Journal of Cellular Biochemistry 51:345–352 (1993)

Binding and Subcellular Distribution of Cyclosporine in Human Fibroblasts

David A. Tipton and Mustafa Kh. Dabbous

Dental Research Center and Departments of Periodontology (D.A.T., M.Kh.D.) and Biochemistry (M.Kh.D.), The University of Tennessee, Memphis, Memphis, Tennessee 38163

Abstract The uptake, binding, and subcellular sites of accumulation of [^{3}H]-cyclosporine (CS) in two human gingival fibroblast strains, GN 23 and GN 54, have been examined. GN 23 responds to CS treatment with a decrease in collagenolysis, while GN 54 does not. Binding of the drug was determined using [^{3}H]-CS concentrations ranging from 10^{-5} to 10^{-8} M in the absence or presence of excess unlabeled CS (1 mM). The binding of the drug to both strains was specific and reached a plateau within 10 min, remaining at that level for up to 1 h. Scatchard analysis of the specific binding of [^{3}H]-CS to the responsive GN 23 strain revealed two dissociation constants: $K_D = 5 \times 10^{-8}$ M (1.2×10^7 sites/cell) and $K_D = 1.4 \times 10^{-6}$ M (2.2×10^8 sites/cell). GN 54, on the other hand, had only one class of low affinity binding site ($K_D = 0.47 \times 10^{-6}$ M [1.2×10^8 sites/cell]). Unlabeled CS (0.01–1 mM) inhibited the binding of [^{3}H]-CS in a dose-dependent manner to both strains, as did the calmodulin antagonist W-7, to a lesser extent. However, W-7 inhibited CS binding much more efficiently in GN 54 than in GN 23, suggesting that calmodulin may be the predominant CS receptor in GN 54. In both strains, 70% of the drug accumulated in the crude nuclear fraction after a 1 min incubation, with very little ($\leq$ 4%) being membrane associated, and the remainder was in the cytosol. In GN 23, CS levels in the crude nuclear fraction reached 80% by 20 min, and remained at this level for up to 1 h. In contrast, in GN 54, at incubation times of more than 1 min, the drug did not selectively accumulate in the crude nuclear fraction, but appeared to be in equilibrium between the nuclear and cytosolic fractions. These data show that the CS resistance of human gingival fibroblasts was not due to their inability to take up and bind CS. Rather, the different effects of CS on the collagenolysis of the responder and non-responder fibroblast strains may be related to the types of CS receptors they possess and differences in the cellular metabolism of CS occurring after binding, including the subcellular sites of drug accumulation.

Key words: gingiva, fibrosis, intracellular drug metabolism, receptors, calmodulin antagonist

Cyclosporine (CS) is a potent immunosuppressant used to prevent allograft rejection [1,2], the precise mechanism of which is not yet clear. However, it appears that CS affects T lymphocyte subsets responsible for graft rejection, inhibiting T cell activation, maturation of cytolytic effector cells, and interleukin-2 (IL-2) production [3]. There have been many studies investigating the existence and cellular location of receptors for CS on T lymphocytes. Early work suggested that CS competed for mitogen and alloantigen receptors on the T cell membrane [4–6]. Subsequent studies, however, showed that it did not compete for T lymphocyte surface molecules such as HLA-DR and receptors for phytohemagluttinin A, concanavalin A, OKT3, or IL-2, and that CS exhibited temperature-dependent, saturable binding to these cells [7–11]. Other work suggested that CS did not bind to specific cell membrane receptors but rather that its membrane binding was passive due to its hydrophobicity [12]. It now appears that T lymphocytes do indeed have saturable receptors for CS, most of which are located in the cytoplasm and bind CS after it diffuses through the cell membrane. These receptors include cyclophilin, a 17-kilodalton protein found in T lymphocytes and other cell types [13,14], the prolactin receptor [15,16], calmodulin [17], and perhaps other calcium-dependent hydrophobic proteins [17,18].

While the majority of studies have investigated T lymphocytes as the cellular target for CS, it can affect other cells as well. For example, CS can exert a cytostatic activity on non-lym-

Received May 4, 1992; accepted October 24, 1992.

Address reprint requests to David A. Tipton, D.D.S., Ph.D., The University of Tennessee, Memphis, 894 Union Avenue, Room 204, Nash Building, Memphis, TN 38163.

phoid cell lines [19 and references therein]. CS also inhibits antigen presentation and production of interleukin-1β (IL-1β) and tumor necrosis factor (TNF) by macrophages [20–30]. We have also shown that CS inhibits monocyte/macrophage production of both of these cytokines, depending on the dose of the drug [31]. Work in this laboratory further suggests that human gingival fibroblasts may be grouped into responder and non-responder strains with respect to the effect of CS on collagenase activity. CS directly affects some of them by decreasing or increasing their collagenase activity, depending on the dose of the drug and the individual strain; other strains are not affected by CS [32]. Such direct action of CS on gingival fibroblast collagen metabolism may be responsible in part for the development of fibrous gingival enlargement, a side effect of this drug which occurs in approximately one-third of the patients taking it [1,33–35].

To further investigate the mechanisms of CS-induced gingival fibrosis and the direct action of CS on fibroblasts, we have examined the binding of [^{3}H]-CS and its subcellular sites of accumulation in two gingival fibroblast strains. One of these is representative of fibroblast strains found to be refractile to CS, while the other is representative of strains which respond to the drug with a decrease in collagenase activity [32]. In the present report, we provide evidence that the different collagenolytic responses of the two strains to CS does not appear to be related to a lack of uptake of CS by the non-responsive strain, but may be related to differences in the types of CS receptors in the two strains, and the subsequent intracellular metabolism of CS, including its subcellular distribution.

MATERIALS AND METHODS

Fibroblasts

The human gingival fibroblast strains (designated GN 23 and GN 54) used in this study were derived from gingival explants from different healthy individuals with non-inflamed gingiva using standard techniques as described earlier [31,32]. The cells used in the experiments described below were used between passages 2 and 10.

Binding Assay

The binding of [^{3}H]-CS to fibroblasts was performed as described by Ryffel et al. [9,36]. The fibroblasts were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco; Grand Island, NY) supplemented with 100 μg/ml gentamicin (Gibco) and newborn calf serum (Gibco) (complete medium) at 37°C in 5% CO_2 in air. The cells were removed from their flasks by trypsinization with 0.25% trypsin (Gibco). After counting in a hemacytometer, the cells were suspended at 2.8×10^6 cells/ml in Hanks balanced salt solution (HBSS) (Gibco) containing 25 mM HEPES (N-2 hydroxyethyl piperazine-N' 2-ethanesulfonic acid) (Sigma; St. Louis, MO) and 5 mg/ml bovine serum albumin (U.S. Biochemical; Cleveland, OH), referred to as buffer solution.

Cells (5×10^5) in 200 μl final volume in Eppendorf tubes were incubated with [^{3}H]-CS (Amersham Corp.; Arlington Heights, IL; specific activity 11.0 Ci/mmol) (10^{-8}–10^{-5} M) in the absence (total binding) or presence of 1 mM unlabeled CS (nonspecific binding) (stock solution prepared as described earlier [31]; Sandoz, Ltd., Basel, Switzerland) for 60 min at 37°C. Alternatively, 5×10^5 fibroblasts were incubated with 1.3×10^{-6} M [^{3}H]-CS for periods of 1–60 min at 37°C with or without 1 mM unlabeled CS. The tubes were centrifuged and an aliquot of the supernatant was counted to determine free ligand concentration. The supernatant was completely aspirated, and the cells were washed twice with 1 and 0.5 ml ice-cold buffer solution. Cell-bound radioactivity was counted in a liquid scintillation spectrometer in triplicate for each [^{3}H]-CS concentration. Specific binding was defined as binding of [^{3}H]-CS in the absence of unlabeled CS minus the binding in the presence of unlabeled CS [37].

Inhibition of [^{3}H]-CS Binding to Fibroblasts by W-7 and CS

CS and the calmodulin antagonist W-7 (N-(6-aminohexyl)-5-chloro-1 naphthaline sulfonamide) (Sigma) were tested for inhibition of the cellular uptake of [^{3}H]-CS by GN 23 and GN 54. These experiments were performed essentially as described above for the binding assay; the cells were incubated for 1 h at 37°C with [^{3}H]-CS ($\sim 1.3 \times 10^{-6}$ M) in the absence or presence of CS or W-7 (1, 0.5, 0.1, 0.05, or 0.01 mM). A stock solution of W-7 was prepared at 4.2×10^{-2} M in 50% ethanol [38]. Cell-bound radioactivity was counted in a liquid scintillation spectrometer in triplicate for each concentration of W-7 or CS.

Fractionation of Cells to Determine Subcellular Location of [^{3}H]-CS

In the absence or presence of unlabeled CS (1 mM), 5 × 10^5 fibroblasts were incubated with [^{3}H]-CS at 1.3 × 10^{-6} M for periods of 1–60 min. The cells were then fractionated into nuclear, membrane, and cytosolic fractions as described by Merker and Handschumacher [14]. Briefly, after the incubation period with [^{3}H]-CS, the cells were centrifuged and washed with ice-cold buffer as described above. The cells were then suspended in ice-cold 10 mM Tris containing 1 mM $MgCl_2$, pH 7.45, and left for 10 min on ice. Tonicity was restored with 10 × HBSS, and the solution was forced three times through a tuberculin syringe with a 26-gauge needle, which disrupts 85–95% of the cells [14]. This suspension was centrifuged at 800*g* for 20 min to obtain a crude nuclear pellet. The resultant supernatant was centrifuged at 100,000*g* for 60 minutes to obtain a crude membrane fraction, and the supernatant was the cytosolic fraction. The sediments were dissolved in 1 N KOH for counting, and cytosol was counted directly in the liquid scintillation spectrometer.

Determination of DNA Content of Subcellular Fractions

The DNA content of the crude nuclear, membrane, and cytosolic fractions was determined using a modification of the method of Burton [39]. The cells were fractionated as described above, and the nuclear and membrane pellets were dissolved in 1 N KOH. Aliquots of the crude nuclear, membrane, and cytosolic fractions were placed in tubes and brought to 0.5 ml total volume with 0.1 M NaOH. To each tube was added 1 ml of diphenylamine (DPA) reagent (Fischer Scientific Co., Fair Lawn, NJ) (1 g DPA/100 ml glacial acetic acid containing 0.5 ml of 2% (v/v) acetaldehyde and 2.75 ml 12 M sulfuric acid). The tubes were incubated in the dark overnight at room temperature. Absorbance was read at 600 nm in a Uvikon 860 spectrophotometer (Kontron Instruments, Zurich, Switzerland). DNA standards using calf thymus DNA (Sigma) were prepared for calibration.

Statistical Analysis

The data are expressed as mean ± standard deviation and were analyzed using a one-way analysis of variance (ANOVA).

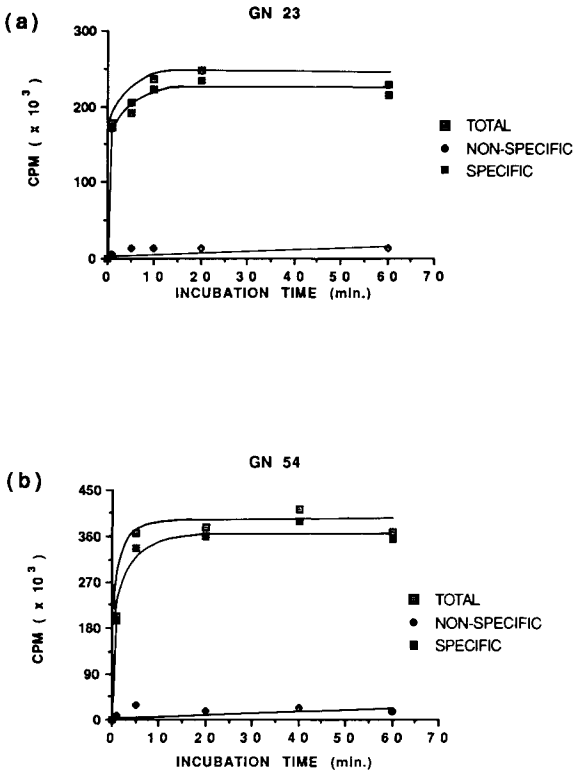

Fig. 1. Time course of binding of [^{3}H]-CS to human gingival fibroblast strains GN 23 (**a**) and GN 54 (**b**). In the absence (total binding) or presence (non-specific binding) of 1 mM CS, 5 × 10^5 cells/200 μl were incubated at 37°C with 1.3 × 10^{-6} M [^{3}H]-CS. The mean value of triplicate measurements is indicated.

RESULTS

Cellular Uptake of [^{3}H]-CS

The cellular uptake of [^{3}H]-CS by both strains was determined by incubating 5 × 10^5 fibroblasts with 1.3 × 10^{-6} M [^{3}H]-CS (~ 1.6 μg/ml) at 37°C for time periods of 1–60 min (Fig. 1a,b). Uptake of [^{3}H]-CS in both strains reached a plateau within 10 min which remained constant for 1 h, the longest time tested. The total, specific, and non-specific uptake of [^{3}H]-CS by GN 23 and GN 54 is shown in Figure 1a,b and demonstrates that the uptake of the drug by both strains was specifically blocked when coincubated with an excess (1 mM) of unlabeled CS.

Specific Binding of [^{3}H]-CS and Scatchard Analysis

Saturation curves demonstrating specific, saturable binding of [^{3}H]-CS to GN 23 and GN 54 are shown in Figure 2a,b. Specific drug binding was defined as the total moles bound minus the moles that could not be displaced by 1 mM unlabeled CS. Saturation of specific binding for

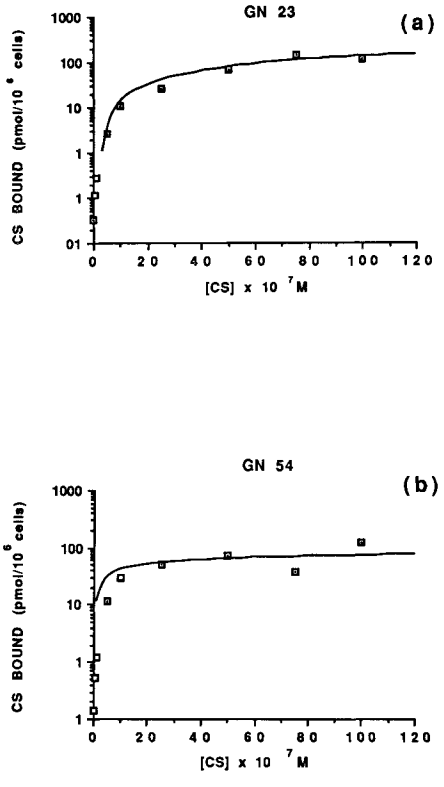

Fig. 2. Specific binding of [^{3}H]-CS to human gingival fibroblast strains GN 23 (a) and GN 54 (b) as a function of ligand concentration For 1 h, 5 × 10^5 cells/200 μl were incubated at 37°C with concentrations of [^{3}H]-CS ranging from 10^{-5} M to 10^{-8} M Specific binding was the amount of [^{3}H]-CS bound in the absence of unlabeled CS minus the amount bound in the presence of 1 mM unlabeled CS The mean value of triplicate measurements is indicated

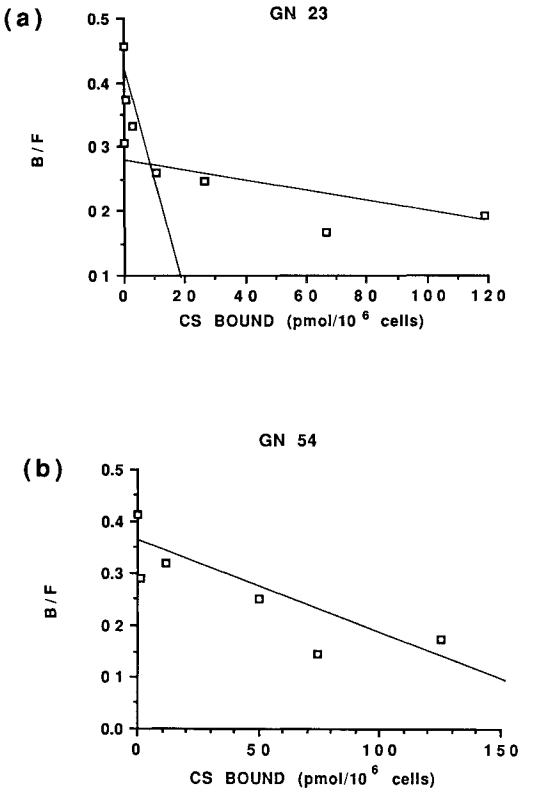

Fig. 3. Scatchard analysis of the specific binding of [^{3}H]-CS to human gingival fibroblast strains GN 23 (a) and GN 54 (b). For 1 h, 5 × 10^5 cells/200 μl were incubated at 37°C with concentrations of [^{3}H]-CS ranging from 10^{-5} M to 10 ^{8} M Specific binding was the amount of [^{3}H]-CS bound in the absence of unlabeled CS minus the amount bound in the presence of 1 mM unlabeled CS The mean value of triplicate measurements is indicated For GN 23 (a), Scatchard analysis yielded a curved line, suggesting two classes of binding sites K_D 5 × 10^{-8} M (1 2 × 10^7 sites/cell) and K_D 1 4 × 10^{-6} M (2 2 × 10^8 sites/cell), calculated by linear regression analysis For GN 54 (b), Scatchard analysis produced a straight line, indicating a single class of binding site (0 47 × 10^{-6} M, 1 2 × 10^8 sites/cell)

GN 23 and GN 54 was at 8.3 × 10^{-6} M and 7.5 × 10^{-6} M, respectively.

Scatchard analysis of the specific binding of [^{3}H]-CS to GN 23 and GN 54 is shown in Figure 3a,b. In the case of GN 23, Scatchard analysis revealed the presence of two classes of CS binding sites: a low affinity site with a calculated K_D of 1.4 × 10^{-6} M, and a high affinity site with a calculated K_D of 5 × 10^{-8} M. It was also calculated from this data that there were 1.2 × 10^7 high affinity binding sites per cell and 2.2 × 10^8 low affinity binding sites per cell.

In contrast to GN 23, Scatchard analysis of [^{3}H]-CS binding to GN 54 revealed a single class of low affinity binding site (K_D 0.47 × 10^{-6} M), with 1.2 × 10^8 binding sites/cell.

Inhibition of Binding of [^{3}H]-CS to Fibroblasts by CS and W-7

Consistent with earlier experiments (Fig. 1), unlabeled CS inhibited the binding of [^{3}H]-CS to both strains, with 1 mM CS almost completely blocking binding to GN 23 (~ 7% of control) and to GN 54 (~ 5% of control) (Fig. 4a,c). However, this inhibition appeared to be more efficient in GN 54 than in GN 23. For example, in GN 54, 1 × 10^{-5} M CS inhibited [^{3}H]-CS binding by ~ 50%, whereas the same concentration inhibited the binding to GN 23 by ~ 13%.

While blocking [^{3}H]-CS binding to both strains, W-7 inhibited its binding less efficiently than did unlabeled CS (Fig. 4). As was the case with unlabeled CS, W-7 caused greater inhibition of binding of [^{3}H]-CS to GN 54 than GN 23 at all concentrations tested: all concentrations of W-7 inhibited the binding of CS to GN 54 (Fig. 4d), while in GN 23, only the two highest W-7 concentrations significantly inhibited CS binding (Fig. 4b).

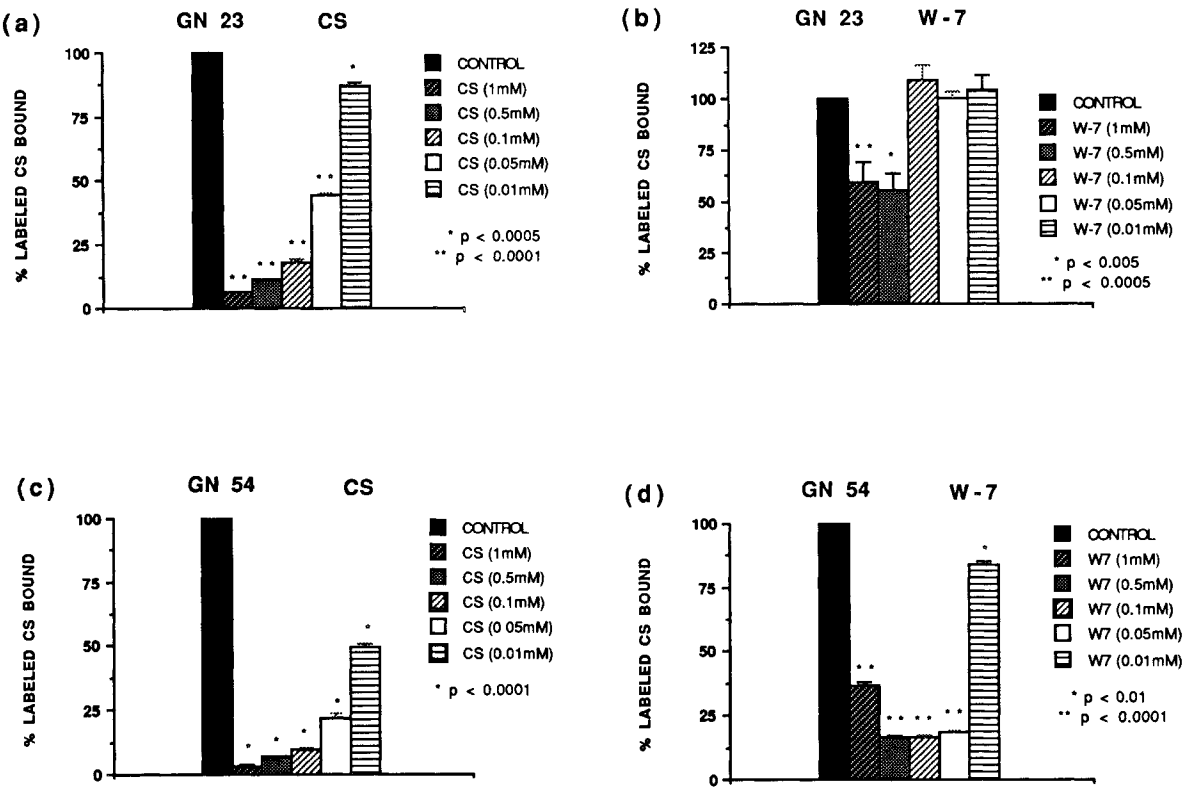

Fig. 4. Inhibition of [^{3}H]-CS binding to fibroblasts by W-7 and CS. Fibroblasts were incubated for 1 h at 37°C with 1.3×10^{-6} M [^{3}H]-CS in the absence or presence of CS or W-7 (0.01–1 mM). **a:** GN 23/CS. **b:** GN 23/W-7. **c:** GN 54/CS. **d:** GN 54/W-7. The data represent the means and standard deviations of triplicate cultures, and statistical analysis was by one-way analysis of variance (ANOVA).

Subcellular Localization of [^{3}H]-CS

The percentages of cell-bound [^{3}H]-CS found in the crude nuclear, cytosolic, and membrane fractions of GN 23 and GN 54 are shown in Figures 5 and 6, respectively. (To assess the efficiency of the cellular fractionation, the DNA content of the three fractions was determined; 93% of the total DNA was found in the low-speed sediment, or crude nuclear fraction, none in the cytosol, and the remainder in the membrane fraction (data not shown). In both GN 23 and GN 54, at all time periods, there was almost no label associated with the membrane fraction ($\leq$ 4%). After a 1 min incubation of [^{3}H]-CS with GN 23, approximately 70% of the label was found in the crude nuclear fraction, and most of the remainder was associated with the cytosol (Fig. 5). The percentage of the [^{3}H]-CS associated with the crude nuclear fraction of this strain gradually increased to a maximum of 80% at 20 min, which remained unchanged at 1 h incubation, and there was a corresponding decrease in the cytosolic label.

In GN 54, most of the [^{3}H]-CS rapidly accumulated in the crude nuclear fraction, but in contrast to GN 23, the level quickly decreased to an equilibrium distribution between the nuclear and cytoplasmic fractions at 50% (Fig. 6).

DISCUSSION

This study describes the uptake, binding, and subcellular localization of [^{3}H]-CS by two types of human gingival fibroblast strains: GN 23, representing a group which responds to CS with a decrease in collagenolysis, and GN 54, representing another group which is resistant to the drug [32]. Direct action of CS on gingival fibroblast collagen synthesis [40] and breakdown [32] may be responsible in part for the development of the fibrous gingival enlargement occurring in some patients taking CS. The purpose of this work was to determine if the CS sensitivity and CS resistance of GN 23 and GN 54, respectively, were correlated with differences in uptake, specific binding, and/or subcellular sites of CS accumulation.

There was specific, saturable binding of CS to GN 23 and GN 54. Therefore, the basis for the CS resistance of GN 54 does not appear to be due to lack of specific uptake. Consistent with

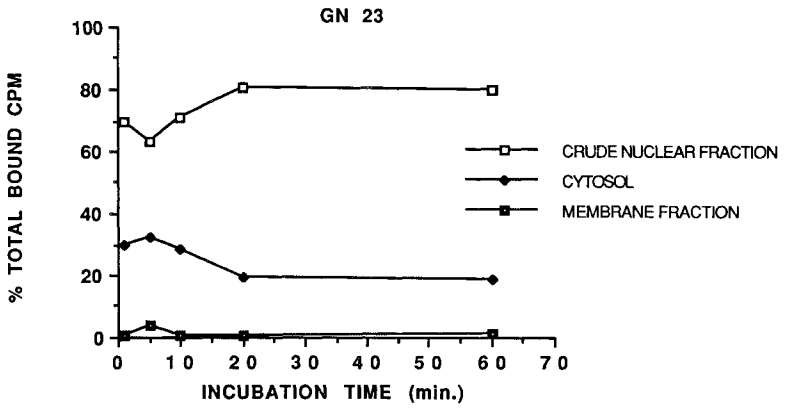

Fig. 5. Subcellular localization of [^{3}H]-CS in the CS-sensitive gingival fibroblast strain GN 23 In the absence or presence of 1 mM CS, 5×10^5 cells/200 μl were incubated at 37°C with [^{3}H]-CS at 1.3×10^{-6} M for periods of 1–60 min The cells were disrupted and fractionated into crude nuclear, membrane, and cytosolic fractions, and associated radioactivity was counted

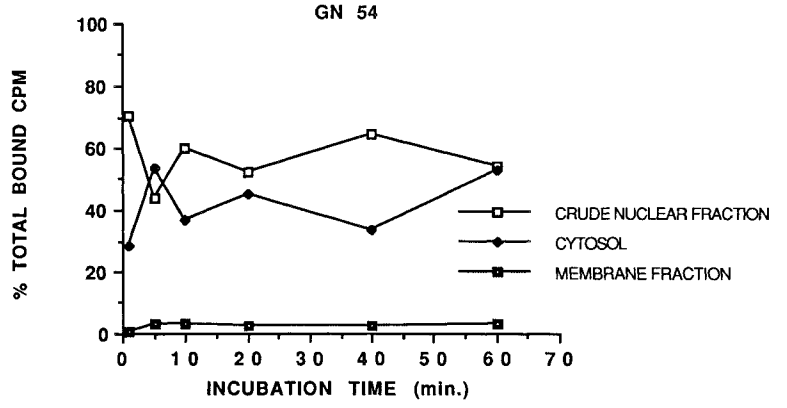

Fig. 6. Subcellular localization of [^{3}H]-CS in the CS-resistant gingival fibroblast strain GN 54 See legend for Fig 5

the results of this study, Koponen and Loor [41] described T cell clones which bound CS equally well but were functionally either sensitive or resistant to it. On the other hand, Hess and Colombani described human T cell populations which displayed an inverse correlation of CS binding with sensitivity and resistance [42]. Moreover, cell strains from different donors can differ in their sensitivity to CS [40,43,44], and our demonstration of CS-resistant and sensitive human gingival fibroblast strains derived from different individuals [32] supports these studies. Maximum uptake of CS by both strains occurred rapidly and was constant for at least 1 h. This is similar to the findings of others using a malignant T cell line [14] and RAJ1 cells (a human Burkitt lymphoma cell line) [45]. Scatchard analysis of CS binding to GN 23 revealed two dissociation constants, similar to evidence that lymphocytes display two CS binding constants [12]. On the other hand, Scatchard analysis of CS binding to GN 54 yielded a straight line, suggesting one dissociation constant.

CS-specific receptors have also been detected in thymocytes, T lymphocytes, granulocytes, monocytes, canine gingival fibroblasts, and human kidney cells [9,11,12,35,46]. Current evidence suggests that there may be several receptors for CS, the majority of which are intracellular. CS appears to diffuse through the cell membrane into the cytoplasm where it reacts with its receptors, including calmodulin, cyclophilin, and perhaps other less well-characterized proteins [3,17,18,47]. The calmodulin antagonist W-7 inhibited CS binding to GN 23 and GN 54 (Fig. 4b,d). Colombani et al. [17] showed that W-7 competitively inhibited the binding of fluorescent CS to T lymphocytes, and Hess et al. [48] provided direct evidence for the binding of CS to calmodulin in T cells. Other work has suggested that calmodulin does not bind to CS [49]; Foxwell et al. found that in

human lung fibroblasts, CS bound to several proteins, but not to calmodulin [50]. However, our results suggest that calmodulin acts as a CS receptor in both the sensitive and resistant human gingival fibroblast strains, and support the findings of Colombani et al. and Hess et al. cited above. In GN 54, all W-7 concentrations significantly inhibited CS binding, suggesting that calmodulin may be the primary receptor. The single class of binding site for CS in GN 54 had a K_D (0.47×10^{-6} M) within the reported range for CS-calmodulin binding (10^{-7} to 10^{-6} M) [13,17]. In GN 23, on the other hand, only the two highest W-7 concentrations significantly inhibited CS binding, suggesting the presence of other receptor(s) not blocked by W-7, consistent with the presence of two classes of CS binding sites in this strain.

The initial association of 70% of the CS with the crude nuclear fraction of both strains is in contrast to the findings of Merker and Handschumacher [14], who found 70% of total cell-bound CS associated with the cytosol of a T lymphoma cell line. This difference may be due to the type of cell used (fibroblast vs. T cell), the species from which the cells were obtained (human vs. mouse), or the state of transformation of the cells (normal vs. malignant). A small percentage of total cell-bound CS was associated with the membrane fractions of both strains ($\leq$ 4%), on the order of that reported by others ($\leq$ 8%) [14]; CS has recently been shown to bind to a 170 kDa membrane glycoprotein [51], and another study suggests the presence of a membrane-bound cyclophilin-related receptor in lymphocytes [52]. The putative difference in the types of receptors in the two strains as discussed above may be related to the finding that CS accumulates in the nuclear fraction of GN 23, while in GN 54, the drug initially appears to enter the nucleus but apparently fails to bind to intranuclear domains. It should be noted that a crude nuclear pellet isolated from disrupted cells by centrifugation at 600*g* for 10 min will contain nuclei as well as large plasma membrane sheets and unbroken cells [53]. However, the fractionation method of Merker and Handschumacher used in this study disrupts 85–95% of the cells, and our work and that of others [14] suggest that there is very little binding of CS to cellular membranes. Furthermore, we found that the pattern of CS distribution among the different subcellular fractions was reproducible. Therefore, it is likely that the differences in binding of CS to the crude nuclear and cytosolic fractions of the two fibroblast strains was not significantly affected by the presence or variation in the amounts of non-nuclear material associated with the crude nuclear fractions, and that these are true differences which reflect the actual distributions of the cell-bound CS. CS binds to both calmodulin and cyclophilin, and CS-cyclophilin complexes bind calcineurin as well as a complex of calcineurin and calmodulin. CS may affect cellular processes that these molecules regulate via association of any or all of these complexes with cytoplasmic and nuclear targets in the cell [54–58].

The characterization of the binding of CS to human gingival fibroblasts in this study is consistent with that of other cell types in several respects: 1) rapid uptake of the drug, reaching a maximum within 10 min and remaining undiminished for up to an hour [14,7]; 2) the presence of both low and high affinity receptor sites and similar K_D values [9,11,12,36,43,45]; 3) similar estimates of the numbers of low and high affinity binding sites [12,51]; and 4) little membrane-bound CS [14]. It is also consistent with a proposed model of subcellular binding of CS, describing intranuclear accumulation of receptor-bound CS, and explaining resistance or sensitivity of cells to CS not due to a lack of CS binding, but rather to distinct cellular physiologic mechanisms occurring after the binding of the drug [3,59]. For example, the sensitivity or resistance of T cells to CS may be affected by the relative intracellular concentrations of CS receptors such as calmodulin and cyclophilin [49]. Foxwell et al. [50] suggested that the CS resistance of a lung fibroblast strain was due to different intracellular targets for CS and/or the different accessibility of cyclophilin to CS than in sensitive cells; alternatively, the CS-binding capacity of cyclophilin may differ among various cell types.

We do not know conclusively whether the GN 23 and GN 54 gingival fibroblast strains do indeed differ in the presence and/or amounts of calmodulin, cyclophilin, and perhaps other CS-binding proteins. However, the results of this study do show that the resistant and sensitive strains are both able to take up and bind CS and suggest that they differ in the types of receptors they possess and in the events which occur after binding, including subcellular distribution. With further study, these differences may shed light on interindividual variations in sensitivity to CS and in the development of CS-induced fibrous gingival enlargement.

ACKNOWLEDGMENTS

The secretarial assistance of Ms. Kathy Walker is gratefully acknowledged. This work was supported by the University of Tennessee College of Dentistry and by Biomedical Research Support Grant BRSG-91-02.

REFERENCES

1 Daley TD, Wysocki GP J Periodontol 55 708–712, 1984
2 Borel JF Transplant Proc 15 2219–2229, 1983
3 Hess AD, Colombani PM In Borel JF (ed) "Progress in Allergy Cicloporin," Vol 38 Basel, Switzerland Karger, 1986, pp 198–221
4 Palacios R Immunol Rev 63 73–110, 1982
5 Britton S, Palacios R Immunol Rev 65 5–22, 1982
6 Palacios R J Immunol 128 337–342, 1982
7 Colombani PM, Donnenberg AD, Robb A, Hess AD Transplant Proc 17 1413–1416, 1985
8 Hess AD, Colombani PM, Donnenberg AD, Fischer AC, Ryffel B Transplant Proc 17 1419–1427, 1985
9 Ryffel B, Gotz V, Heuberger B J Immunol 129 1978–1982, 1982
10 Kahan BD, Kerman RH, Agostino AF, LeGrue SJ In White DJG (ed) "Cyclosporin A " Amsterdam Elsevier, 1982, pp 281–293
11 Ryffel B, Willard-Gallo KE, Tammi K, Loken MR Transplantation 37 276–280, 1984
12 Legrue SJ, Friedman AW, Kuhan BD J Immunol 131 712–718, 1983
13 Handschumacher RE, Harding MW, Rice J, Drugge R Science 226 544–547, 1983
14 Merker MM, Handschumacher RE J Immunol 132 3064–3070, 1984
15 Larson DF In Borel JF (ed) "Progress in Allergy Cyclosporin " Vol 38 Basel Karger, 1986, pp 222–238
16 Russell DH, Matrisian L, Kibler R, Larson DF, Poulos B, Magun BE Biochem Biophys Res Commun 121 899–906, 1984
17 Colomboni PM, Robb A, Hess AD Science 228 337–339, 1985
18 Benson A, Ziegler HK Transplantation 47 696–703, 1989
19 Ramirez-Bosca A, Kanitakis J, Thivolet J Cancer 66 936–940, 1990
20 Esa AH, Hess AD Fed Proc 44 4521, 1985
21 Bunjes D, Hardt C, Rollinghoff M, Wagner H Eur J Immunol 11 657–661, 1981
22 Yoshimura N, Oka T, Ohmori Y, Aikawa I, Fukuda M, Kahan BD Transplant Proc 19 (1, Pt 2) 1194–1198, 1987
23 Yoshimura N, Kahan B, Matsui S, Kita M, Hamashima T, Oka T Transplant Proc 20 (2, Suppl 2) 69–74, 1988
24 Andrus L, Lafferty K Scand J Immunol 15 449–458, 1982
25 Yoshimura N, Matsui S, Hamashima T, Kita M, Oka T Transplantation 45 157–162, 1988
26 Abbud-Filho M, Kupiec-Weglinski J, Araujo J, Heidecke C, Tilney N, Strom T J Immunol 133 2582–2586, 1984
27 Espevik T, Figari IS, Shalaby MF, Lackides GA, Lewis GD, Shepard M, Palladino MA J Exp Med 166 571–576, 1987
28 Remick D, Nguyen D, Eskandari M, Strieter R, Kunkel S Biochem Biophys Res Commun 161 551–555, 1989
29 Nguyn D, Eskandari M, DeForge L, Raiford C, Strieter R, Kunkels, Remick D J Immunol 144 3822–3828, 1990
30 McKenna R, Szturm K, Jeffrey F, Rush D Transplantation 47 343–348, 1989
31 Tipton DA, Pabst MJ, Dabbous MKh J Cell Biochem 44 253–264, 1990
32 Tipton DA, Stricklin GP, Dabbous MKh J Cell Biochem 46 152–165, 1991
33 Calne R, Rolles K, White DJ, Thiru S, Evans D, McMaster P, Dunn D, Graddock G, Henderson R, Aziz S, Lewis P Lancet 2 1033–1036, 1979
34 Rateitschak-Pluss E, Hefti A, Lortscher R, Thiel G J Clin Periodontol 10 237–247, 1983
35 Rostock MH, Fry HR, Turner JE J Periodontol 57 294–299, 1986
36 Ryffel B, Donatach P, Gotz U, Tschopp M Immunology 41 913–919, 1980
37 Hollenberger MD, Cuatrecasas P In O'Brien RD (ed) "The Receptor," Vol 1 New York Plenum, 1979, p 193
38 Tanabe K, Izumo A, Kato M, Miki A, Doi S J Protozool 36 139–143, 1989
39 Burton K In Grossman L, Modave K (eds) "Methods in Enzymology," Vol 12, Part B New York Academic Press, 1968, pp 163–206
40 Hassell TM, Romberg E, Sobhani S, Leskol L, Douglas R Transplant Proc 20 (Suppl 3) 993–1002, 1988
41 Koponen M, Loor F Ann Immunol 134 171–189, 1983
42 Hess AD, Colombani PM Transplantation 46 615–675, 1988
43 Klintmalm G, Sawe J, Ringden O, von Bahr C, Magnusson A Transplantation 39 132–139, 1985
44 Sander B, Brigati C, Moller E Scand J Immunol 23 435–440, 1986
45 Fabre J, Fabre G, Lena N, Lano J Biochem Pharm 35 4261–4266, 1986
46 Srinivasan G, Bashirelahi N, Hassell T, Sauk JJ J Dent Res 66 236 (abstract), 1987
47 Caroni P, Rothenfluh A, McGlynn E, Schneider C J Biol Chem 266 10739–10742, 1991
48 Hess AD, Tuszynski T, Engel P, Colombani P, Farrington J, Wenger R, Ryffel B Transplant Proc, 1985
49 Hait W, Harding M, Handschumaker R Science 233 987–988, 1986
50 Foxwell B, Woerly G, Husi H, Mackie A, Quesniaux V, Hiestand P, Wenger R, Ryffel B Biochim Biophys Acta 1138 115–121, 1992
51 Ryffel B, Woerly G, Rodriguez C, Foxwell B J Receptor Res 11 675–686, 1991
52 Cacalano NA, Chen B-X, Cleveland WL, Erlanger BF Proc Natl Acad Sci USA 89 4353–4357, 1992
53 McNamee MG Biotechniques 7 466–475, 1989
54 Randak C, Brabletz T, Hergenrother M, Sobotta I, Serfling E EMBO J 9 2529–2536, 1990
55 Zipfel PF, Irving SG, Kelly K, Siebenlist U Mol Cell Biol 9 1041–1048, 1989
56 Liu J, Farmer JD, Lane WS, Friedman J, Weissman I, Schreiber S Cell 66 807–815, 1991
57 Friedman J, Weismann I Cell 66 799–805, 1991
58 McKeon F Cell 66 823–826, 1991
59 Hess AD, Colombani PM In Gupta S, Paul W, Fauci A (eds) "Advances in Experimental Medicine and Biology Mechanisms of Lymphocyte Activation and Immune Regulation" Vol 213 New York Plenum, 1987, pp 309–330