Studying fatty aldehyde metabolism in living cells with pyrene-labeled compounds

Markus A. Keller,* Katrin Watschinger,* Karsten Lange, Georg Golderer,* Gabriele Werner-Felmayer,* Albin Hermetter, Ronald J. A. Wanders,** and Ernst R. Werner*†

Division of Biological Chemistry,* Biocenter, Innsbruck Medical University, 6020 Innsbruck, Austria; Organic Chemistry Department,† Bergische Universität Wuppertal, 42097 Wuppertal, Germany; Institute of Biochemistry,** Graz University of Technology, 8010 Graz, Austria; and Laboratory Genetic Metabolic Diseases,** Academic Medical Center at the University of Amsterdam, 1105 AZ Amsterdam, The Netherlands

Abstract  The lack of fatty aldehyde dehydrogenase function in Sjögren Larsson Syndrome (SLS) patient cells not only impairs the conversion of fatty aldehydes into their corresponding fatty acids but also has an effect on connected pathways. Alteration of the lipid profile in these cells is thought to be responsible for severe symptoms such as ichthyosis, mental retardation, and spasticity. Here we present a novel approach to examine fatty aldehyde metabolism in a time-dependent manner by measuring pyrene-labeled fatty aldehyde, fatty alcohol, fatty acid, and alkylglycerol in the culture medium of living cells using HPLC separation and fluorescence detection. Our results show that in fibroblasts from SLS patients, fatty aldehyde is not accumulating but is converted readily into fatty alcohol. In control cells, in contrast, exclusively the corresponding fatty acid is formed. SLS patient cells did not display a hypersensitivity toward hexadecanal or hexadecanone, but 3-fold lower concentrations of the fatty alcohol than the corresponding fatty aldehyde were needed to induce toxicity in SLS patient and in control cells.—Keller, M. A., K. Watschinger, K. Lange, G. Golderer, G. Werner-Felmayer, A. Hermetter, R. J. A. Wanders, and E. R. Werner. Studying fatty aldehyde metabolism in living cells with pyrene-labeled compounds. J. Lipid Res. 2012. 53: 1410–1416.

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The autosomal, inherited disease Sjögren Larsson Syndrome (SLS; OMIM: 270200) is caused by mutations in the ALDH3A2 gene (1). This gene codes for the enzyme fatty aldehyde dehydrogenase (FALDH; [EC 1.2.1.48]), which catalyzes the irreversible, NAD-dependent conversion of a wide range of different fatty aldehydes into their corresponding fatty acids (2, 3). This enzyme is active as a homodimer (4) and is bound to membranes by its hydrophobic carboxy-terminus (5). More than 70 mutations are known that impair fatty aldehyde dehydrogenase function (6), thereby having a severe impact on the metabolism of lipids in cells (7). Accumulating fatty aldehydes in SLS patients are suspected to cause a wide range of symptoms, such as ichthyosis, mental retardation, and spasticity (8), by chemical interaction with free amino-groups of lipids and proteins (9).

The crucial role of fatty aldehyde dehydrogenase in multiple lipid pathways is not only emphasized by the diversity of symptoms but also by the targeted localization of fatty aldehyde dehydrogenase splice variants to the endoplasmatic reticulum and to peroxisomes, respectively (5), and its role in diabetes induced lipid peroxidation (10, 11). Fatty aldehydes are produced in fatty alcohol metabolism (2). They are also formed in the enzymatic cleavage of ether lipids by alkylglycerol monooxygenase (12) and in leukotriene B4 degradation (13).

The role of fatty aldehyde dehydrogenase in lipid metabolism has been studied by quantification of different lipid species in SLS patient cells by organic phase extraction of cell pellets and analysis with thin-layer chromatography and high-performance thin layer chromatography (7, 9, 14). Another approach was to use radioactively labeled substrates, which are quantified by scintillation spectrometry, subsequent to organic phase extraction, separation by thin layer chromatography, identification of bands of interest, and scraping off selected bands (7, 9, 14).

Here, we present a sensitive method that allowed us to follow the fate of four different fluorescent labeled substrates in the culture medium of living cells. We used this
method to monitor the metabolism of fatty aldehyde and fatty alcohol in SLS patients and in control fibroblasts.

MATERIALS AND METHODS

Materials

Pyrenedecanol was synthesized from pyrenedecanoic acid, and 1-O-pyrenedecyl-ω-glycerol (1-O-pyrenedecyglycerol) was synthesized from pyrenedecanol and glycerol as described elsewhere (15). Pyrenedecanal was purchased from Ramidus AB (Lund, Sweden).

Synthesis of n-hexadecanal

n-Hexadecanal (2.0 g) was dissolved in 30 ml of dry CH₂Cl₂. Then, 1.3 ml dry DMSO was added, and mixture was cooled to 0°C in an ice bath, and 2.35 g P₂O₅ was added. The mixture stirred for 2 h without cooling. After completion of the reaction ( TLC control), the mixture was cooled to 0°C, and 4.25 ml of triethylamine was added dropwise, followed by 1 h of stirring at room temperature. Then, the reaction mixture was mixed with 20 ml of water, and 18% (w/v) HCl solution was added dropwise until pH 2 was obtained. The phases were separated, the organic layer was dried over Na₂SO₄ and filtered, and the solvent was removed under reduced pressure. The crude product was purified by a short column chromatography (silica gel) using a solvent mixture of hexane/ethylacetate 10:1 (v:v). After removing the solvents, a colorless solid was obtained (yield, 1.8 g [92%]; mp, 32°C) (16, 17). Product purity was confirmed by ¹H- and ¹³C-NMR.

Cells

Fibroblasts from three different SLS patients were provided by the Laboratory of Prof. J. A. Ronald Wanders (Genetic Metabolic Diseases, Academic Medical Center at the University of Amsterdam, The Netherlands) (13, 18). The patients displayed the typical symptoms of SLS. Lack of enzymatic activity had been confirmed by measurement of FALDH activity in fibroblasts, and the mutations in the ALDH3A2 gene were identified. Additional information on SLS patient cells can be found in Table 1. Human dermal fibroblasts from three healthy individuals served as controls and were kindly provided by Christine Heufler (Department of Dermatology, Innsbruck Medical University). Informed consent and institutional approval of the studies was obtained at the respective institutions. Chinese hamster ovary K1 cells (CHO-K1) used for transfection experiments were purchased from LG Promochem (Wesel, Germany).

Cell culture conditions

If not stated otherwise, all cells were grown at 37°C, at 100% humidity, and in an atmosphere of 5% CO₂. Normal human fibroblasts and SLS patient fibroblast cells were maintained in 75 cm² flasks in (DMEM containing GlutaMAX I, 1,000 mg/l D-glucose and sodium pyruvate (Gibco, Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) heat-inactivated fetal calf serum (PAN Biotech, Aidenbach, Germany). CHO-K1 cells were grown in F12K medium with 10% (v/v) fetal calf serum and penicillin-streptomycin (Sigma, Vienna, Austria).

Quantification of fluorescent lipid metabolites in the culture medium of living cells

Fibroblasts were plated in 24-well plates at a density of 10⁵ cells per well in 2 ml of medium and allowed to adhere for 24 h. The cells were incubated with one of the following fluorescent compounds: pyrenedecanal, pyrenedecanol, pyrenedecanoic acid, or 1-O-pyrenedecyglycerol. These compounds were added to the medium from a sterile 100-fold stock solution in ethanol:water (1:7; v:v), resulting in a final concentration of 5 µM. After 24 h of incubation, 10 µl of the culture medium was transferred into a 1.5 ml tube, and 30 µl of methanol was added. The mixture was mixed and centrifuged at 13,000 × g for 10 min. A 10-µl sample of the mixture was injected into the HPLC system, and fluorescent compounds were quantified as described below. Peaks were identified by spiking of samples with standard solutions of the fluorescent compounds. The quantification was done by referring to the total fluorescence of the initially applied substrate.

For time course experiments, a 10-µl sample was taken under sterile conditions after 5 min, 30 min, and 1, 4, 10, and 24 h. The samples were analyzed as described above.

Quantification of fluorescent metabolites in cell pellets

After taking the sample for the culture medium assay, the remaining medium was removed by suction. Adherent cells were washed with 1 ml Dulbecco’s PBS buffer (Serva, AL-Laborotechnik, Amstetten, Austria) and then detached with 500 µl trypsin/EDTA (Sigma, Vienna, Austria) for 5 min at 37°C. Subsequently, 500 µl PBS was added, and the cell suspension was transferred into a 1.5 ml tube. Cells were pelleted by centrifugation for 10 min at 5,000 × g, and 30 µl of methanol was added to the pellets followed by shaking at 900 rpm and room temperature for 1 h to lyse the cells and extract the fluorescent labeled lipids. The mixture was then centrifuged at 13,000 × g for 10 min, and 10 µl of the supernatant was injected into the HPLC system.

Because lipids were extracted from cell pellets, quantification was not possible by referring to the initial substrate amount. Instead, data were normalized by the total fluorescence of pyrene-labeled lipids in each sample. Controls showed that there was no carryover of fluorescent metabolites by adherence on plastic ware.

Cytotoxic effects of long-chain fatty aldehydes, alcohols, and acids on SLS and control cells

Cytotoxic effects were studied with a method adapted from James and Zoeller (9). Cells were plated in 96-well plates at a density of 500 cells/well in 200 µl of DMEM. Cells were allowed to adhere for 24 h, and 0, 25, 62.5, 125, 250, 500, 750, and 1250 µM of hexadecanal, hexadecanol, or hexadecanoic acid or 5 µM of pyrene-labeled aldehyde, alcohol, alkylglycerol, and fatty acid were added from a 40× stock solution in ethanol (triplicates for each condition). The percentage of ethanol added to the

| TABLE 1. Sjögren Larsson Syndrome patient fibroblast cells used for this study |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cells | Specific activity* | % of Control | Allele 1 | Allele 1 | Allele 2 | Allele 2 |
| | pmol/min/mg | % | Nucleotide | Translation | Nucleotide | Translation |
| SLS 1 | 4.7 ± 0.62 | 15.5 ± 2.1 | c.487_488insA | p.1163fs | c.487_488insA | p.1163fs |
| SLS 2 | 4.5 ± 0.58 | 14.9 ± 1.9 | c.943C>T | p.315S | c.1297_1298delGA | p.433fs |
| SLS 3 | 3.1 ± 0.32 | 10.4 ± 1.7 | c.178G>T | p.E60X | c.471delG | p.32-157del |

*Data are from Keller et al. (4).
individual wells was 2.5% (v/v), which had no influence on cell viability, as was shown by comparison of ethanol-only treated controls with untreated cells.

Cells were incubated with the compounds for 24 h. The medium was replaced with compound-free DMEM, and cells were allowed to grow out for another 96 h. Cell viability was measured using the MTT assay (Promega, Mannheim, Germany). Ethanol-only treated samples served as reference.

Solubility of hexadecanoic acid in DMEM medium containing 2.5% (v/v) ethanol was limited to concentrations below 750 µM for hexadecanal and hexadecanol to concentrations below 1250 µM as judged by visual inspection of turbidity. Therefore, the analysis was only performed in those concentrations where compounds were soluble (up to 500 µM).

### High-performance liquid chromatography analysis

Fluorescent substrates and their metabolites were quantified on an Agilent 1200 Series HPLC system using a Zorbax XDB-C8 USP-L7 column (Agilent Technologies, Vienna, Austria) as described in (4). Briefly, after treating the sample with a 3-fold excess of methanol, 10 µl of the mixture was injected into the HPLC system and eluted with 10 mM potassium phosphate (pH 6.0) containing 81.25% (v/v) methanol at a flow rate of 1.0 ml/min. After 8 min, a linear gradient to 100% methanol at 8.5 min was applied. From 12.5 to 13 min, the initial mobile phase composition was restored. Fluorescent detection was performed with excitation at 340 nm and emission at 400 nm. The identity of pyrene-labeled compounds was confirmed by spiking with a standard solution of the respective compound. For fatty aldehyde dehydrogenase activity measurements, peaks were quantified via their peak area in comparison to an external 100 nM standard solution of synthetic pyrenedecanoic acid.

### Transfection of CHO-K1 cells

Expression plasmids of candidate genes were obtained from OriGene Technologies Inc. (Rockville, MD; ALDH1A1: SC321535; ALDH2: SC119703; ALDH3A1: SC321516; ALDH3B1: SC119707; ALDH3B2: SC119708). CHO-K1 cells were grown in F12-K medium (Gibco, Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum and penicillin-streptomycin. For transfection, 1412 Journal of Lipid Research

### Fatty aldehyde dehydrogenase activity measurement

For activity measurement, monolayers of transfected CHO-K1 were collected by trypsinization, washed with Dulbecco’s PBS buffer (Serva), and resuspended in distilled water containing 0.5% (w/v) CHAPS (Roth, Lactan, Graz Austria) and 1% (v/v) of a protease inhibitor mixture (GE Healthcare, Vienna, Austria). Cells were opened by rapid freeze thawing.

Fatty aldehyde dehydrogenase activity was measured as previously published (4). Briefly, the assay mix contained 20 mM sodium pyrophosphate (pH 8.0), 1% Triton-X100 (v/v, reduced form, Serva), 50 µM pyrene, and 1 mM NAD (Sigma). The reaction was started by the addition of the reaction mixture to 3 µl of sample, corresponding to 15–25 µg of total protein. Samples were incubated in air-tight tubes at 37°C in a total volume of 10 µl for 10 min. No loss of volume was observed under these conditions. The reaction was terminated by the addition of 30 µl methanol. The amount of pyrenedecanoic acid formed was determined by HPLC with fluorescence detection as described above. To control for variations in the extraction efficiency and for errors in sampling, the total area of all fluorescent peaks was monitored. This parameter was stable. Typical variation coefficients observed were 3.1% within run, 11.9% day to day.

### Statistical analysis

Unless otherwise stated, data are presented as means ± SD. Statistical significance was determined by two-way ANOVA for the quantification of fluorescent labeled metabolites or one-way ANOVA for transfection experiments with Bonferroni as posthoc analysis using GraphPad Prism 5.01 (GraphPad Software, Inc., San Diego, CA).

### RESULTS

HPLC-based quantification of lipid metabolites formed from fluorescent labeled compounds in living cells

Our aim was to study the fatty aldehyde and fatty alcohol metabolism in living cells. Therefore, we established a novel protocol that allows us to follow the fate of different fluorescent labeled compounds in the culture medium of cells. We used the following four pyrene-labeled substrates (Fig. 1): 1-O-pyrenedecylglycerol ([1], peak 1), which is converted into pyrenedecanal ([2], peak 2) by alkylglycerol monoxygenase (12). The formed fatty aldehyde is further

**Fig. 1.** Monitoring of fatty alcohol and aldehyde metabolism with fluorescent labeled substrates. A: 1-O-pyrenedecylglycerol [1] is cleaved by alkylglycerol monoxygenase (AGMO), forming pyrene, and a glycerol derivative. The fatty aldehyde is then converted into pyrenedecanoic acid [3] by fatty aldehyde dehydrogenase (FALDH) or into pyrenedecanol [4] by a fatty alcohol dehydrogenase (ADH). B: Living fibroblasts were incubated with 5 µM of one of the fluorescent substrates [1–4]. For the chromatograms shown here, pyrenedecanal [2] was used. After 24 h, 10 µl of the culture medium were mixed with 30 µl methanol. The mixture was centrifuged, and fluorescent compounds were quantified using HPLC and fluorescence detection. The metabolite composition in normal human fibroblasts is shown in the upper trace. The lower trace depicts the composition in SLS patient fibroblasts. Two representatives of 32 chromatograms are shown.
metabolized into pyrenedecanoic acid ([3], peak 3) by FALDH (4) or into pyrenecanol ([4], peak 4) by a fatty alcohol dehydrogenase (18). Several further unidentified fluorescent peaks appeared in the chromatogram in addition to the four metabolites quantified (Fig. 1B). The four metabolites accounted for a major portion (74.5 ± 10.5% [mean ± SD]; n = 44) of the total area of all fluorescent peaks detected.

SLS patient fibroblasts (SLS cells; Table 1) of three different individuals were incubated with one of the fluorescent compounds at a time. Fibroblasts from three healthy individuals served as controls. Our HPLC method was used to quantify the metabolites that are formed during incubation. Fig. 1B shows typical chromatograms for the incubation of normal human fibroblast (upper trace) and SLS patient fibroblasts (lower trace) with pyrenedecanal. Comparison of the chromatograms shows that there are big differences in the pyrene-labeled metabolite composition of SLS and control cells. However, the original substrate pyrenedecanal (peak 2) is metabolized to a large extent in both SLS and control cells.

The detection limit for pyrenedecanoic acid was 10 fmol. Due to the longer retention times and broadening of peaks, the detection limits of pyrenedecanol, pyrenecanolin, and 1-O-pyrenedecylglycerol were 10 times higher (100 fmol). The 5 µM of fluorescent substrates we used were not cytotoxic to the cells. Viable cell numbers as determined by the MTT assay in the presence of 5 µM pyrene-labeled aldehyde, alcohol, alkylglycerol, and acid in comparison to solvent-only treated cells (mean ± SD; n = 3) were 107.5 ± 17.5%, 106.9 ± 17.5%, 101.6 ± 21.7%, and 97.5 ± 15.4%, respectively, for control fibroblasts. For SLS patient fibroblasts, the corresponding viable cell numbers were 110.9 ± 12.3, 108.3 ± 13.0, 99.6 ± 12.9, and 96.1 ± 7.4, respectively.

**Fluorescent metabolite composition in the culture medium of living fibroblasts**

Fig. 2A shows the metabolite composition in the culture medium of cells after incubation with pyrenedecanal. The control cells and the SLS cells were able to readily metabolize the fatty aldehyde. Under our experimental conditions, more than three quarters of the added pyrenedecanal were metabolized. Control cells converted it almost exclusively into the corresponding fatty acid and formed only marginal amounts of fatty alcohol. In contrast, in SLS cells the most prominent species found was the fatty alcohol. Fatty acid was also formed to some extent by SLS cells, yielding up to about one-third of the levels found in the culture medium of control cells. We detected no accumulation of pyrene-labeled alkylglycerol in SLS or control cells, although fatty alcohols are precursors for ether lipid synthesis. This may be due to conversion of alkylglycerols to ether phospholipids in the cells.

The ratio of fatty acid to fatty alcohol after incubation with pyrenedecanal was a reliable marker for the ability of cells to oxidize fatty aldehydes to the corresponding acid. In the culture medium of SLS patient cells, the ratio was R_{acid/alcohol} = 0.6 ± 0.2 (n = 17; range: 0.2–1.0). For control cells, the measured mean ratio was about 46-fold higher: \( R_{acid/alcohol} = 28 ± 11 \ (n = 17; \ range: 14–50; \ when \ no \ fatty \ alcohol \ was \ detectable, \ the \ detection \ limit \ was \ used \ for \ data \ analysis). \ R_{acid/alcohol} \ Was \ not \ dependent \ on \ incubation \ time, \ as \ was \ shown \ by \ time \ course \ experiments.

The metabolite composition in the culture medium of living cells after the incubation with pyrenedecanol is shown in Fig. 2B. Control cells were able to convert approximately half of the added fatty alcohol into the corresponding fatty acid. SLS cells, in contrast, metabolized less than a quarter of the added fatty alcohol. In addition, less than 15% of pyrenedecanoic acid was formed by SLS as compared with control cells. These differences were highly significant (\( P < 0.001 \)). Interestingly, no accumulation of pyrene-labeled fatty aldehyde was observed in control or in SLS fibroblasts.

The metabolite compositions after incubation with pyrenedecanoic acid and 1-O-pyrenedecylglycerol are shown in Figs. 2C and 2D, respectively. These substances were not metabolized to compounds detectable in our chromatograms in control and in SLS cells. A major part of the added compounds was still present in the culture media after the incubation period.

**Fluorescent metabolite composition in control and SLS cell pellets**

After measuring metabolites in the culture medium, we collected the respective cell pellets, extracted the lipid components, and analyzed them with the same HPLC method (see Material and Methods). The results for these measurements are presented in Fig. 3. Incubation with
readily metabolized into fatty alcohols in SLS cells, we compared the cytotoxic effect of fatty aldehyde, fatty alcohol, and fatty acid. We measured the LD$_{50}$ values of hexadecanal, hexadecanol, and hexadecanoic acid (Table 2). We found no significant difference between the cytotoxic effect of these compounds on control and SLS cells ($P > 0.05$). LD$_{50}$ values for hexadecanol (SLS cells: 45.7 ± 1.5 µM; controls: 38.6 ± 6.6 µM) were about three times lower than the values for hexadecanal (SLS cells: 128 ± 10 µM; controls: 122 ± 15 µM) ($P < 0.001$).

Substrate specificity of fatty aldehyde dehydrogenase

Our results show that SLS cells are to some extent able to convert fatty aldehydes into fatty acids. To check whether aldehyde dehydrogenases other than fatty aldehyde dehydrogenase are able to metabolize pyrenedecanal, we transfected different aldehyde dehydrogenases into CHO-K1 cells to measure their capability to catalyze this reaction. The results are shown in Fig. 5. One-way ANOVA analysis revealed that only fatty aldehyde dehydrogenase shows a significantly higher enzymatic activity than the green fluorescent protein transfected controls ($P < 0.05$). LD$_{50}$ values for hexadecanol (SLS cells: 45.7 ± 1.5 µM; controls: 38.6 ± 6.6 µM) were about three times lower than the values for hexadecanal (SLS cells: 128 ± 10 µM; controls: 122 ± 15 µM) ($P < 0.001$).

**Table 2. Cytotoxicity of hexadecanal, hexadecanol, and hexadecanoic acid toward control and SLS patient fibroblasts**

|                      | SLS Cells     | Control Cells |
|----------------------|---------------|---------------|
| LD$_{50}$ (hexadecanal) | 128 ± 10 µM   | 122 ± 15 µM   |
| LD$_{50}$ (hexadecanol) | 45.7 ± 1.5 µM | 38.6 ± 6.6 µM |
| LD$_{50}$ (hexadecanoic acid) | > 500 µM     | > 500 µM      |
tient cells gave rise to the accumulation of fatty alcohols because of the lack of fatty aldehyde dehydrogenase function in SLS patients. Pyrenedecanal and pyrenedecanol differ strongly. The ability to convert the pyrenedecanal substrate into pyrenedecanoic acid was measured in the cell pellet as described by Keller et al. (4). Values are shown as mean ± SD. n = 6 for FALDH and control; n = 3 for all other transfections.

(51% protein sequence homology), ALDH1A1 (28% protein sequence homology), and ALDH2 (28% protein sequence homology).

DISCUSSION

In previous work we showed that pyrenedecanal can be used to quantify fatty aldehyde dehydrogenase activity in cell and tissue homogenates (4). The method we present here extends the range of application of this compound to monitor fatty aldehyde metabolism in the culture media of living cells. Our approach enables measurement of the accumulation or depletion of metabolites formed from pyrenedecanal, pyrenedecanoic acid, and 1-0-pyrenedecyl glycerol in a time-dependent manner in cells grown in a 24-well culture dish. We demonstrate that the four quantified fluorescent metabolites appear in comparable relative amounts in the cell pellets and in the culture medium. This shows that the transport of the added substrates and the quantified metabolites through the cell membrane is not a limiting factor. Further metabolites, such as esterified compounds (e.g., triglycerides, phospholipids, and cholesterol esters), might be formed from the fluorescent labeled compounds and might not elute from our column at 100% methanol. Balance calculations indicate that we recovered 65% to 85% of the initially added fluorescent label in the chromatograms. Thus, the four metabolites we measure appear to constitute a major portion of the metabolites formed under our experimental conditions.

For our measurements, we used 5 µM of fluorescent substrate, which was not cytotoxic to the cells. Because only small amounts of the culture medium and no cell material are required, this analysis can be easily combined with other assays.

Our experiments demonstrate that the relative amount of fluorescent labeled metabolites measured after incubation of cultivated, living SLS patient and control cells with pyrenedecanal and pyrenedecanoic acid differed strongly. The lack of fatty aldehyde dehydrogenase function in SLS patient cells gave rise to the accumulation of fatty alcohols from fatty aldehydes, whereas in control cells almost exclusively the nontoxic fatty acid was formed. Although control cells were able to convert fatty alcohols into the corresponding fatty acid, they remained unmetabolized by SLS patient cells. These results are in line with previous findings by Rizzo et al. (7, 19), who detected elevated fatty alcohol levels in serum and fibroblasts of SLS patients and decreased ability of SLS patient cells to oxidize long-chain fatty alcohols to acid.

The inability of patient cells to metabolize fatty aldehydes and the formation of Schiff base adducts with lipids and proteins is suspected to be responsible for the symptoms of SLS (9). Surprisingly, we found in our time course experiments that control and SLS cells were able to metabolize fatty aldehydes at the same rate. Despite the sensitive fluorescent detection system, we found no accumulation of fatty aldehydes in all cells at any time point when incubated with fatty alcohol, fatty acid, or alkylglycerol. Although we cannot exclude that the failure to find pyrene-labeled fatty aldehyde when cells were incubated with pyrenedecanol could be due to its unmeasured stable Schiff base formation with phosphatidylethanolamine, more alcohol remains unmetabolized in SLS as compared with control fibroblasts (Fig. 2B). Thus, the deficiency to oxidize the fatty aldehyde to the acid in SLS fibroblasts apparently impairs the metabolism of the fatty alcohol. As is evident from the results of experiments feeding pyrenedecanal to SLS fibroblasts, the equilibrium between fatty aldehyde and fatty alcohol lies on the fatty alcohol side in these cells (Fig. 2A).

In contrast to previous findings in which SLS patient fibroblasts were described to be hypersensitive to fatty aldehydes (9), we could not detect significantly different LD₅₀ values for hexadecanal, hexadecanoic acid, or hexadecanoic acid in SLS patient and control cells. Cells were tolerant toward the fatty acid up to the highest soluble concentrations, and a LD₅₀ value was not assessable. Interestingly, in our setting fatty alcohols were more toxic than fatty aldehydes in SLS and in control cells, as is reflected by the 3 times lower LD₅₀ value for hexadecanol. Our data suggest that particular attention should be paid to the biochemical action and toxicity of fatty alcohols when interpreting SLS symptoms.

In addition to the accumulation of fatty alcohols in SLS patient fibroblasts, we observed the simultaneous formation of fatty acids. To determine to what extent other aldehyde dehydrogenases are responsible for the residual fatty aldehyde dehydrogenase activity, we transfected FALDH, ALDH1A1, ALDH2, ALDH3A1, ALDH3B1, and ALDH3B2 into CHO-K1 cells. FALDH was found to be the only aldehyde dehydrogenase able to significantly increase the conversion of pyrenedecanal to pyrenedecanoic acid when compared with controls. However, there was a clear trend that transfected ALDH3A1 was able to partially take over the chemical action and toxicity of fatty aldehydes when cells were incubated with pyrenedecanol could be due to its unmeasured stable Schiff base formation with phosphatidylethanolamine, more alcohol remains unmetabolized in SLS as compared with control fibroblasts (Fig. 2B). Thus, the deficiency to oxidize the fatty aldehyde to the acid in SLS fibroblasts apparently impairs the metabolism of the fatty alcohol. As is evident from the results of experiments feeding pyrenedecanal to SLS fibroblasts, the equilibrium between fatty aldehyde and fatty alcohol lies on the fatty alcohol side in these cells (Fig. 2A).

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of ALDH3A1, ALDH3B1, and ALDH1A1 were 5-, 10-, and 20-fold lower. This suggests that, in SLS patient fibroblasts, ALDH3A1 could only partially substitute for the fatty aldehyde dehydrogenase function. The ability of ALDH3A1 to metabolize fatty aldehydes may play a more prominent role in other SLS patient tissues with high ALDH3A1 expression levels. ALDH3A1 has been mainly studied in cornea (21) but also shows high expression in lung, esophagus, and stomach (22). Further investigations are needed to gain a complete understanding of the interplay between different aldehyde dehydrogenase enzymes.

Because in many tissues fatty aldehydes are produced by cleavage of allylglycerols (15), we were also interested in the ability of fibroblasts to degrade this lipid species. With the method we describe here, we monitored allylglycerol metabolism in living SLS patient and control cells. We detected only marginal amounts of fatty acid and no fatty aldehyde after incubation with 1-O-pyreneacyl glycerol. The only enzyme known to be able to cleave the ether bond of allylglycerols is allylglycerol monooxygenase. However, we were able to measure allylglycerol monooxygenase activities in other cell lines, such as RAW 264.7 (data not shown), in which the enzyme is expressed (12). Also, Rizzo et al. (14), by the use of radioactive labeled allylglycerols, described the formation of only minute levels of fatty acids (1.4%) in normal human fibroblasts. Altogether, our data suggest that allylglycerols may play only a minor role as a fatty aldehyde source in fibroblasts.

We present an efficient approach to monitor fatty aldehyde dehydrogenase activity in intact cultured cells by analyzing 10 µl of the cell culture medium. The pyrene label is an attractive alternative to radiolabeling of lipids because it is widely accepted by enzymes metabolizing long aliphatic side chains (23) and can be detected with high sensitivity due to its intense fluorescence. As with other labeling techniques, however, our method presented here gives no information on endogenous levels of the respective lipids.

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