Pergamon

Biochemical Pharmacology, Vol. 49, No. 3, pp. 367–373, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(94)00423-4

GROWTH INHIBITORY EFFECTS OF FK506 AND CYCLOSPORIN A INDEPENDENT OF INHIBITION OF CALCINEURIN

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(*Received* 6 *May* 1994; *accepted* 9 *September* 1994)

Abstract—The ability of the immunosuppressive agent FK506 to affect growth of the epidermal growth factor-receptor (EGF-R) overexpressing cell line, A431, was compared with that of the structurally unrelated immunosuppressive compound, cyclosporin A (CyA). Both were shown to inhibit growth, although neither of them caused down-regulation of the EGF-R or affected epidermal growth factor (EGF)-induced tyrosine phosphorylation of the EGF-R. Inhibition of growth was not specific to EGF-R pathways, as both FK506 and CyA also inhibited EGF-and platelet-derived growth factor (PDGF)-induced DNA synthesis in fibroblasts. In all assays FK506 was less potent than CyA even though it is 10–100 times more potent as an immunosuppressive agent. The role of calcineurin in CyA- or FK506-induced growth inhibition was investigated using the synthetic pyrethroid insecticides: cypermethrin, deltamethrin and fenvalerate, which are known calcineurin inhibitors. Failure of these agents to block cell growth or influence growth factor-induced mitogenesis indicated that the biochemical pathway(s) by which CyA or FK506 inhibited cell growth did not depend solely on inhibition of calcineurin.

Key words: cyclosporin A; FK506; pyrethroid insecticides; calcineurin; epidermal growth factor-receptor

CyA‡ and FK506 are structurally unrelated compounds (a cyclic undecapeptide and a cyclic ester, respectively) that share similar immunosuppressive properties. The more recently discovered compound, FK506, is 10–100 times more active, as an immunosuppressant, than CyA [1]. Both agents prevent the activation of T-cells that normally occurs following antigen binding to the T-cell receptor and which ultimately leads to T-cell proliferation [2]. A number of intracellular binding proteins has been identified which bind to CyA and FK506. These proteins, which belong to the immunophilin superfamily [reviewed in 3, 4], have distinct specificities for CyA and FK506 and are known as cyclophilins and FKBPs, respectively [4, 5]. Although the immunophilins possess PPIase activity [5, 6] the immunosuppressive effect of the drugs does not appear to be due to inhibition of the PPIase as some drug analogues that block this enzyme activity fail to suppress the immune response [4, 7]. Rather, evidence suggests that the drug–receptor complexes bind to and inactivate components of intracellular signalling pathways. One such target for the immunosuppressive drug complex has been identified as calcineurin [8, 9], a calcium/calmodulin-dependent serine/threonine phosphatase (Type 2B) [10]. Inhibition of calcineurin appears to block nuclear translocation of the transcription factor NF-AT which is necessary for activation of cytokine genes during T-cell activation [11, 12].

CyA is used in the treatment of psoriasis [13], a condition characterized by over-proliferation of keratinocytes. Cultured keratinocytes express EGF-R [13] and synthesize the EGF-R ligands TGFα [14] and amphiregulin [15], indicating that epidermal proliferation *in vivo* may be regulated by an autocrine loop or loops. While the mechanism of epidermal hyperproliferation in psoriasis has not been established, psoriatic epidermis displays increased EGF-R levels [16] and overproduction of TGFα [17], suggesting that overstimulation of the EGF-R may play a role in the pathogenesis of the disease. In addition to modifying T-cell-mediated skin reactions, CyA has been shown to inhibit keratinocyte growth *in vitro* [18] and *in vivo* [19] and may, therefore, modulate mitogenic signalling via the EGF-R. Although CyA-induced growth inhibition of keratinocytes is not associated with decreased expression of TGFα *in vitro* [13], the effect of CyA on down-regulation of the EGF-R is controversial. The drug has been reported to cause substantial down-regulation of EGF-R in cultured keratinocytes [20] but other workers have failed to reproduce this effect *in vitro* [13] or *in vivo* [21]. FK506 has also been shown to be effective as a treatment for psoriasis and pyoderma gangrenosum [22]; however, its beneficial effects may be more related to suppression of T-cell involvement than inhibition of keratinocyte proliferation [23].

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‡ Abbreviations: EGF, epidermal growth factor; TGFα, transforming growth factor alpha; EGF-R, epidermal growth factor receptor; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; IGF-1, insulin-like growth factor-1; CyA, cyclosporin A; FKBP, FK506-binding protein; PPIase, peptidyl-prolyl *cis-trans* isomerase; DMEM, Dulbecco's modified Eagle's medium; FBS, foetal bovine serum; FUdR, 5′-fluoro-deoxyuridine; IUdR, 5′-iodo-deoxyuridine; TCA, trichloroacetic acid; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol.

In this study, we have probed growth factor-stimulated signal transduction pathways using CyA and FK506. We have compared the effect of FK506 and CyA on the growth of the malignant keratinocyte cell line, A431, that expresses high levels of EGF-R [24], and have investigated whether growth inhibition specifically involves blockade of EGF-R activation or of pathways downstream of the receptor. In addition, we have used a series of Type II synthetic pyrethroid insecticides that are reported to be potent inhibitors of calcineurin [25] to determine whether inactivation of calcineurin is sufficient for growth inhibition.

MATERIALS AND METHODS

Materials. Recombinant hEGF was purchased from Life Technologies (Paisley, U.K.). [^{125}I]EGF was prepared using Iodo-beads (Pierce, Luton, U.K.) according to the manufacturer's instructions. Partially purified porcine PDGF was purchased from Bioprocessing. A431 cells were obtained from The European Collection of Animal Cell Cultures, Centre for Applied Microbiology and Research (Porton Down, Salisbury, U.K.). NR6/HER cells (NR6 cells transfected with human EGF-R) were a gift from Dr G. Panayatou, Ludwig Institute for Cancer Research, (London, U.K.). Biotinylated mouse monoclonal anti-phosphotyrosine antibody (PY20) was purchased from ICN Biochemicals (Thame, U.K.). Synthetic pyrethroid insecticides were purchased from Calbiochem-Novabiochem, (Nottingham, U.K.). FK506 was a gift from Fujisawa Pharmaceutical Co., Ltd (Osaka, Japan). Cell culture reagents were purchased from Life Technologies, with the exception of Ultraculture serum-free medium which was purchased from Bio-Whittaker (Reading, U.K.). CyA and FK506 were dissolved in dimethyl sulphoxide at 10 mM and further diluted in assay buffer. Dilutions of the vehicle alone had no effect on cell growth or DNA synthesis.

Cell culture. Cells were grown routinely in DMEM containing 10% FBS, 2 mM glutamine, non-essential amino acids, 10 U/mL penicillin and 10 μg/mL streptomycin at 37° in a humified atmosphere of air containing 5% CO_2.

Cell proliferation assay. Methylene blue dye was used to assess the cell density of adherent cell cultures [26]. A431 cells were trypsinized and resuspended in 10% FBS/DMEM. A total of 5×10^3 cells/well was aliquoted into sterile 96-well tissue culture plates and incubated at 37° for 5 hr to allow the cells to adhere to the plate. The medium was then pipetted off and replaced with 100 μL/well of triplicate samples of test solution in serum-free medium (Ultraculture). The plates were incubated for 4 days; the medium was removed by pipette each day and replaced with fresh medium to minimize exhaustion or degradation of the test solution.

To measure cell density, the medium was removed and the cells were fixed in 200 μL/well of formol saline (4% formaldehyde, 0.15 M NaCl) at room temperature for a minimum of 1 hr. The fixative was then tipped off and the plates were blotted on absorbent paper. The cells were stained with 100 μL/well of methylene blue solution (1% methylene blue in 10 mM disodium tetraborate, pH 8.5) for 30 min at room temperature. Excess dye was washed from the plates with 10 mM disodium tetraborate, pH 8.5, and the plates were blotted. Methylene blue was extracted from the cells by incubation with 100 μL/well of acidified ethanol (1 : 1 0.1 M HCl/ethanol) for 30 min at room temperature. The absorbance at 650 nm of individual wells was determined using a microplate photometer (Dynatech, Billingshurst, U.K.). An $A_{650\,nm}$ of 1.0 was equivalent to 2.25×10^4 cells/well.

Measurement of mitogenic activity. NR6/HER cells were trypsinized and resuspended in 10% FBS/DMEM. A total of 5×10^3 cells/well was aliquoted into sterile 96-well trays and incubated at 37° for 3 days. The medium was then changed to 80 μL/well of 1% FBS/DMEM and the cells were incubated for a further 24 hr at 37°. Test substances, standards and controls were prepared on sterile 96-well trays at appropriate dilutions in buffer consisting of equal volumes of PBS (9.2 mM Na_2HPO_4, 0.01 M NaH_2PO_4, 150 mM NaCl) and DMEM containing 25 mM HEPES, pH 7.5, 2% BSA, 8 μg/mL insulin and 480 μg/mL transferrin. Eighty microlitres of test sample was then transferred from the reagent tray to the cell tray and the cells were incubated at 37° for 22 hr. Fifty microlitres of DMEM containing 92.5 KBq/mL [^{125}I]UdR (sp. act. 74 TBq/mmol) and 2 μM FUdR was added to each well and the cells were incubated for a further 2 hr at 37°. The medium was then pipetted off the cells and discarded. The cells were fixed with 200 μL/well of 5% TCA at 4° for 10 min. The fixed cells were washed twice with 200 μL/well of 5% TCA followed by two washes with 200 μL/well of methanol. The trays were left to dry at room temperature. Remaining acid-insoluble material was dissolved in 100 μL/well of 0.2 M NaOH, transferred to plastic tubes and counted for 1 min/tube in a gamma counter.

Measurement of EGF-receptor phosphorylation. A431 cells were seeded into sterile 96-well plates and grown to 80% confluence in 10% FBS/DMEM. Cells were then incubated with 10% FBS/DMEM alone or containing 10 μM CyA or FK506 at 37° for 24 hr. The medium was then removed and the cells were incubated with 100 ng/mL EGF for 10 min. The cells were washed twice with PBS containing 100 mM NaF, 1 mM $NaVO_4$, 1 mM PMSF, 5 μg/mL leupeptin and 1 μg/mL aprotonin (this cocktail of inhibitors was included in all subsequent washes and incubations), solubilized with 100 μL/well of 80 mM Tris, pH 6.8, containing 0.1 M DTT, 2% SDS, bromophenol blue and 10% glycerol, and the samples boiled for 2 min.

Proteins were separated by SDS–PAGE [27] on a 7.5% acrylamide gel and transferred to nitrocellulose membranes (Hybond C, Amersham, Little Chalfont, U.K.) by electrophoresis. Immunoblotting was performed after incubating the membranes in blocking buffer (PBS/3% BSA) for 1 hr at 22°. Membranes were incubated with PY20 anti-phosphotyrosine antibody at 1/2000 for 4 hr at 22°, washed, and then incubated with streptavidin biotinylated-horseradish peroxidase conjugate (1/500) for 1 hr at 22°. Bound antibody was detected

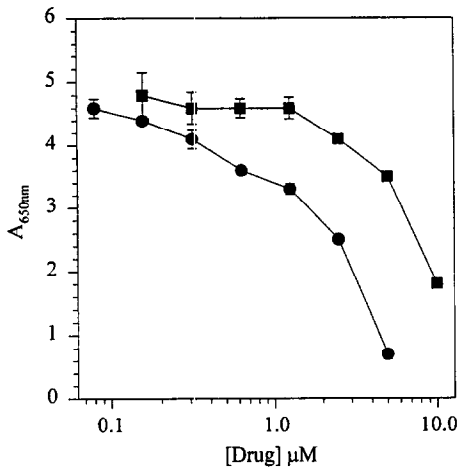

Fig. 1. Effect of FK506 or CyA on A431 cell growth. Cell growth was measured by Methylene Blue uptake of the cell culture, as described in Materials and Methods after a 4 day growth period in serum-free medium alone or plus FK506 (■) or CyA (●) at the doses shown. The $A_{650\,nm}$ of the cell cultures on day 0 was subtracted from the $A_{650\,nm}$ on day 4. Data points are the means ± SEM (N = 3). The corrected $A_{650\,nm}$ on day 4 for cells grown in serum-free medium without immunosuppressant was 4.8 ± 0.1.

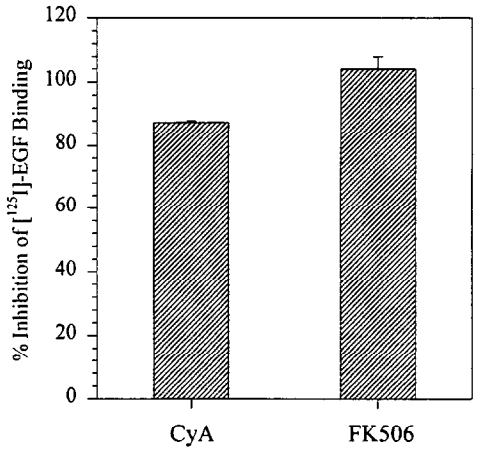

Fig. 2. Effect of FK506 or CyA on the level of cell surface EGF-R. Membane EGF-R on A431 cells was measured by binding of [^{125}I]EGF as described in Materials and Methods. Treated cells were incubated with 10 μM FK506 or CyA for 24 hr before addition of [^{125}I]EGF. Specific binding of [^{125}I]EGF is expressed as a % of that measured in untreated cells. Data points are the means ± SD of two individual experiments.

using enhanced chemiluminescence and autoradiography according to the manufacturer's instructions (Amersham, U.K.).

[^{125}I]*EGF binding assay.* A431 cells were seeded into sterile 96-well plates and grown to 80% confluence in 10% FBS/DMEM. Cells were then incubated with 10% FBS/DMEM alone or containing 10 μM CyA or FK506 at 37° for 24 hr. The medium was then removed from the cells and replaced with 100 μL/well of DMEM containing 1% BSA and 0.02% sodium azide. Following a 30 min incubation at 22° the medium was replaced with 100 μL/well of 10 ng/mL [^{125}I]EGF (sp. act. 0.75 MBq/μg) in DMEM/1% BSA/0.02% azide and incubated at 22° for 4 hr. Under these conditions there was no internalization of EGF-R. The radiolabelled cells were then washed with PBS/0.1% BSA. One set of wells was fixed with formol saline and stained according to the methylene blue protocol above to determine cell number/well. A second set of wells was dried and the cells were redissolved in 100 μL/well 0.5 M NaOH. The samples were aliquoted into tubes and counted for 1 min/tube in a gamma counter. Non-specific binding of [^{125}I]EGF was measured in the presence of 2 μg/mL unlabelled EGF, and was <5% of total binding.

RESULTS

Effect of FK506 and CyA on A431 cell growth

CyA and FK506 were tested for their ability to inhibit proliferation of A431 cells in serum free medium over a 4 day period, and the results are shown in Fig. 1. As previously reported [18, 28], CyA blocked cell growth at μM concentrations (IC_{50} 2.5 μM). By comparison, FK506 was a less potent inhibitor of growth (approximately three times less active than CyA) even though it is 10–100 times more immunosuppressive than CyA. Similar results were obtained when the assay was performed in 10% FBS/DMEM (results not shown).

Effect of CyA and FK506 on EGF-R

To determine whether CyA or FK506 had any direct effect on the EGF-R, the immunosuppressants were assayed for their ability to down-regulate the EGF-R or inhibit ligand-induced receptor autophosphorylation. When A431 cells were pre-incubated with CyA or FK506 in 10% FBS/DMEM for 24 hr and cell surface EGF-R was subsequently assayed by [^{125}I]EGF binding, it was found that treatment of cells with 10 μM CyA in 10% FBS/DMEM resulted in only a 10% reduction in cell surface EGF-R (Fig. 2), even though this concentration of drug was cytostatic. Similarly, 10 μM FK506, which reduced cell growth in 10% FBS/DMEM by 62% over 4 days, had no effect on the level of cell surface EGF-R (Fig. 2).

To determine the effect of CyA or FK506 on EGF-stimulated tyrosine phosphorylation of its receptor, A431 cells were pretreated with immunosuppressant drug as above and then stimulated with EGF and the level of EGF-R tyrosine phosphorylation determined by western blot analysis of solubilized cells. As shown in Fig. 3, following stimulation by EGF a band corresponding to the tyrosine-phosphorylated 170 kDa EGF-R was observed (Fig. 3b). Twenty-four hour pretreatment of cells with either CyA or FK506 did not significantly alter the level of EGF-R phosphorylation observed after ligand stimulation (Fig. 3c, d, respectively).

Effect of FK506 and CyA on growth factor-induced mitogenesis

To assess the specificity of growth inhibition, the

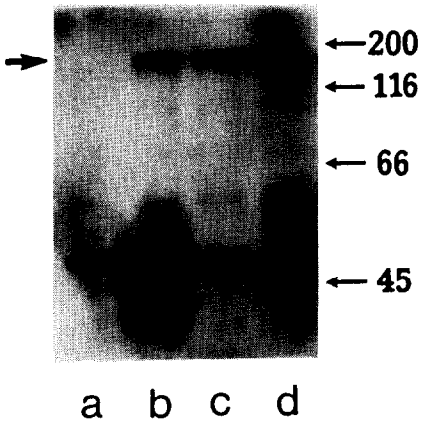

Fig. 3. Effect of FK506 or CyA on EGF-R tyrosine phosphorylation. Control A431 cells or cells pre-incubated with 10 μM FK506 or CyA for 24 hr were stimulated with 100 ng/mL EGF. Tyrosine phosphorylation of cell proteins was determined by SDS–PAGE and western blotting as described in Materials and Methods. The lanes show: (a) unstimulated cells; (b) cells stimulated by EGF; cells stimulated by EGF following incubation with (c) CyA or (d) FK506. The position of mol. mass markers (kDa) are shown on the right and the 170 kDa band corresponding to the EGF-R is indicated by an arrow on the left.

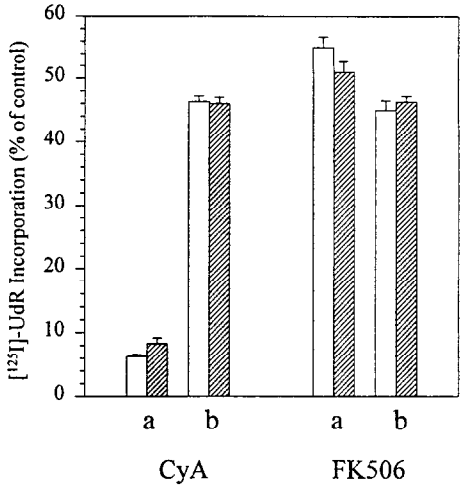

Fig. 5. Effect of FK506 or CyA on growth factor-induced mitogenesis when added at different time points. The mitogenesis assay was performed as described in the legend for Fig. 4. DNA synthesis was stimulated by 1 ng/mL EGF (unhatched bars) or 2.5 IU/mL PDGF (hatched bars). 12.5 μM CyA or FK506 was added (a) with the growth factor or (b) cells were incubated with growth factor alone for 22 hr and the inhibitor was added with the [125I]UdR. Incorporation of [125I]UdR in the presence of growth factor plus CyA or FK506 is expressed as a percentage of incorporation in the presence of growth factor alone. Data points are the means ± SEM (N = 3).

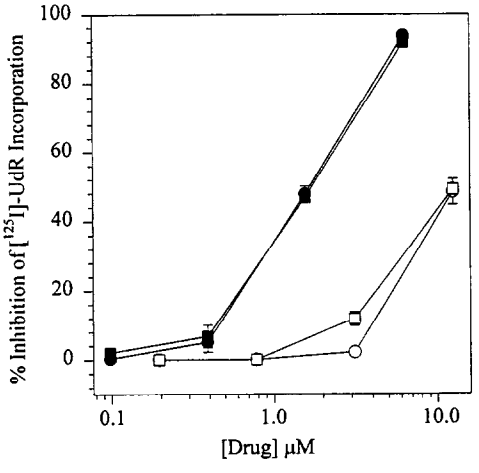

Fig. 4. Effect of FK506 (white symbols) or CyA (black symbols) on mitogenesis induced by 1 ng/mL EGF (○,●) or 2.5 IU/mL PDGF (□, ■). DNA synthesis was measured by incorporation of [125I]UdR into NR6/HER fibroblasts during S-phase as described in Materials and Methods. Data points are the means ± SEM (N = 3).

ability of FK506 and CyA to inhibit the mitogenic activity of EGF or PDGF was assayed using NR6/HER fibroblasts which possess receptors for both EGF and PDGF. CyA blocked both EGF- and PDGF-induced mitogenesis with similar IC_{50}s (approx. 1.5 μM) (Fig. 4). FK506 was also inhibitory but was approximately 10 times less active than CyA (Fig. 4).

The inhibitory effect of FK506 and CyA was maximal when they were added to the quiescent cells together with growth factor; however, some inhibition was also observed if the cells were stimulated with growth factor alone for 22 hr and the immunosuppressant was added in S-phase during the [125I]UdR pulse-labelling step of the assay (Fig. 5). For CyA, there was almost complete inhibition of DNA synthesis when added with the growth factor; this was reduced to approximately 55% inhibition when the drug was added at 22 hr. The inhibitory effect of FK506 was similar at both time points. This suggested that, in these assays, the drugs were affecting either uptake or utilization of thymidine by the cells.

Effect of the pyrethroid insecticides on A431 cell growth

As neither FK506 nor CyA showed specific inhibitory effects towards EGF as opposed to PDGF, this suggested that they might share a common intracellular target. Thus, as both CyA and FK506 inactivate calcineurin [8, 9], experiments where performed to determine whether inhibition of calcineurin alone was responsible for growth inhibition. However, incubation of A431 cells with the potent calcineurin inhibitors cypermethrin, deltamethrin and fenvalerate (IC_{50} for calcineurin inhibition: 40 pM, 100 pM and 2–4 nM, respectively [25] or permethrin, a weak calcineurin inhibitor [25],

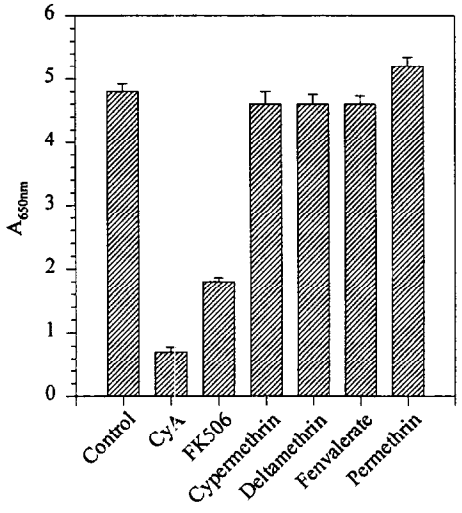

Fig. 6. Effect of FK506, CyA and synthetic pyrethroid insecticides on A431 cell growth. Cell growth was measured by Methylene Blue uptake of the cell culture, as described in Materials and Methods, after a 4 day growth period in serum-free medium alone or plus 10 μM CyA, 10 μM FK506, or 50 nM pyrethroid. The $A_{650\,nm}$ of the cell cultures on day 0 was subtracted from the $A_{650\,nm}$ on day 4. Data points are the means ± SEM (N = 3).

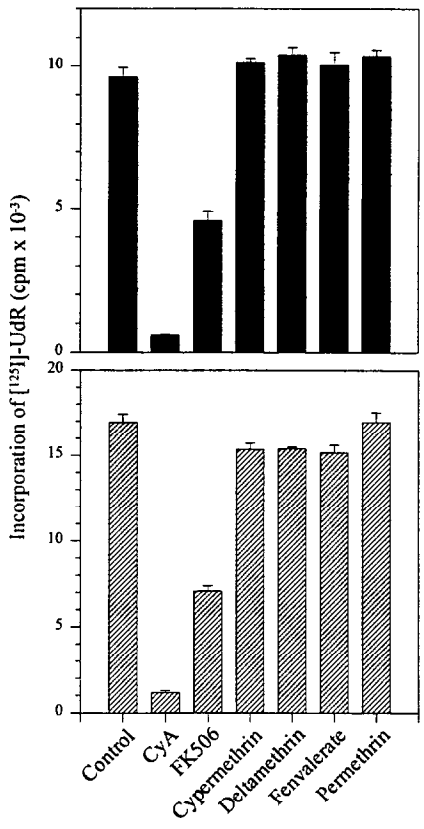

Fig. 7. Effect of FK506, CyA and pyrethroids on growth factor-induced mitogenesis. DNA synthesis in NR6/HER cells stimulated by 1 ng/mL EGF (solid bars) or 2.5 IU/mL PDGF (hatched bars) alone or in the presence of 12.5 μM CyA, 12.5 μM FK506 or 250 nM pyrethroid was assayed as described in the legend for Fig. 4. Data points are the means ± SEM (N = 3).

over a range of concentrations (500 pM–50 nM) had no significant effect on growth in serum-free medium (Fig. 6) or in 10% FBS/DMEM (results not shown).

Effect of the pyrethroid insecticides on growth factor-induced mitogenesis

Consistent with the lack of effect of the pyrethroid insecticides on A431 cells, EGF- or PDGF-induced mitogenesis using NR6/HER cells was also insensitive to inhibition by these compounds when assayed from 250 pM to 250 nM (Fig. 7).

DISCUSSION

In this study we have shown that FK506, like CyA, is able to inhibit proliferation of transformed keratinocytes as well as EGF- and PDGF-induced DNA synthesis in cultured fibroblasts. As previously reported [13, 18, 29], we found that the IC_{50} for CyA inhibition of keratinocyte growth *in vitro* was in the μM range. FK506 was found to be three times less active than CyA. This observation is in direct contrast to the comparative immunosuppressive activity of the two drugs where the IC_{50} values for inhibition of T-cell proliferation *in vitro* are 5–50 nM (6–60 ng/mL) and 0.5 nM (0.4 ng/mL) for CyA and FK506, respectively [7, 8]. During the course of this study, Duncan [23] reported that FK506 at doses between 10 and 1000 ng/mL (i.e. up to 1.2 μM) had no effect on proliferation of normal keratinocytes. These results are consistent with our observations using malignant A431 cells, as doses of FK506 greater than 1.2 μM were required to achieve growth inhibition.

It has been suggested that the effectiveness of CyA as a treatment for psoriasis could, in part, be attributable to a direct effect on keratinocyte growth as well as to inhibition of helper T-cell proliferation and resulting cytokine release [29]. Up-regulation of EGF-R and TGFα in psoriatic epidermis [17], together with the observation that CyA inhibits EGF-induced DNA synthesis, suggests that the drug may modulate signalling via the EGF-R. However, it has been shown that the growth inhibitory effects of CyA are not limited to keratinocytes but are also observed with fibroblasts and endothelial cells [28–30]. Moreover, CyA can inhibit growth induced by other growth factors such as PDGF, IGF-1 and bFGF [28, 30]. In the present work, we have further shown that neither CyA nor FK506 inhibits the ability of EGF to stimulate receptor autophosphorylation; we have also shown that neither drug caused substantial EGF-R down-regulation, in agreement with the observations of Khandke and co-workers [13]. These results indicate that the immunosuppressants act on a pathway common to a number of growth factors and downstream of receptor phosphorylation.

Following stimulation by growth factors such as

EGF and PDGF, a similar series of events has been identified within target cells. These include changes in phosphorylation of cellular signalling proteins, activation of ras GTPase, changes in ion fluxes and phosphoinositide metabolism and activation of transcription [31]. The intracellular binding proteins for CyA and FK506 are PPIases (cyclophilins and FKBPs, respectively) and, in T-cells, the pathway leading to immunosuppression in both cases involves the drug/PPIase complex binding to, and inactivating, calcineurin. Calcineurin is a calcium/calmodulin-dependent serine/threonine phosphatase (Type 2B) [10] whose inhibition blocks nuclear translocation of the transcription factor NF-AT, which is necessary for activation of cytokine genes during T-cell activation [11, 12]. To determine whether inactivation of calcineurin could account for the growth inhibition or blockade of mitogen-induced DNA synthesis by CyA or FK506, we examined the effects on growth of the type II synthetic pyrethroid insecticides which are potent calcineurin inhibitors [25], as well as neuronal sodium channel modifiers [32]. It was found that even high concentrations of inhibitors failed to induce growth inhibition in A431 cells or modulate the response of fibroblasts to EGF or PDGF. Assuming that the pyrethroids reached their intracellular target, this indicated that the effect of CyA and FK506 was unlikely to result from inhibition of calcineurin alone. The lack of effect of the pyrethroids is consistent with observations in yeast, where generation of calcineurin-deficient mutants has demonstrated that calcineurin is dispensible for growth under normal conditions [33] even though it is necessary for recovery from α-mating factor arrest [34].

Although binding of a growth factor to its receptor stimulates a rapid cascade of intracellular signals, in order to generate a full mitogenic response, the growth factor needs to be present for the first 10–12 hr of the pre-replicative phase [35]. Thyberg and Hansson [30] have shown that addition of CyA 4–6 hr after growth factor, compared with simultaneous addition, did not reduce the inhibitory effect of the compound on growth factor-stimulated DNA synthesis in cultured cells. This effect was apparently not associated with a decrease in growth factor-induced RNA and protein synthesis. More recently, rapamycin, a macrolide structurally related to FK506 which also binds FKBPs, has been shown to cause G1 arrest when added to osteosarcoma cells 4–6 hr after addition of serum growth factors. This appears to be due to inhibition of a cyclin-dependent kinase activity and a cyclin D1-cdk association during early G1 [36]. In the present experiments, the effect of CyA or FK506 on growth factor-induced DNA synthesis was observed not only when the drug was added with the mitogen but also, to a lesser degree in the case of CyA, when the drug was added 22 hr after addition of mitogen when the cells were in S-phase. As DNA synthesis is generally measured by the incorporation of radiolabelled nucleotides into new DNA, the possibility exists that the drugs interfered with uptake and phosphorylation of thymidine by the cells; thus, interpretation of any experiments utilizing this protocol for determining the effect of CyA on proliferation should be viewed with caution. However, as growth inhibition can also be observed in proliferation assays based directly on cell number, it is conceivable that another part of the growth inhibitory activity of CyA and FK506 results from interference with DNA synthesis itself.

If calcineurin is not a key target for growth inhibition by CyA or FK506 in keratinocytes and fibroblasts, this suggests that there may be other endogenous targets for cyclophilin- or FKBP-drug complexes. For example, it has been proposed that FKBP25, a 25 kDa FK506 binding protein which has the ability to associate with casein kinase II, may regulate growth by modulating the function of casein kinase II [37]. Other FKBPs have also been described which associate with steroid hormone receptors via heat shock proteins [38, 39]. Alternatively, the inhibition of PPIase activity, which appears to have no causative role in immunosuppression, may contribute to the inhibition of growth factor signalling pathways. For example, it could prevent conformational changes in signalling proteins or cause improper folding or trafficking of cellular proteins. The differences in activity between CyA and FK506 may be due to differences in the specificities and comparative activities of their corresponding PPIase receptors within the target cells.

Acknowledgements—This work was supported by the Cancer Research Campaign, U.K.

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