Effect of the Δ^6-Desaturase Inhibitor SC-26196 on PUFA Metabolism in Human Cells

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ABSTRACT: The objective of this study was to determine the effect of 2,2-diphenyl-5-(4-{[(1*E*)-pyridin-3-yl-methylidene]-amino}piperazin-1-yl)pentanenitrile (SC-26196), a Δ^6-desaturase inhibitor, on PUFA metabolism in human cells. SC-26196 inhibited the desaturation of 2 μM [1-^{14}C]18:2n-6 by 87–95% in cultured human skin fibroblasts, coronary artery smooth muscle cells, and astrocytes. By contrast, SC-26196 did not affect the conversion of [1-^{14}C]20:3n-6 to 20:4 in the fibroblasts, demonstrating that it is selective for Δ^6-desaturase. The IC_{50} values for inhibition of the desaturation of 2 μM [1-^{14}C]18:3n-3 and [3-^{14}C]24:5n-3 in the fibroblasts, 0.2–0.4 μM, were similar to those for the inhibition of [1-^{14}C]18:2n-6 desaturation, and the rates of recovery of [1-^{14}C]18:2n-6 and [3-^{14}C]24:5n-3 desaturation after removal of SC-26196 from the culture medium also were similar. SC-26196 reduced the conversion of [3-^{14}C]22:5n-3 and [3-^{14}C]24:5n-3 to DHA by 75 and 84%, respectively, but it had no effect on the retroconversion of [3-^{14}C]24:6n-3 to DHA. These results demonstrate that SC-26196 effectively inhibits the desaturation of 18- and 24-carbon PUFA and, therefore, decreases the synthesis of arachidonic acid, EPA, and DHA in human cells. Furthermore, they provide additional evidence that the conversion of 22:5n-3 to DHA involves Δ^6-desaturation.

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FA Δ^6-desaturation is a key reaction in mammalian PUFA metabolism (1,2). It is the rate-limiting step in the conversion of linoleic acid (18:2n-6) to arachidonic acid (20:4n-6), the n-6 PUFA necessary for eicosanoid biosynthesis (1). Δ^6-Desaturation is also the initial reaction in the conversion of α-linolenic acid (18:3n-3) to EPA (20:5n-3), the n-3 analog of arachidonic acid (1,2). In addition, Δ^6-desaturation is required for the conversion of tetracosapentaenoic acid (24:5n-3) to DHA (3,4), the n-3 PUFA required for normal development and function of the central nervous system (5). Human and rat Δ^6-desaturases have been cloned (6,7), and the human gene, designated FA desaturase-2 (FADS2), has been localized to a 1.4-mb region of human chromosome 11q12-q13.1 (8). The FA Δ^5-desaturase, a second desaturase necessary for the production of arachidonic acid and EPA from their respective PUFA precursors (1,2), also has been cloned (9). This gene, designated FA desaturase-1 (FADS1), is present in the same 1.4-mb segment of human chromosome 11q12-q13.1 and is located 11.3 kb distant from FADS2 (8).

Numerous potent and selective inhibitors of mammalian Δ^6- and Δ^5-desaturases have been developed recently (10,11). One of these compounds, 2,2-diphenyl-5-(4-{[(1*E*)-pyridin-3-yl-methylidene]amino}piperazin-1-yl)pentanenitrile (SC-26196), inhibits the Δ^6-desaturation of linoleic acid in isolated rat liver microsomes with an IC_{50} of 0.2 μM (11). The IC_{50} of SC-26196 for Δ^5- and Δ^9-desaturation in these microsomes is >200 μM, indicating that the inhibitor is highly selective for Δ^6-desaturation. SC-26196 also inhibited the desaturation of linoleic acid when it was administered to mice. This produced an anti-inflammatory effect, presumably because eicosanoid production was reduced due to the lower availability of arachidonic acid (10,11).

Because the rodent studies indicated that SC-26196 is potentially useful as an anti-inflammatory agent, we wished to determine whether this compound is a potent and selective Δ^6-desaturase inhibitor in human tissues. We also investigated whether SC-26196 is effective against n-3 PUFA, including 24:5n-3, which is thought to be an intermediate in the conversion of 22:5n-3 to DHA (3,4). Human skin fibroblasts (HSF) were utilized for most of the studies because previous work demonstrated that these cells readily desaturate 18- and 24-carbon n-3 and n-6 PUFA substrates (12,13).

MATERIALS AND METHODS

Cell culture. Eagle's minimum essential medium (MEM) was modified by the addition of L-glutamine, gentamicin (Life Technologies, Grand Island, NY), nonessential amino acids, HEPES, and basal medium Eagle (BME) vitamins (Sigma, St. Louis, MO) (13). Normal HSF were obtained from stock cultures maintained by the University of Iowa Cardiovascular Center Tissue Culture Laboratory (Iowa City, IA) and were grown in 75 cm^2 vented flasks at 37°C in the modified Eagle's MEM containing 10% FBS (HyClone, Logan, UT) (13). Human coronary artery smooth muscle cells (CC-2576) were obtained from Clonetics (BioWhittaker, Walkersville, MD) and grown in Clonetics smooth muscle cell medium (CC-3182) supplemented with 5% FBS. Human astrocytes also

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Abbreviations: AM, acetoxymethyl ester; BME, basal medium Eagle; FADS, fatty acid desaturase; HSF, human skin fibroblasts; MEM, minimum essential medium; SC-26196, 2,2-diphenyl-5-(4-{[(1*E*)-pyridin-3-yl-methylidene]amino}piperazin-1-yl)pentanenitrile.

were obtained from Clonetics (CC-2567) and were grown in Clonetics astrocyte medium (CC-3186) supplemented with 5% FBS. Cell viability was determined using the commercially available calcein acetoxymethyl ester (AM) reagent obtained from Molecular Probes (Eugene, OR) (14,15).

Incubation and analysis. HSF from stock cultures were suspended by incubation with trypsin-EDTA solution (Sigma) and seeded in 10 cm^2 wells for each experiment (16). The HSF were incubated with modified Eagle's MEM containing 10% FBS in humidified 5% CO_2 chambers at 37°C until they reached 85–90% confluence. The cultures were then incubated for 3 h at 37°C with modified Eagle's MEM containing 2% FBS and either SC-26196, a Δ^6-desaturase inhibitor kindly provided by the Pharmacia Corporation (10), dissolved in DMSO, or an equivalent amount of DMSO alone. In one experiment, SC-26196 was replaced by anthranilicanilide, a Δ^5-desaturase inhibitor also provided by Pharmacia Corporation (11), dissolved in DMSO. Radiolabeled FA was added, and the incubation was continued for 24 h unless indicated otherwise. [1-^{14}C]18:2n-6, [1-^{14}C]18:3n-3, [1-^{14}C]γ-linolenic acid (18:3n-6), and [1-^{14}C]dihomo-γ-linolenic acid (20:3n-6) were purchased from American Radiolabeled Chemicals (St. Louis, MO), and the corresponding unlabeled FA were obtained from Cayman Chemical (Ann Arbor, MI). [3-^{14}C]Docosapentaenoic acid (22:5n-3 and 22:5n-6), [3-^{14}C]tetracosapentaenoic acid (24:5n-3), [3-^{14}C]tetracosahexaenoic acid (24:6n-3), and the corresponding unlabeled FA were generously provided by Dr. Howard Sprecher, Department of Medical Biochemistry, Ohio State University (Columbus, OH).

The human coronary artery smooth muscle cells and astrocytes were treated similarly and tested when the cultures were 90% confluent. These cultures were incubated with 2 μM [1-^{14}C]18:2 in the respective Clonetics medium containing 5% FBS. At the end of the incubation, the medium was removed and centrifuged at 1000 × *g* to sediment any cell debris. The supernatant solution was removed, acidified to pH 4 with formic acid, and extracted twice with 4 vol of ethyl acetate. An aliquot of the combined ethyl acetate extracts was dried under N_2, dissolved in scintillator solution, and assayed for radioactivity in a liquid scintillation spectrometer (16). Quenching was monitored with the external standard.

The cell monolayer was washed with ice-cold Dulbecco's PBS solution immediately after the medium was removed. The washed cells then were scraped with a rubber policeman into 2 mL ice-cold methanol containing 1% acetic acid; after 4 mL chloroform and 1.5 mL acidified NaCl were added, the chloroform phase was isolated (16). After the solvent was evaporated under a stream of N_2, the lipid residue was suspended in 0.5 mL of a 2:1 mixture of chloroform and methanol, and the radioactivity contained in an aliquot of this mixture was measured by liquid scintillation spectrometry (16).

Chromatography. The radioactive FA contained in the medium and cell lipid extracts were separated by HPLC (17,18). Aliquots of the medium and cell lipid extract containing 20,000–50,000 dpm were dried under N_2, transesterified with 12% BF_3 in methanol at 95°C for 45 min, and extracted with *n*-heptane. The FAME were then separated on an Alltech (Deerfield, IL) 3-μm Adsorbosphere 4.6 × 150 mm reversed-phase C18 column (18), using a Gilson (Middleton, WI) dual pump gradient HPLC system equipped with an automatic sample injector (17). The solvent consisted of acetonitrile and water adjusted to pH 4.0 with formic acid. For most separations, the gradient began at 76% acetonitrile and was increased stepwise over 10 min to 86% acetonitrile. It remained at 86% acetonitrile for 25 min and was then increased over 2 min to 100% acetonitrile, where it was maintained for an additional 23 min. The flow rate was 0.7 mL/min. To separate 18:3n-3 from 22:6n-3, the initial 76% acetonitrile concentration was maintained for 45 min, increased over 1 min to 100% acetonitrile, and then maintained for 14 min (12). The effluent was mixed with 2.1 mL of BudgetSolve liquid scintillation solution (RPI, Mt. Prospect, IL), and the radioactivity was assayed by passing the mixture through an in-line flow scintillation detector (IN/US Systems, Tampa, FL). Radiolabeled FAME standards were included with each set of chromatograms.

RESULTS

Effect of SC-26196 on 18:2n-6 metabolism. To evaluate the effectiveness of SC-26196 in human cells, we initially investigated its ability to inhibit the Δ^6-desaturation of 18:2n-6 (1,2). Control cultures and those treated with 2 μM SC-26196 were incubated for 24 h in a medium containing 2 μM [1-^{14}C]18:2n-6. The presence of SC-26196 did not affect the total amount of radiolabeled FA taken up by the cells. However, as shown by the HPLC analyses in Figure 1, major differences were observed in the amounts of radiolabeled products that were formed. In the control HSF cultures, 49% of the cell lipid radioactivity was contained in two main products, 20:3 and 20:4 (Fig. 1A). By contrast, 20:3 and 20:4 accounted for only 3.5% of the radioactivity incorporated into the cell lipids when 2 μM SC-26196 was present, a 93% reduction, and 88% remained as 18:2 (Fig. 1B). Unmodified 18:2 was the main radiolabeled FA contained in the medium after the 24-h incubation in both sets of HSF cultures. In the control cultures, 20:3 accounted for 4.9% of the radioactivity in the medium, but no radiolabeled 20:3 or other Δ^6-desaturation products were detected in the medium when SC-26196 was present (data not shown).

To determine the extent to which SC-26196 was effective in other human cells, we tested its ability to inhibit the desaturation of 2 μM [1-^{14}C]18:2n-6 in the human coronary artery smooth muscle and astrocyte cultures. Substantial amounts of radiolabeled 20:3 and 20:4 and a small amount of 22:4 were present in the smooth muscle cell lipids after incubation for 24 h (Fig.1C). The formation of these products was almost completely inhibited when 2 μM SC-26196 was present in the incubation medium (Fig. 1D). Similarly, substantial amounts of radiolabeled 20:3, 20:4, and 22:4 were present in the human astrocytes after a 24-h incubation with [1-^{14}C]18:2n-6

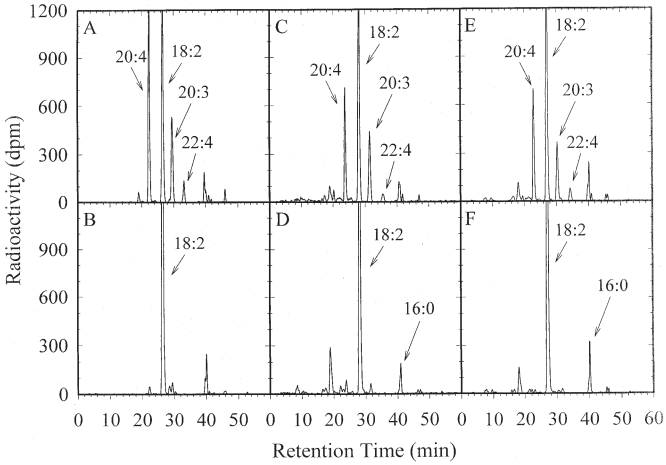

FIG. 1. Effect of SC-26196 on the metabolism of linoleic acid. Experimental cultures were incubated initially for 3 h with 2 μM SC-26196 dissolved in DMSO at 37°C, and control cultures were incubated with an equivalent amount of DMSO alone. At the end of the 3-h incubation, 2 μM [1-^{14}C]18:2n-6 was added, and the incubation was continued for 24 h at 37°C. SC-26196 remained in the medium of the experimental cultures during the incubation with the [1-^{14}C]18:2n-6. After the cells were washed, the lipids were extracted and methylated, and the radiolabeled FAME were assayed by RP-HPLC with an on-line flow scintillation detector. The HPLC tracings shown are from: (A) a control HSF culture, and (B) an HSF culture treated with SC-26196; (C) a control human coronary artery smooth muscle culture, and (D) a smooth muscle cell culture treated with 2 μM SC-26196; (E) a control human astrocyte culture, and (F) an astrocyte culture treated with 2 μM SC-26196. The HSF were incubated in a medium containing modified Eagle's MEM and 2% FBS, and the smooth muscle and astrocyte cultures were incubated in their respective Clonetics media containing 5% FBS. Abbreviations: SC-26196, 2,2-diphenyl-5-(4-{[(1*E*)-pyridin-3-yl-methylidene]amino}piperazin-1-yl)pentanenitrile; HSF, human skin fibroblasts; MEM, minimum essential medium.

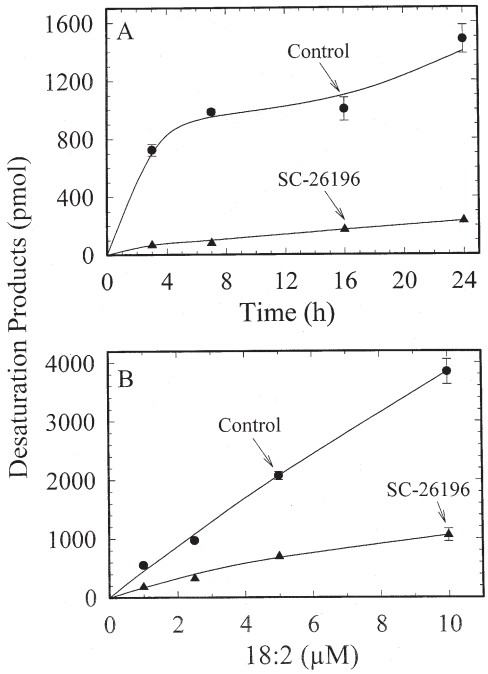

FIG. 2. Effect of time of incubation and FA concentration on the inhibition of linoleic acid desaturation produced by SC-26196 in HSF. The experimental design, analysis, number of replicates, and reproducibility were as described in Figure 1. In A, the culture medium contained 2 μM SC-26196, and the time of incubation after addition of 2 μM [1-^{14}C]18:2n-6 varied from 3 to 25 h; in B, the cultures were incubated with 1 μM SC-26196 and 1–10 μM [1-^{14}C]18:2n-6. Each data point represents the sum of the radiolabeled desaturation products contained in the hydrolyzed cell lipid extract as determined by HPLC, and the pmol values were calculated using the specific activity of the [1-^{14}C]18:2n-6 added to the culture medium. Standard error bars are shown where they are larger than the size of the data point. See Figure 1 for abbreviations.

(Fig. 1E), and the formation of these products also was almost completely inhibited by 2 μM SC-26196 (Fig. 1F). The vascular smooth muscle cells and astrocytes released a small amount of radiolabeled 20:3 and 20:4 into the culture medium, but this did not occur when SC-26196 was present in the incubation medium (data not shown). These results demonstrate that the effectiveness of SC-26196 in human cells is not limited to skin fibroblasts and suggest that the inhibitor may have a wide spectrum of action in human tissues.

The effect of time of incubation and FA concentration on the inhibition produced by SC-26196 in the HSF is shown in Figure 2. Each point represents the total amount of desaturation products detected by HPLC analysis of the hydrolyzed cell lipid extract. In the time-dependent study (Fig. 2A), the medium contained 2 μM SC-26196 and 2 μM [1-^{14}C]18:2n-6. A substantial reduction in the formation of desaturation products occurred within 3 h, the earliest time tested. More desaturation products were formed as the incubation progressed, but the percentage reduction produced by SC-26196 relative to the control cultures was similar throughout the 24-h period.

To determine whether the inhibition could be overcome by raising the FA concentration, the HSF were incubated for 24 h with 1 μM SC-26196 and increasing amounts of [1-^{14}C]18:2n-6. Although the total amount of desaturation products increased as the FA concentration was raised from 1 to 10 μM, the cultures incubated with SC-26196 converted substantially less of the 18:2 to desaturation products than the corresponding control cultures at each FA concentration (Fig. 2B). Therefore, a 10-fold excess of 18:2 was not sufficient to overcome the inhibitory effect of 1 μM SC-26196.

In additional experiments, the effect of SC-26196 on HSF viability was assessed to determine whether the decrease in the formation of Δ^6-desaturase products might be due to cytotoxicity. The calcein AM assay was used as an index of cell viability (14,15). Compared with control HSF cultures incubated for 24 h, those incubated under the same conditions with 2.5 and 10 μM SC-26196 exhibited a 5.1 ± 0.7 and 6.6 ± 0.9% reduction in viability, respectively (mean ± SEM, n = 8). Thus, the decrease in formation of 18:2n-6 desaturation products far exceeded the small reduction in cell viability produced by the inhibitor.

Selectivity of SC-26196 for Δ^6-desaturase. The conversion of 18:2n-6 to 20:4 requires two additional reactions after the initial Δ^6-desaturation, chain elongation, and Δ^5-desaturation

(1,2). It was not possible to determine whether SC-26196 had any effect on these reactions when the cells were incubated with [1-^{14}C]18:2n-6 because conversion to these products was almost completely eliminated. Therefore, we tested the effect of the inhibitor directly on the utilization of radiolabeled 18:3n-6 and 20:3n-6 in HSF.

The HSF cultures incubated for 24 h with 2 μM [1-^{14}C]-18:3n-6 converted 78% of the radioactivity incorporated into the cell lipids to 20-carbon products. In the corresponding incubation with 2 μM SC-26196, the 20-carbon products contained 82% of the incorporated radioactivity, indicating that the inhibitor did not affect the chain-elongation reaction (data not shown).

SC-26196 did not inhibit the conversion of 20:3n-6 to 20:4 (Fig. 3). Control HSF incubated for 24 h with 2 μM [1-^{14}C]-20:3n-6 converted 33% of the radioactivity present in the cell lipids to 20:4n-6 (Fig. 3A), and those treated with SC-26196 converted 35% (Fig. 3B). To be certain that this Δ^5-desaturation reaction was susceptible to inhibition in HSF, we tested the effect of anthranilicanilide on the conversion of 20:3n-6 to 20:4. Anthranilicanilide is a selective Δ^5-desaturase inhibitor that has an IC_{50} of 0.04 μM in rat liver microsomes (10,11). The addition of 2 μM anthranilicanilide reduced the conversion of 2 μM [1-^{14}C]20:3n-6 to 20:4 to 2.7% (Fig. 3C), a decrease of >90% compared with the control cultures. Thus, like Δ^6-desaturation, the Δ^5-desaturation reaction is susceptible to inhibition in HSF, but it is not affected by SC-26196. Taken together, these results demonstrate that SC-26196 reduces the conversion of 18:2n-6 to 20:4 in HSF by inhibiting the Δ^6-desaturase reaction.

Effect of SC-26196 on n-3 PUFA metabolism. To determine whether SC-26196 was effective against n-3 PUFA substrates, we investigated its effect on the metabolism of 18:3n-3 in HSF. The results are shown in Figure 4. When control HSF were incubated for 24 h with 2 μM [1-^{14}C]18:3n-3, 57% of the cell lipid radioactivity was present in three products, 20:5, 22:5, and 22:6 (Fig. 4A). The conversion of 18:3n-3 to these products requires Δ^6-desaturation (1,2). The addition of 2 μM SC-26196 reduced the amount of [1-^{14}C]18:3n-3 radioactivity converted to these products to 12%, an 80% reduction, whereas the amounts remaining in 18:3 and converted to its chain-elongated product, 20:3, increased threefold (Fig. 4B).

The effect of SC-26196 on 22:5n-3 metabolism also was investigated. The main radiolabeled products synthesized from 2 μM [3-^{14}C]22:5n-3 by the control HSF were 20:5 and 22:6, accounting for 22 and 20%, respectively, of the radioactivity contained in the cell lipids (Fig. 4C). When 2 μM SC-26196 was added, only 5% of the cell lipid radioactivity was converted to 22:6, a 75% reduction. By contrast, the amount retroconverted to 20:5 increased to 27% (Fig. 4D).

To determine whether the reduction in 22:6 was due to inhibition of the Δ^6-desaturation of the 24-carbon intermediate that forms when n-3 PUFA precursors are converted to DHA (1–4), we investigated the effect of SC-26196 on the metabolism of 24:5n-3. The HPLC results are shown in Figure 5.

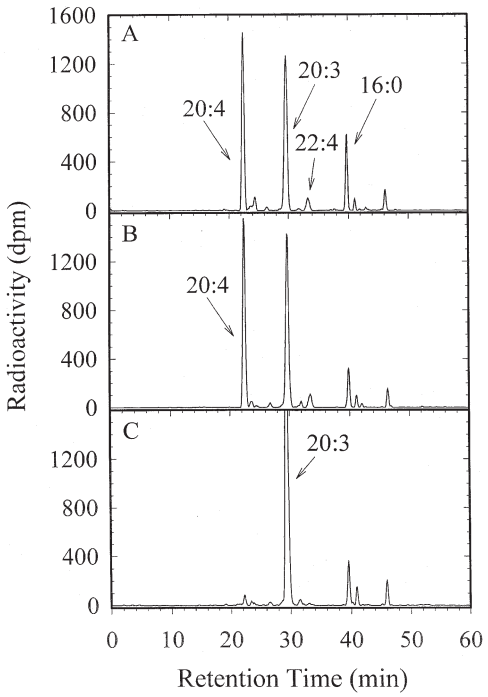

FIG. 3. Effect of Δ^6- and Δ^5-desaturase inhibitors on the metabolism of dihomo-γ-linolenic acid. The experimental design was as described in Figure 1, except that the HSF were incubated with 2 μM [1-^{14}C]-20:3n-6. The HPLC tracings shown are from cultures incubated with: (A) 20:3n-6 alone; (B) 20:3n-6 plus 2 μM SC-26196; and (C) 20:3n-6 plus 2 μM anthranilicanilide (CP-74006). Single tracings are shown, but similar chromatograms were obtained from two additional cultures in each case. See Figure 1 for abbreviation.

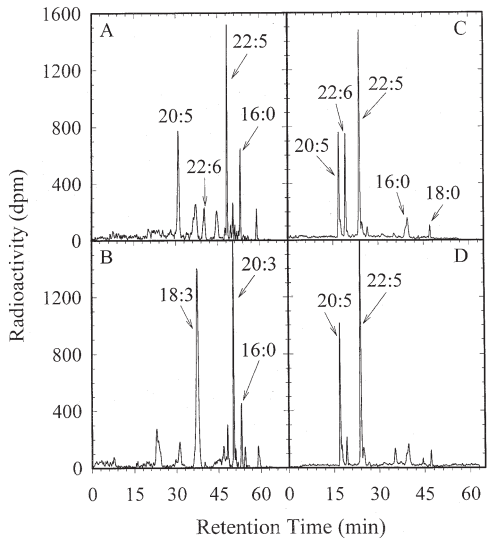

FIG. 4. Effect of SC-26196 on the metabolism of α-linolenic and docosapentaenoic acids in HSF. The experimental design and analysis were as described in Figure 1, except that the cells were incubated with: (A) 2 μM [1-^{14}C]18:3n-3; (B) 2 μM [1-^{14}C]18:3n-3 plus 2 μM SC-26196; (C) 2 μM [3-^{14}C]22:5n-3; and (D) 2 μM [3-^{14}C]22:5n-3 plus 2 μM SC-26196. Single tracings are shown, but similar chromatograms were obtained from two additional cultures in each case. See Figure 1 for abbreviations.

When the HSF were incubated for 24 h with 2 μM [3-^{14}C]-24:5n-3, 55% of the radioactivity incorporated into the cell lipids was converted to 22:6 (Fig. 5A). When 2 μM SC-26196 was added, only 9% of the 24:5n-3 was converted to 22:6, an 84% decrease (Fig. 5B). To determine whether the inhibitory effect occurred on the desaturation of 24:5n-3 or the retroconversion of the resulting product, we also investigated the effect of SC-26196 on the conversion of 24:6n-3 to 22:6. Control cultures incubated for 24 h with 2 μM [3-^{14}C]24:6n-3 converted 68% of the radioactivity incorporated into the cell lipids to 22:6 (Fig. 5C), whereas 64% of the incorporated radioactivity was converted to 22:6 by the corresponding cultures containing 2 μM SC-26196 (Fig. 5D). Taken together, these results indicate that SC-26196 reduces the conversion of 22:5n-3 to 22:6 by inhibiting the Δ^6-desaturation of 24:5n-3, not the retroconversion of 24:6.

Effect of SC-26196 concentration. The effectiveness of SC-26196 in inhibiting the desaturation of the 18- and 24-carbon PUFA substrates was compared, and the results are shown in Figure 6. The HSF cultures were incubated with 2 μM [1-^{14}C]18:3n-3 (Fig. 6A) and 2 μM [3-^{14}C]24:5n-3 (Fig. 6B) in the presence of increasing concentrations of SC-26196. Each point represents the sum of the Δ^6-desaturated FA products contained in the HSF, as determined by HPLC analysis of the cell lipid extract. More inhibition occurred as the SC-26196 concentration increased, and the calculated IC_{50} values were 0.4 and 0.2 μM for the incubations with 18:3n-3 and 24:5n-3, respectively. In a similar experiment with 2 μM [1-^{14}C]18:2n-6, the calculated IC_{50} value was 0.1 μM (data not shown). These findings indicate that the effectiveness of SC-26196 in HSF is approximately similar for 18- and 24-carbon PUFA substrates.

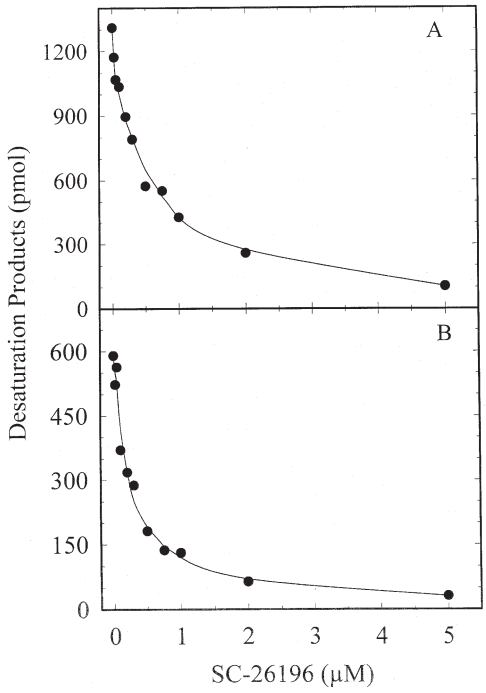

FIG. 6. Effect of SC-26196 concentration on the formation of radiolabeled FA desaturation products from α-linolenic and tetracosapentaenoic acids in HSF. The experimental design and analysis were as described in Figure 1, except that the SC-26196 concentration varied from 25 nM to 5 μM. The HSF were incubated with: (A) 2 μM [1-^{14}C]-18:3n-3; or (B) 2 μM [3-^{14}C]24:5n-3. Each data point represents the sum of the radiolabeled desaturation products in the hydrolyzed and methylated cell lipid extract determined by HPLC analysis. The pmol values were calculated from the specific activity of the radiolabeled FA added to the culture medium. See Figure 1 for abbreviations.

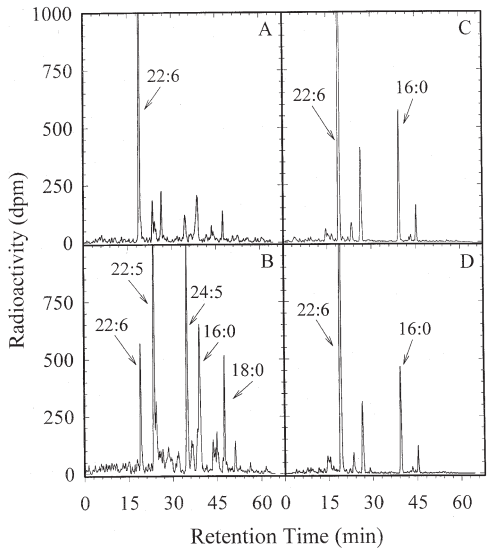

FIG. 5. Effect of SC-26196 on the metabolism of tetracosapentaenoic and tetracosahexaenoic acids in HSF. The experimental design and analysis were as described in Figure 1, except that the cells were incubated with: (A) 2 μM [3-^{14}C]24:5n-3; (B) 2 μM [3-^{14}C]24:5n-3 plus 2 μM SC-26196; (C) 2 μM [3-^{14}C]24:6n-3; and (D) 2 μM [3-^{14}C]24:6n-3 plus 2 μM SC-26196. Single tracings are shown, but similar chromatograms were obtained from two additional cultures in each case. See Figure 1 for abbreviations.

Recovery of Δ^6-desaturation capacity. Additional studies were done to assess the recovery of Δ^6-desaturation after exposure of the HSF to SC-26196. The cultures were incubated with a medium containing 2 μM SC-26196 for 3 h; after the inhibitor was removed, the incubation was continued for varying times in fresh medium containing 2% FBS. The Δ^6-desaturation activity was measured by adding radiolabeled FA at the end of the recovery period; each point in Figure 7 represents the sum of the radiolabeled Δ^6-desaturation products contained in the cell lipid extract as determined by HPLC. Two separate experiments were done, one with [1-^{14}C]18:2n-6 in which recovery was measured over a 24-h period after removal of the inhibitor, and the other with [3-^{14}C]24:5n-3 in which the recovery period was extended to 48 h. In both cases, a progressive increase in the formation of Δ^6-desaturation products occurred during the recovery period. When 2 μM [1-^{14}C]18:2n-6 served as the tracer, the formation of Δ^6-desaturation products by the HSF that had been exposed to SC-26196 was 65% as much at the end of the 24-h recovery period as in corresponding control cultures (Fig. 7A). When 2 μM [3-^{14}C]24:5n-3 served as the tracer, the recovery reached 75% of the control value at the end of the

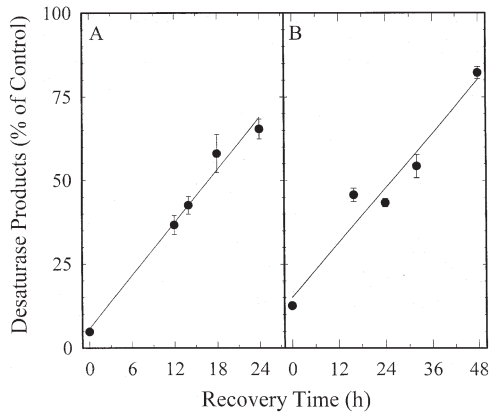

FIG. 7. Recovery of Δ^6-desaturation activity in HSF after removal of SC-26196 from the incubation medium. The data points indicate the percentage of desaturation products formed at various recovery times, relative to the amount formed by the control cultures that were not exposed to SC-26196. In A, the tracer used to measure Δ^6-desaturation was 2 μM [1-^{14}C]18:2n-6 and recovery was followed for 24 h; in B, the tracer used was 2 μM [3-^{14}C]24:5n-3 and recovery was followed for 48 h. The experimental design was the same in both cases. Three cultures that had not been exposed to the inhibitor were incubated for 24 h with the radiolabeled FA, and the desaturation products were determined by HPLC analysis. These results were taken as the control (100%) value. Three additional cultures were incubated with 2 μM SC-26196, and the radiolabeled FA was added to these cultures immediately, without any period of recovery. The desaturation products formed by these cultures were calculated as a percentage of the control values and are the points shown at "0" recovery time on the *x*-axis. The remaining cultures were incubated for 3 h with 2 μM SC-26196. After the medium containing the inhibitor was removed, sets of three cultures were incubated in fresh medium containing 2% FBS for each of the recovery times shown on the *x*-axis. At the end of the recovery period for each set of cultures, 2 μM radiolabeled FA was added and the incubation continued for 24 h. The sum of the radiolabeled desaturation products formed during this 24-h incubation was determined by HPLC, and the values are presented as a percentage of the control value. Each point is the mean of values obtained from three separate cultures, and the error bars indicate the SEM. See Figure 1 for abbreviations.

48-h recovery period (Fig. 7B). Thus, the HSF recovered the capacity to perform Δ^6-desaturation after removal of the inhibitor, but the recovery occurred slowly.

DISCUSSION

Obukowicz *et al*. (10) showed that SC-26196 is a potent and selective inhibitor of linoleic acid Δ^6-desaturation in rat liver microsomes and that it inhibits the conversion of linoleic acid to arachidonic acid in the liver when it is administered to mice. The present results extend these findings to human cells and provide additional information about the properties and effectiveness of the inhibitor. They demonstrate that SC-26196 does not inhibit the conversion of dihomo-γ-linolenic acid to arachidonic acid, a reaction mediated byΔ^5-desaturase. This indicates that SC-26196 is a selective inhibitor of Δ^6-desaturation in intact human cells, a finding that is consistent with the results in isolated rat liver microsomes (10). The present data show that SC-26196 also is effective against α-linolenic acid, the n-3 analog of linoleic acid, and that it inhibits the Δ^6-desaturation of the 24-carbon intermediate formed in the conversion of docosapentaenoic acid to DHA (3,4). Therefore, in addition to inhibiting arachidonic acid formation, SC-26196 will decrease the synthesis of EPA and DHA from n-3 PUFA precursors. The fact that SC-26196 is effective in three different kinds of human cells suggests that it most likely has a wide spectrum of action in human tissues.

The studies of Obukowicz *et al*. (10) demonstrated that SC-26196 had a direct inhibitory effect on the Δ^6-desaturase in isolated rat liver microsomes. We obtained IC_{50} values in HSF in the same range as those reported for SC-26196 in the incubations with microsomes, suggesting that a similar inhibitory process occurred in the intact cells. The slow recovery of Δ^6-desaturation after removal of SC-26196 from the culture medium suggests that the inhibitor either binds irreversibly to the enzyme or dissociates very slowly after it binds. Alternatively, the inhibitor may be resistant to inactivation or excretion by HSF and, as a result, remains active in the cells for a prolonged period even though it is no longer available in the extracellular fluid.

The finding that SC-26196 reduced the desaturation of α-linolenic acid to almost the same extent as linoleic acid is consistent with a large body of evidence indicating that the same Δ^6-desaturase acts on both of these 18-carbon PUFA (1,6). On the basis of previous studies with rat liver homogenates, it was concluded that the same enzyme catalyzes the Δ^6-desaturation of the 24-carbon intermediates formed in PUFA metabolism (19,20). In agreement with this conclusion, we observed previously that the desaturation of the 18- and 24-carbon PUFA was reduced to the same extent in mutant HSF deficient in Δ^6-desaturase (13). The present finding that SC-26196 inhibits the Δ^6-desaturation of 24:5n-3 in HSF with an IC_{50} value in the same range as those for linoleic and α-linolenic acids also suggests that the 18- and 24-carbon PUFA substrates are acted on by a single Δ^6-desaturase. Similarly, the observation that the recovery of Δ^6-desaturation after removal of the inhibitor occurred at roughly similar rates when either [1-^{14}C]18:2n-6 or [3-^{14}C]24:5n-3 was used as the tracer provides further support for the conclusion that the same enzyme acts on the 18- and 24-carbon PUFA substrates.

In contrast to these results, data obtained with two human malignant cell lines have been interpreted to indicate that separate Δ^6-desaturases act on the 18- and 24-carbon PUFA (21). Molecular evidence also suggests the possibility that there may be two human Δ^6-desaturases. A third PUFA desaturase gene designated FA desaturase-3 (FADS3) is present in the 1.4-mb region of human chromosome 11q12-q13.1, which contains the Δ^6- and Δ^5-desaturase genes (8). The possibility that FADS3 is a second Δ^6-desaturase gene was discounted initially because the coding sequence has only 60–70% homology with FADS2 (8). However, if the FADS3 gene product is selective for 24-carbon PUFA substrates, this difference might be explained by the considerable difference in size between the 18- and 24-carbon PUFA. Although the present results do not exclude the possibility of separate Δ^6-desaturases

for 18- and 24-carbon PUFA, the fact that the SC-26196 IC_{50} values obtained for the 18- and 24-carbon PUFA are so similar makes it unlikely because the two enzymes would have to interact almost identically with the inhibitor.

The fact that SC-26196 inhibits the synthesis of EPA and DHA, the biologically active members of the n-3 PUFA series, is unlikely to cause any serious problems in mammalian organisms. Although EPA can be converted to eicosanoids that have anti-inflammatory and antithrombotic properties (22,23), very little EPA is normally present in the tissues and it likely is not biologically essential (24). On the other hand, DHA is necessary for normal development and function of the central nervous system (3,25–33). Some DHA is synthesized from n-3 PUFA precursors in the liver (34–37), microvascular endothelium (38), and astrocytes (39,40). However, these sources may not be essential because mammalian cells readily incorporate DHA when it is available in the extracellular fluid (41–43). Similarly, the brain can effectively utilize DHA present in the diet (44–46).

In conclusion, the present results demonstrate that SC-26196 is a potent, selective, and long-acting Δ^6-desaturase inhibitor that is effective against 18- and 24-carbon PUFA substrates in human cells. In addition to suppressing the conversion of linoleic acid to arachidonic acid, SC-26196 inhibits the conversion of n-3 PUFA precursors to EPA and DHA. The similarity in IC_{50} values for these substrates is consistent with data indicating that a single enzyme catalyzes the Δ^6-desaturation of 18- and 24-carbon PUFA (13,20). Furthermore, the fact that this selective Δ^6-desaturase inhibitor considerably reduces the conversion of docosapentaenoic acid to DHA provides additional support for the mechanism proposed by Sprecher and co-workers that the Δ^4-double bond in DHA is formed by the Δ^6-desaturation of a 24-carbon intermediate (1–4).

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