vazguez, J., Long, A., Albert, S. and Brennan, M. (1973) J. Natl. Cancer Inst. 51, 1409–1416.
[23] Bergmeyer, C. (1984) Methods Enzym. Anal. 3, 171–172.
[24] Wyllie, A.H., Shen, W., Kamendulis, L.M., Ray, S.D. and Corcoran, G.B. (1980) Nature 284, 555–556.
[25] Shen, W., Kamendulis, L.M., Ray, S.D. and Corcoran, G.B. (1991) Toxicol. Appl. Pharmacol. 111, 242–254.
[26] Ray, S.D., Sorge, C.L., Kamendulis, L.M. and Corcoran, G.B. (1992) J. Pharmacol. Exp. Ther. 263, 387–394.
[27] Richards, G.M. (1974) Anal. Biochem. 57, 369–376.
[28] Burton, K. (1956) Biochem. J. 62, 315–323.
[29] Davies, P.J., Murtaugh, M.P., Moore Jr., W.T., Johnson, G.S. and Lucas, D. (1985) J. Biol. Chem. 260, 5166–5174.
[30] Oberhammer, F., Wilson, J.W., Dive, C., Morris, I.D., Hickman, J.A., Wakeling, A.E., Walker, P.R. and Sikorska, M. (1993) EMBO J. 12, 3679–3684.
[31] Arends, M.J., Morris, R.G. and Wyllie, A.H. (1990) Am. J. Pathol. 136, 593–608.
[32] Kiguchi, K., Glesne, D., Chubb, C.H., Fujiki, H. and Huberman, E. (1994) Cell Growth Differ. 5, 995–1004.
[33] Tohda, H., Yasui, A., Yasumoto, T., Nakayasu, M., Shima, H., Nagao, M. and Sugimura, T. (1994) Biochem. Biophys. Res. Commun. 203, 1210–1216.
[34] Suedhoff, T., Birckbichler, P.J., Lee, K.N., Conway, E. and Patterson, M.K. (1990) Cancer Res. 50, 7830–7834.
[35] Ledda Columbano, G.M., Coni, P., Curto, M., Giacomini, L., Faa, G., Oliverio, S., Piacentini, M. and Columbano, A. (1991) Am. J. Pathol. 139, 1099–1109.
[36] Mastino, A., Grelli, S., Piacentini, M., Oliverio, S., Favalli, C., Perno, C.F. and Garci, E. (1993) Cell Immunol. 152, 120–130.
[37] Lu, X., Xie, W., Reed, D., Bradshaw, W.S. and Simmons, D.L. (1995) Proc. Natl. Acad. Sci. USA 92, 7961–7965.
[38] Boe, R., Gjertsen, B.T., Vintermyr, O.K., Houge, G., Lanotte, M. and Doskeland, S.O. (1991) Exp. Cell Res. 195, 237–246.
[39] Gentile, V., Thomazy, V., Piacentini, M., Fesus, L. and Davies, P.J. (1992) J. Cell Biol. 119, 463–474.
[40] Schimke, R.T., Kung, A., Sherwood, S.S., Sheridan, J. and Sharma, R. (1994) Phil. Trans. R. Soc. Lond. B 345, 311–317.
[41] Walker, P.R., Kwast-Welfeld, J., Gourdeau, H., Leblanc, J., Neugebauer, W. and Sikorska, M. (1993) Exp. Cell Res. 207, 142–151.