Cancer Chemother Pharmacol (1991) 28: 102–104
034457049100064W

Cancer Chemotherapy and Pharmacology

© Springer-Verlag 1991

Role of protein kinase C in Adriamycin-induced erythroid differentiation of K562 cells

Richard Hoffman* and Edward S. Newlands

CRC Laboratories, Charing Cross Hospital, Fulham Palace Road, London, W5 8RF, UK.

Received 2 December 1990/Accepted 23 January 1991

Summary. Modulators of protein kinase C (PKC) were used to investigate the role of this enzyme during Adriamycin-induced erythroid differentiation of K562 cells. Adriamycin (0.1 μM) induced erythroid differentiation in 60% ± 10% of K562 cells. Phorbol myristate-12-acetate, an activator of protein kinase C, was strongly anti-proliferative to K562 cells (IC_{50}, 8 nM) but did not induce erythroid differentiation. Staurosporine inhibited PKC from K562 cells (IC_{50}, 8 nM) and blocked Adriamycin-induced erythroid differentiation, but only at concentrations marginally below those that inhibited proliferation (IC_{50}, 81 nM). 1-(5-Isoquinolinylsulphonyl)-2-methylpiperazine (H-7) inhibited K562 PKC (IC_{50}, 26 μM) but reduced Adriamycin-induced differentiation by <50% at concentrations of up to 600 μM. These data argue against a major role for PKC during Adriamycin-induced erythroid differentiation in K562 cells.

Introduction

The induction of tumour cell differentiation represents an attractive alternative to cytotoxic chemotherapy for the treatment of a wide range of malignancies [14]. Several agents induce differentiation in the cell line K562, which was derived from a patient with chronic myeloid leukaemia [8]. The cytotoxic agent Adriamycin induces erythroid-like differentiation in K562 cells [5], whereas the phorbol ester phorbol myristate-12-acetate (PMA) induces megakaryocytic-like differentiation [1].

Although Adriamycin is traditionally considered to exert its anti-proliferative effect through DNA intercalation, it has been proposed that increased inositol lipid metabolism and activation of protein kinase C (PKC) may be additional sites of action of this drug [13, 19]. A good deal of evidence implicates PKC in leukaemia cell differentiation. For example, the human promyelocytic cell line HL60 differentiates into monocyte/macrophage-like cells on exposure to PMA (an activator of PKC) [11], and inhibitors of PKC block PMA-induced differentiation of HL60 cells [9, 10]. Since PMA does not induce erythroid differentiation in K562 cells [7], whereas adriamycin does, we undertook this study to investigate the question as to whether PKC is involved in the Adriamycin-induced erythroid differentiation of K562 cells.

Materials and methods

Materials. Tissue-culture medium RPMI 1640 and foetal calf serum were purchased from Gibco Ltd. (Paisley, Scotland). 1-(5-Isoquinolinylsulphonyl)-2-methylpiperazine (H-7) was supplied by Sigma (Poole, Dorset) and staurosporine was obtained from Novabiochem Ltd. (Nottingham, UK).

Cell culture. K562 cells were grown at 37° C in RPMI 1640 medium containing foetal calf serum (10%), glutamine (2 mM), penicillin (100 IU/ml) and streptomycin (100 μg/ml) under an atmosphere of 5% CO_2 in air. All agents added to the cells were first sterilized through 0.2-μM filters. Treated cells were grown for 5 days. After this time, differentiation was assessed following benzidine staining [18] by counting between 200–300 cells/data point. Cell number was determined using a haemocytometer and cell viability was determined by trypan blue exclusion. Concentrations of agents required to cause 50% inhibition of cell proliferation (IC_{50}) were determined using the computer programme developed by Chou and Chou [2].

Protein kinase C. K562 cells were harvested in the late-log growth phase and total PKC (soluble plus membrane-bound) was extracted by sonicating the cells in extraction buffer comprising TRIS buffer (pH 7.2, 20 mM) containing sucrose (0.25 M), ethylene glycol-bis (β-amino ethyl-ether)N,N,N′,N′-tetraacetic acid (EGTA, 10 mM), ethylenediamine-tetraacetic acid (EDTA, 2 mM). Tween-20 (0.1%, v/v) and leupeptin (40 μg/ml) at 4° C. The sonicate was equilibrated for 30 min and centrifuged (100,000 *g*, 1 h). PKC was partially purified on a DE52 column (Whatman) and assayed by measuring the incorporation of [γ-^{32}P]-adenosine 5'-triphosphate (ATP) into histone III-S [6]. IC_{50} values for PCK were determined according to the dose-effect analysis of Chou and Chou [2].

* *Present address:* Xenova Limited, 545 Ipswich Road, Slough, Berks., SL1 4EQ, UK

Offprint requests to: Richard Hoffman, Xenova Limited, 545 Ipswich Road, Slough, SL1 4EQ, UK

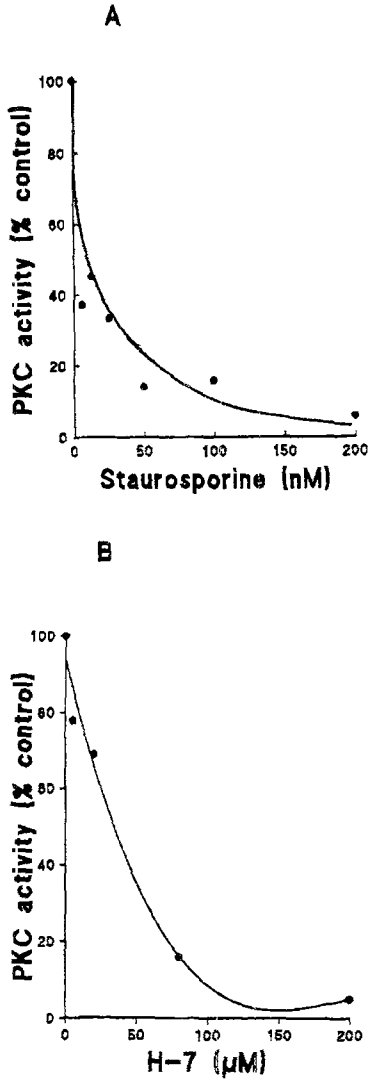

Fig. 1. A, B. Inhibition of protein kinase C from K562 cells by A staurosporine and B H-7. Data represent the means of two experiments. Mean values between experiments varied by <10% (SEM)

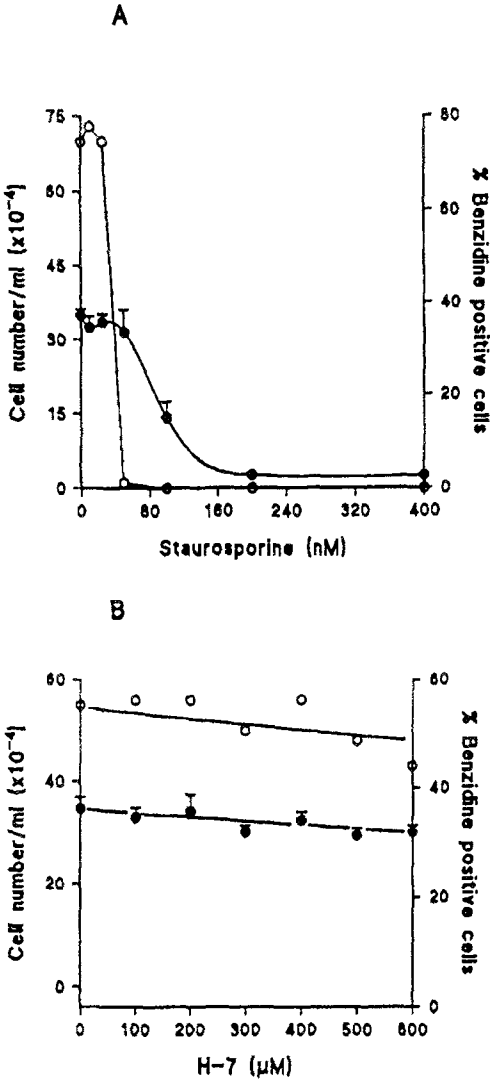

Fig. 2. A, B. Effects of A staurosporine and B H-7 on K562 proliferation (●) (values represent the means + SEM, $n = 4$) and Adriamycin-induced erythroid differentiation (○; mean values of two experiments that varied by <10%)

Results

As assessed by benzidine staining, Adriamycin (0.1 μM) induced erythroid differentiation in 60% ± 10% of K562 cells at 5 days after treatment without causing significant cytotoxicity. Continual exposure of K562 cells to the PKC activator PMA did not induce erythroid differentiation, although this substance was strongly anti-proliferative to the cells, showing an IC_{50} of 8 nM.

Several inhibitors of PKC, which have been reported to block the differentiation of leukaemia cell lines, were used to examine the role of this enzyme during Adriamycin-induced erythroid differentiation of K562 cells. Staurosporine inhibited partially purified PKC from K562, displaying an IC_{50} of 8 nM (Fig. 1 A). At a concentration of 50 nM, staurosporine blocked the ability of Adriamycin to induce erythroid differentiation in K562 cells, but this dose also inhibited the proliferation of the cells (Fig. 2 A). Staurosporine used alone at concentrations of up to 200 nM did not induce erythroid differentiation in K562 cells.

The protein kinase inhibitor H-7 inhibited K562 PKC, exhibiting an IC_{50} of 26 μM (Fig. 1 B). This inhibitor did not significantly reduce Adriamycin-induced differentiation when used at concentrations of up to 600 μM (Fig. 2 B), nor did it induce differentiation on its own. In contrast to staurosporine, H7 showed little anti-proliferative activity against K562 (Fig. 2 B) when the two compounds were compared in terms of the concentration required to inhibit PKC.

Discussion

Our data argue against a major role for PKC during the Adriamycin-induced erythroid differentiation of K562 cells. PMA did not differentiate K562 cells along the erythroid lineage, and the PKC inhibitor H-7 did not block Adriamycin-induced erythroid differentiation. Although staurosporine blocked erythroid differentiation, it only did so at concentrations that inhibited cell proliferation. These

concentrations would prevent the cycling of cells that is necessary for the expression of differentiation markers.

It has been suggested that phagocytic differentiation of HL60 cells may require the activation of both arms of the inositol lipid pathway, namely PKC and an increase in intracellular calcium [11]. However, in K562, the diacylglycerol analogue 1,2-dioctanoyl-*sn*-glycerol did not induce erythroid differentiation either when used alone or when combined with the calcium ionophore A23 187 (unpublished observations).

Both staurosporine and H-7 inhibit several different protein kinases [3, 16]. Hence, these agents can be expected to have multiple effects on cell differentiation and proliferation. Staurosporine is a potent inhibitor of tyrosine kinase [12]. The abl-encoded tyrosine kinase of K562 cells is associated with their proliferative capacity [15] and may conceivably be a site of the anti-proliferative action of staurosporine. The anti-proliferative effects of staurosporine on NIH/3T3 cells probably result from the inhibition of several protein kinases [17]. We have previously demonstrated that quercetin, an inhibitor of both PKC and tyrosine kinases, is also anti-proliferative towards K562 cells [4].

References

1. Alitalo R, Andersson LA, Betsholtz C, Nilsson K, Westermark B, Heldin C-H, Alitalo K (1987) Induction of platelet-derived growth factor gene expression during megakaryoblastic and monocytic differentiation of human leukaemia cell lines. EMBO J 6: 1213
2. Chou J, Chou T-C (1987) Dose effect analysis with microcomputers. Elsevier Biosoft, Cambridge
3. Hidaka H, Inagaki M, Kawamoto S, Sasaki Y (1984) Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. Biochemistry 23: 5036
4. Hoffman R, Graham L, Newlands ES (1989) Enhanced antiproliferative action of busulphan by quercetin on the human leukaemia cell line K562. Br J Cancer 59: 347
5. Jeannesson P, Ginot L, Manfair M, Jardillier J-C (1984) Induction of haemoglobin synthesis in the human leukaemia cell line K562 by Adriamycin. Anticancer Res 4: 47
6. Kitano T, Go M, Kikkawa U, Nishizuka Y (1986) Assay and purification of protein kinase C. Methods Enzymol 124: 349
7. Koeffler HP, Bar-Eli M, Territo MC (1981) Phorbol ester effect on differentiation of human myeloid leukaemia cell lines blocked at different stages of maturation. Cancer Res 41: 919
8. Lozzio CB, Lozzio BB (1975) Human chronic myelogenous leukaemia cell-line with positive Philadelphia chromosome. Blood 45: 321
9. Matsui T, Nakao Y, Koizumi T, Katakami Y, Fujita T (1986) Inhibition of phorbol ester-induced phenotypic differentiation of HL-60 cells by 1-(5-isoquinolinyl)-2-methylpiperazine, a protein kinase inhibitor. Cancer Res 46: 583
10. Merrill AH Jr, Sereni AM, Stevens VL, Hannun YA, Bell RM, Kinkade JM Jr (1986) Inhibition of phorbol ester-dependent differentiation of human promyelocytic leukaemic (HL60) cells by sphinganine and other long-chain bases. J Biol Chem 261: 12610
11. Morin MJ, Kreutter D, Howard R, Sartorelli AC (1987) Disparate effects of activators of protein kinase C on HL-60 promyelocytic leukaemia cell differentiation. J Biol Chem 262: 11758
12. Nakano H, Kobayashi E, Takahashi I, Tamaoki T, Kuzuu Y, Iba H (1987) Staurosporine inhibits tyrosine-specific protein kinase activity of Rous sarcoma virus transforming protein p60. J Antibiot 40: 706
13. Posada J, Vichi P, Tritton TR (1989) Protein kinase C in Adriamycin action and resistance in mouse sarcoma 180 cells. Cancer Res 49: 6634
14. Reiss M, Gamba-Vitalo C, Sartorelli AC (1986) Induction of tumour cell differentiation as a therapeutic approach: preclinical models of haematopoietic and solid neoplasms. Cancer Treat Rep 70: 201
15. Richardson JM, Morla AO, Wang JYJ (1987) Reduction in protein tyrosine phosphorylation during differentiation of human leukaemia cell line K562. Cancer Res 47: 4066
16. Ruegg UT, Burgess GM (1989) Staurosporine, K-252 and UCN-01: potent but non-specific inhibitors of protein kinases. Trends Pharmacol Sci 10: 218
17. Smith CD, Glickman JF, Chang K-J (1988) The antiproliferative effects of staurosporine are not exclusively mediated by inhibition of protein kinase C. Biochem Biophys Res Commun 156: 1250
18. Tisdale M (1985) Induction of haemoglobin synthesis in the human leukaemia cell line K562 by monomethyltriazenes and imidazotetrazinones. Biochem Pharmacol 34: 2077
19. Tritton TR, Hickman JA (985) Cell membranes as a chemotherapeutic target In: Muggia F (ed) Experimental and clinical progress in cancer chemotherapy. Martinus Nijhoff, Boston, p 81