Lipoperoxides in Sebum of Substance Users and Controls*

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ABSTRACT

Sebum was collected from forehead skin in five compulsive heroin and/or cocaine (substance) users and in five controls over three consecutive periods, each lasting three hours. The participants were adult black and white men similar in age and smoking habits, who did not consume alcohol. Lipoperoxides were determined in sebum as malondialdehyde by high performance liquid chromatography. Two participants were excluded in the control group: in both, urinary lipoperoxides were elevated; in one, urine tested positive for Δ-9 tetrahydrocannabinol (THC). All other participants had negative urine drug screening tests. Relative to the controls, all substance users had elevated concentrations of lipoperoxides in urine. Compared to the controls, the rate of sebum excretion in the last collecting period was higher in substance users, but sebum had significantly lower lipoperoxide concentration. It is assumed that compulsive drug use may influence lipoperoxidation of incipient sebum, possibly by altered tissue perfusion.

Introduction

Lipid peroxidation is an oxidative deterioration of unsaturated fatty acids that can involve enzymatic and nonenzymatic mechanisms. In the autocatalytic, free-radical mediated process generated by activated oxygen, unsaturated fatty acids form lipid peroxides.18,10 These subsequently decompose to a variety of products such as low molecular mass hydrocarbons, ketones, alkenals, alkanals, fatty acids, and hydroxy-aldehydes, including malondialdehyde (MDA). Malondi-
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Aldehyde may be detected by high performance liquid chromatography (HPLC) after the reaction of MDA with thio-barbituric acid.\textsuperscript{8,11} Products of MDA have been studied quite extensively in both animals and humans in different conditions.\textsuperscript{1,5,9,12}

Recently, certain illicit drugs have been implicated in enhanced production of lipoperoxides. Cocaine, administered to rat brain mitochondria, directly induced oxygen-derived free radical generation of lipoperoxides,\textsuperscript{4} whereas the degree of lipid peroxidation in mice liver microsomes occurred in proportion to the extent of cocaine oxidative metabolism.\textsuperscript{20}

In contrast, morphine did not promote lipoperoxidation in rat brain mitochondria. Heroin was not evaluated in the same regard as morphine.\textsuperscript{4} Elevated levels of lipoperoxides in urine have been described in otherwise healthy persons who tested positive for cocaine, marijuana, and opiates.\textsuperscript{13}

Several studies have assessed lipid peroxidation in human skin where squalene appears to be the main substrate for the production of MDA.\textsuperscript{9,16,17,19}

In skin, active oxygen can be generated by exposure to ultraviolet light, by microsomal oxidation of endogenous substrates, or by autooxidation of xenobiotics. The subsequently formed squalene peroxide radical decays also to MDA.\textsuperscript{16,17,19} To the best of our knowledge, the relation between the rate of sebum excretion and its lipoperoxide concentration in humans has not yet been evaluated.

In this study, the rate of sebum excretion and MDA in skin lipids of adult compulsive heroin and cocaine (substance) users were compared with those in controls.

Materials and Methods

PARTICIPANTS

After the project was approved by the Institutional Research Board at Veterans Administration Medical Center, Brentwood Research and Development Service, Los Angeles, CA, informed consent was obtained from all adult participants. Five male substance users (four black and one white), initiating a rehabilitation treatment for compulsive use of heroin and/or cocaine, volunteered to participate in the study. Five men (two black and three white) with a negative history of drug use, from two participating institutions volunteered as controls. Participants in both groups were similar in age, smoking habits, and did not consume alcohol. None of the participants had acne or other skin disease at the time of investigation.

SAMPLING

Sebum was collected onto cigarette papers of the same batch.\textsuperscript{*} The gum stripes and the glue at the edges of the papers were removed. Papers were handled with clean forceps, gloved hands, and stored until needed in 60 mL Wheaton wide mouth "400" borosilicate glass bottles with flat-bed and ground-glass hood stoppers.\textsuperscript{†} Sebum collections followed the protocol of Cunliffe and Shuster\textsuperscript{3} and were performed between 7 A.M. and 5 P.M. on two sites of the forehead. The skin was precleaned with isopropyl alcohol, and the samples of the initial three 10 minute collections were discarded. Sebum was then collected in three consecutive three hour intervals.

Sebum quantities were determined by the direct gravimetric technique and served to calculate the rate of sebum excretion as described by Lookingbill and Cunliffe.\textsuperscript{15} The weighing room had a relatively constant temperature (21 to 23°C) and humidity (under 40 percent). Papers were weighed on a Mettler bal-

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\* Top brand, Reynolds Co., Chicago, IL 60640.
\† VWR Scientific (216134), Cerritos, CA 90702.
ance H 54,‡ which reads to 0.01 mg, and has a weighing range of 160 g ± 0.05 mg. Weight allowance change was within 0.05 mg. The rate of sebum excretion was expressed as µg per sq cm per min.³,¹⁵

For safety reasons, HPLC grade ethyl acetate§ was used instead of ether to extract sebum from cigarette papers. Pre-trial Sudan III staining of the papers showed good extraction of lipids with this solvent. The extraction was performed in Pyrex inoculum standardization test tubes (16 x 100 mm) with teflon-lined screw caps.¹¹

After weighing, cigarette papers from both collecting sites were placed into test tubes and vortexed for three minutes in 8 mL ethyl acetate. Dripped-off papers were inspected against light for lipid residues; if found, the extraction was repeated. The solvent was evaporated under a hood in a heat-block at 34°C and under a pure nitrogen stream. Residues were redissolved in 400 µL ethyl acetate, vortexed, the samples halved, and the solvent evaporated. These samples were coded and stored at −20°C until needed for analysis. Blank samples, to which the laboratory was blinded, included empty test tubes, evaporated solvent, and residues from extracted clean cigarette papers.

Urine samples were obtained on the day of sebum collection for both the drug screening test and the determination of lipoperoxide levels. Urine was voided into assigned 60 mL wide mouth Wheaton glass bottles and was not treated prior to the MDA-TBA reaction. No attempt was made to prevent tampering of the samples. For lipoperoxide determination, 10 mL of urine was pipetted into the test tubes; the remainder served for the drug screening test. Samples were coded and stored at −20°C until needed for analysis. In two controls, a second determination of MDA in urine was done two weeks later.

**ANALYSES**

Urine samples were screened for drugs by the standard fluorescent polarization immunoassay.¶ Urine creatinine (Cr) concentrations (mg per dL) were determined with an Astra chemistry analyzer.**

Lipoperoxides in sebum and in urine were determined as the malondialdehyde-thiobarbituric acid adduct (MDA-TBA) by HPLC,†† using a modified plasma method.¹⁴ The MDA-TBA were determined in 600 µL of urine, in sebum samples, in 0.36, 1.45, and 2.42 nmol per 0.6 mL of 1,1,3,3-tetraethoxypropane (TEP)‡‡ standards, and in the blank samples. To each of the samples 3.0 mL of (one percent) phosphoric acid solution (grade 85.9 percent)§ and one mL of (0.6 percent) TBA‡‡ were added.

To both sebum and blank samples 1.0 mL of distilled water was added; 400 µL of distilled water was added to the remaining samples. Capped test tubes were vortexed for 20 s, and placed in a boiling water bath for 60 min. The samples were then stored at 3°C until analysis by HPLC. Because MDA-TBA adduct is unstable at neutral or alkaline pH, the boiled samples were neutralized individually within 30 minutes before injection onto the HPLC column. From boiled samples, 0.5 mL was pipetted into 1.5 mL

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¶ Abbott Diagnostics Inc., Abbott Park, IL 60064.
** Beckman Instruments, Inc., Fullerton, CA 92634.
†† Waters Assoc. (WISP Model 710B autoinjector, 50 µL injection/6 min) Milford, MA 01757.
‡‡ Sigma Chemical Co., St. Louis, MO 63178.
¹ Mettler Instruments Co., Hightstown, NJ 08520.
polypropylene microtubes§§ that contained 0.5 mL of fresh methanol\textsuperscript{11} \textsuperscript{14} NaOH§ solution (9 mL of one mol per L NaOH pipetted into a 50 mL volumetric flask with methanol q.s. to the mark).

Capped microtubes were vortexed and centrifuged at 9500 \times g for 90 seconds to sediment any precipitated proteins. The concentration of MDA was determined by injecting 50 \mu L of each neutralized, centrifuged sample into the adjusted and equilibrated HPLC. The apparatus was adjusted to the following settings: (a) solvent rate 2.0 mL per min; (b) spectrophotometer wave length = 532 nm, range (AUFS) = 0.05, time constant = 1.0, coarse zero = 0; and (c) recorder chart speed = one cm per min, and absorbance recording time of each effluent = six min. The MDA-TBA adduct has a retention time of about 4.2 min. Concentration of MDA of a sample was computed from the peak height of the MDA-TBA adduct by reference to the calibration curve prepared from both the TEP standards, where TEP under hydrolysis liberates stoichiometric amounts of TBA, and the blank (distilled water) sample. The results of urine samples were corrected to a volume of 1.0 mL, and the lipoperoxide concentration was expressed as nmol MDA per mg Cr.\textsuperscript{13,14} For skin lipids, lipoperoxide concentration was expressed as nmol MDA/mg sebum.

Statistical computations: the mean, 1SD, one-way ANOVA (F-test), Student’s two-tailed \textit{t} test, and Pearson determination momentum were performed, for orientational purpose, on Stratgraphics software V 4.0.¶¶

Results

The participants in both groups were adult men, similar in age and smoking habits, who did not consume alcohol. All substance users were recent compulsive users of heroin and/or cocaine. Participants in the control group had a negative history for drug abuse, but two in this group were excluded because of test results indicative of drug use (table I).

Urine

Results of the fluorescent polarization immunoassay drug screen were negative in all substance users and in all but one control where the result was positive for \textit{\&}-9 tetrahydrocannabinol and was not evaluated. Urinary lipoperoxides were elevated in all substance users, ranging from 2.34 to 3.90 nmol MDA per mg Cr.\textsuperscript{13} Concentrations of MDA in three controls were in the normal reference range (0.89 ± 0.35 nmol MDA per mg Cr) for adults.\textsuperscript{14} Malondialdehyde was repeatedly elevated in two controls, but only in one was the urine positive for tetrahydrocannabinol. Results of these two participants were not evaluated.

Sebum

The amounts of sebum collected on the left and right forehead sites in both the

\begin{table}[h]
\centering
\caption{General Characteristics of Participants}
\begin{tabular}{llll}
\hline
 & Substance & Users & Controls \\
\hline
Age: year (mean ± 1 SD) & 36 (3) & 40 (2) \\
Race: Black & 4 & 2\textsuperscript{a} \\
 & White & 1 & 3\textsuperscript{b} \\
No. participants using: &  &  &  \\
Heroin & 3 & 0 \\
Cocaine & 5 & 0 \\
Marijuana & 5 & 1\textsuperscript{c} \\
Cigarettes (1 pack/day) & 5 & 3 \\
Alcohol & 0 & 0 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a,b} Elevated urine MDA in one participant each; results not evaluated.
\textsuperscript{c} Tetrahydrocannabinol detected in urine; results not evaluated.

\textsuperscript{§} J. T. Baker Chemical Co., Phillipsburg, NJ 07840.
\textsuperscript{§§} Eppendorf, FR Germany.
\textsuperscript{13,14} Mallinkrodt Spec. Chem., Paris, KY 40361.
\textsuperscript{¶¶} STSC Inc., Rockville, MD 20852.
substance users and in the controls had a good correlation throughout the study (r = 0.93; p = 0.05). The rate of sebum excretion was the highest in both groups during the first three hours and lower thereafter. The rate of sebum excretion in substance users remained stable in the second and third collection whereas it decreased to very low levels in the control group (table II).

**LIPOPEROXIDES IN SEBUM**

The residues from extracted clean cigarette papers contained traces of MDA that were subtracted from the results of sebum samples. The remaining blank samples were negative for lipoperoxides. As presented in table II, the substance users had significantly lower MDA concentrations in the third collecting period (0.62 ± 0.2 nmol MDA per mg sebum) as compared to the control group (1.6 ± 0.7 nmol MDA per mg sebum [t = 3.1, P < 0.05]).

**Discussion**

The intent of this study was to compare the rate of sebum excretion and the lipoperoxide concentrations in skin lipids of substance users with those in controls. Nonenzymatic oxidative lipoperoxidation of unsaturated fatty acids, including squalene, can be assessed by measuring malondialdehyde concentrations, a product of peroxidation. Based on published data on MDA in other biological samples, increased lipoperoxide concentrations were anticipated in sebum of substance users.

Sebocytes are unique in their differentiation and formation of their end-product, sebum. Their contact with the basement membrane, and hence the blood supply, is limited to the first few days of differentiation. At this time, intracellular sebum production is low. During the subsequent centripetal migration of sebocytes toward the pilosebaceous duct, the cell lipid components are progressively converted to squalene, wax esters, and triacylglycerols with unusual fatty acids. After cell rupture (holocrine excretion), sebum flows toward the skin surface. The average time between the synthesis of sebum and its secretion onto the skin surface is eight days. During the transit of sebum to the skin surface, the epidermis serves as a reservoir and influences its composition. Sustained continuous sampling on skin surface may deplete this reservoir.

In our study, the rate of sebum excretion was determined by the gravimetric method from the amounts of skin lipids collected on the cigarette papers. The correlation between the rates of sebum excretion on both sites of the forehead was good in both groups throughout the study, indicating a close control of the possible confounding variables and ade-

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Rates of Sebum Excretion and Lipoperoxide Concentrations in Substance Users and in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance Users (n = 5)</td>
<td>Controls (n = 3)</td>
</tr>
<tr>
<td>3 hours</td>
<td>6 hours</td>
</tr>
<tr>
<td>RSE (μg/sq.cm/min)</td>
<td>1.6 ± 0.9</td>
</tr>
<tr>
<td>MDA (nmol/mg sebum)</td>
<td>1.7 ± 0.4</td>
</tr>
</tbody>
</table>

RSE = rates of sebum excretion

MDA = lipoperoxide concentrations
quate adherence to the protocol. In the control group, more sebum was collected during the first three hour period than in subsequent periods. Throughout the study, the rates of sebum excretion in this group were in accordance with published data of earlier workers who used the same method. In both our groups, the rates of sebum excretion in the first and in the second period were similar. In the control group, the rate of sebum excretion declined appreciably at the end of the nine hour period whereas it remained stable in substance users. Similarly, in both groups concentrations of MDA in sebum were comparable during the first two periods. However, at the end of the third period, MDA remained unchanged in the control group, but declined to very low values in substance users.

Recently, low concentration of MDA was detected in ischemic myocardial tissue that surged after myocardial reperfusion. Likewise, lipoperoxidation appears to proceed to a limited extent under anaerobic conditions in the skin of otherwise normal individuals. Our results on a limited number of substance users led us to speculate that their much lower concentration of MDA in sebum of the last collecting period may have been a consequence of drug induced alterations in tissue perfusion. Heroin and cocaine reduce tissue perfusion by venu- dilatation and vasoconstriction, respectively. Compulsive drug use may have perpetuated these effects and decreased the peroxidation of the nascent sebum. Since both the concentrations of MDA and the rates of sebum excretion in the first two periods were comparable in both groups, differences in the epidermic reservoir or in sebum composition are less likely to have accounted for the low MDA found in the third period in substance users.

Lipoperoxides have previously been found increased in urine of substance users. In our two groups, urinary lipoperoxides were elevated in all substance users and in two participants of the control group; in one urine was positive for THC, and reticent drug use was assumed in the other participant. Lipoperoxides in urine then may be used ancillary to the standard drug screening tests in otherwise healthy individuals. In conclusion, our results indicate that substance users may have a more sustained rate of sebum excretion. Contrary to our expectations, lipoperoxidation of the incipient sebum appeared reduced in substance users, possibly because of altered perfusion caused by recent compulsive drug use.

Statement

*This study was approved by the Institutional Research Board at Veterans Administration Medical Center, Brentwood Research and Development Service, Wilshire and Sawtelle Blvds., Los Angeles, CA 90073, Project No.: 007; 11/30/90. The study was conducted according to the Declaration of Helsinki and Tokyo on human experimentation.

References


