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A study on photodegradation of methadone, EDDP, and other drugs of abuse in hair exposed to controlled UVB radiation

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The drug content of hair may be affected by washing, chemical or thermal treatments, the use of cosmetics, or exposure to the environment. Knowledge concerning the effect of natural or artificial light on drug content in hair can be helpful to the forensic toxicologist, in particular when investigating drug concentrations above or below pre-determined cut-offs.

The photodegradation of methadone and its metabolite, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) was studied in authentic positive hair samples by comparing drug concentrations determined by liquid chromatrography-high resolution mass spectrometry before and after exposure to UVB light (*in vivo* study). The same approach was applied in order to investigate the light sensitivity of opiates (6-monoacetylmorphine and morphine) and cocainics (cocaine and benzoylecgonine) in true positive hair. The yields of photodegradation were calculated for each drug class in eight different positive hair samples irradiated by UVB at 300 J/cm^2 obtaining averages, ranges and standard deviations. In parallel, the photostability of all the compounds as 10^{-5}–10^{-4} M standard solutions in methanol were examined by means of UVB light irradiation in the range 0–100 J/cm^2 followed by UV/Vis spectroscopic analysis and direct infusion electrospray ionization-high resolution mass spectrometry (*in vitro* study). In hair, methadone was shown to be significantly affected by light (photodegradation of 55% on average), while its metabolite EDDP proved to be more photostable (17%). 6-monoacetylmorphine, morphine, benzoylecgonine, and cocaine were more photostable than methadone *in vivo* (on average, 21%, 17%, 20%, and 11% of degradation, respectively). When irradiated in standard solutions, the target molecules exhibited a larger photodegradation than *in vivo* with the exception of cocaine (photodegradation for methadone up to 70%, 6-monoacetylmorphine and morphine up to 90%, benzoylecgonine up to 67%, cocaine up to 15%). Some factors possibly affecting the yields of photodegradation in hair and partially explaining the differences observed between the *in vivo* and the *in vitro* studies were also investigated, such as the colour of hair (the role of melanin) and the integrity of the keratin matrix.

Additional supporting information may be found in the online version of this article at the publisher's web site.

Keywords: methadone; EDDP; drugs of abuse; hair; light exposure; UVB; photodegradation

Introduction

Hair analysis is a valuable tool in clinical and forensic toxicology when cases of chronic intoxication, use, abuse, or single dose consumption need to be diagnosed in the context of facilitated crimes, withdrawal controls, doping controls, or workplace drug testing.[1,2] The xenobiotic concentrations in hair, however, may be affected by washing, chemical or thermal treatments, the use of cosmetics, or exposure to the environment.[1,3–5]

Among physical factors that affect the concentrations, solar light is unquestionably able to induce changes in molecular structures when irradiated in solution; this decreases the concentration of the original drugs and/or produces new compounds, which sometimes have the same structure as natural metabolites.[6]

Scalp hair is exposed to sunlight and/or artificial light for many hours per day; hence, the action of light on hair could alter the content of drugs/illicit drugs and/or metabolites. Photomodification of xenobiotics incorporated in the keratin may derive from the direct action of light on the molecules or it may be mediated by intermediate radicals and/or reactive oxygen species produced by the drug itself or formed by eumelanin and pheomelanin present in the hair, under the effect of irradiation.

A couple of studies on the sunlight sensitivity of drugs of abuse in hair samples have been published by Skopp *et al.*[7,8] in which tetrahydrocannabinol (THC), cocaine, benzoylecgonine (BZE), 6-monoacetylmorphine (6-MAM), morphine, and dihydrocodeine showed different behaviours. However, to the best of our knowledge, no data have been published on the effect of light on methadone and its metabolite 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in the keratin matrix.

Methadone (RS-dimethylamino-4,4-diphenyl-3 heptanone) is a long-acting synthetic opioid used for the treatment of opiate addiction as it acts as an agonist on the same μ receptors as opioids despite having a different chemical formula. Methadone metabolism

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in humans occurs primarily by N-demethylation to the pharmacologically inactive EDDP, catalyzed predominantly by hepatic cytochrome – P450 enzymes, in particular by CYP2B6, with some urinary excretion of the unchanged drug.[9] It has been shown that high doses of methadone (> 100 mg/day) allow for better control of the consumption of illicit opiates by treated patients.[10] Indeed, methadone is also the recommended pharmacotherapy for pregnant women who are opioid-dependent,[11,12] thus methadone and its main metabolite, EDDP, are markers of methadone exposure in pregnant women's hair.[13–15]

For these reasons, general knowledge on the stability and photostability of the particular target analytes in the biological matrix under investigation must be taken into account for reliable interpretation of the analytical results.

In the present paper, levels of methadone and EDDP were determined by means of liquid chromatography-high resolution mass spectrometry (HPLC-HRMS)[16,17] in authentic hair samples from drugs users, before and after irradiation under UVB light at 300 J/cm^2.

Results were obtained as range and average of relative photodegradation yield, and were compared with those obtained for 6-MAM and cocaine and their respective metabolites, morphine and BZE, in positive hair samples, analogously irradiated with UVB light at 300 J/cm^2.

The composition of sunlight at ground level, per square metre with the sun at its height, is about 527 watts of infrared radiation, 445 watts of visible light, and 32 watts of ultraviolet radiation.[18] Based on wavelength, the UV spectrum is commonly divided into three regions: UVA (400–320 nm), UVB (320–290 nm), and UVC (290–200 nm). The UVC is filtered by the ozone layer, whereas UVA and UVB reach the surface of the Earth. UVA represents more than 90% of the solar UV radiation that reaches the surface of the Earth.[19] However, short-wave UVB photons are far more energetic than long-wave UVA photons. It is also noteworthy that UVB is largely restricted to midday and, in temperate climates, to spring and summer. In contrast, UVA, although most abundant at times of peak UVB irradiance, is present in sunlight all day throughout the whole year.

However, the choice of UVB light for irradiating hair samples lies in the observation that all the compounds under study absorb UVB light when irradiated in solution, and they all undergo photodegradation in those conditions; only cocaine demonstrated a rather small absorption at UVA frequencies. Visible light was neither absorbed nor did it induce modifications in the target compounds.

Although the photodegradation of a molecule not only depends strongly on the wavelength used, but also on the state (solid, liquid), and the environment in which it is irradiated (solvent, polarity, pH, presence of salts, presence of oxygen, presence of other components in the sample), we tested the analytes in methanol solution to gather general information on their behaviour in the presence of light. Pure methanol solutions of all the target analytes were irradiated with UVB light in the range 0–100 J/cm^2 in order to study their kinetics of photodegradation and, by comparison with the *in vivo* obtained results, to understand the effect, if any, of the keratin matrix and melanin on the photodegradation yields in hair.

Experiments

Chemicals

Methadone, EDDP, 6-monoacetlmorphine, morphine, cocaine, and benzoylecgonine were purchased from LGC Promochem Cerilliant (Teddington, Middlesex, UK) as pure solutions in methanol at 1.0 mg/mL. The internal standards (IS) benzoylecgonine-D3, methadone-D3, and morphine D-3 were methanol solutions also from LGC Promochem at 1.0 mg/mL.

Methanol, CH_2Cl_2, acetone and acetonitrile (Merck, Darmstadt, Germany) were high performance liquid chromatography (HPLC) grade. Ammonium acetate, of analytical grade, was from Merck also. Trifluoroacetic acid (TFA) was from Sigma (Sigma-Aldrich, Milan, Italy). HPLC water was prepared using a Milli-Q Plus (Millipore, Molsheim, France) system. All other reagents were from Sigma-Aldrich (St Louis, MO, USA).

Hair samples

Authentic positive hair was selected from samples routinely tested at the laboratory that had previously tested positive for one or more of the following drugs: methadone, opiates, and/or cocaine. Hair samples were collected with scissors from the posterior vertex, and cut as close to the scalp as possible, wrapped in aluminium foil, and kept at room temperature until analysis.

Irradiation procedure

For UVB irradiation, Philips PL-S 9W/12 lamps, mainly emitting at 312 nm, were used. The total energy impacting the sample was monitored using a radiometer (Mod. 97503, Cole-Parmer Instrument Company, Niles, IL, USA), equipped with a 312-CX sensor. The intensity of the UVB radiation was 0.50 J/cm^2/min. The samples were maintained at room temperature during irradiation.

Photolysis experiments in solution (*in vitro* study)

Solutions of the compounds at concentrations ranging from 10^{-5} to 10^{-4} M in methanol were irradiated in 1 cm quartz cuvettes at controlled temperature (25 °C) with increasing UVB doses, up to 100 J/cm^2.

Photolysis was evaluated by UV spectrophotometry (UV-Vis Varian Cary 50 Spectrophotometer) analyzing the change in the original spectrum upon irradiation, as already described,[20] and by high resolution mass spectrometry (HRMS). At selected UV doses, the solutions were diluted to 10^{-6} M in methanol and analyzed by direct injection HRMS, to measure the photodegradation of the analytes by recording the decrease of the ion signal of protonated molecules obtained by electrospray ionization (ESI) of solutions kept in the dark. The results are the mean of at least three experiments.

Photolysis experiments in hair samples (*in vivo* study)

Hairs, 5–7 cm long, were divided into two approximately identical strands: one was kept in the dark and the other was put between two optical glasses 50 × 50 × 1.15 mm and exposed to 300 J/cm^2 of UVB radiation while kept at room temperature. The intensity of the UVB light was 0.16 J/cm^2/min, lower than *in vitro* experiments, due to the fact that the hair samples were placed between two optical glasses that partially shielded the incoming wavelengths. The hair samples were irradiated for 32 h, corresponding to approximately 300 J/cm^2. The glasses used were of a type able to cut wavelengths below 300 nm, thereby omitting other wavelengths that were emitted by the lamp but not present in the incoming irradiation of the sun spectrum, and which could interfere with and/or alter the results.

Hair sample preparation and extraction

Hair samples were decontaminated with 3 mL of CH_2Cl_2 and acetone. The micropulverized method proposed by Favretto *et al.*[16,17] was applied on 10 ± 0.3 mg hair. Briefly, whole strands of hair were placed in a vial with one stainless-steel bullet, 20 μL of acetonitrile, 20 μL of TFA 1 M, 15 μL of IS working solution in methanol, and 145 μL of water, then shaken by an automatic pulverizer MM2000 (Retsch, Haan, Germany) at 30 Hz amplitude for 10 min. After centrifugation and filtration, 10 μL of the clear extracts were directly injected into the HPLC-HRMS.

HRMS

All measurements were performed on an LTQ-Orbitrap (Thermo Fisher Scientific, Bremen, Germany) high accuracy, high resolution mass spectrometer operating in positive ESI mode and equipped with a Surveyor MS Pump.

ESI-HRMS

For the analysis of pure standard solutions, direct injection analysis was performed with a syringe pump delivering solutions at 10 μL/min directly into the ESI source; the positive ion ESI parameters were as follows: capillary voltage 10 V, sheath gas flow rate 20 (arbitrary units, a.u.), auxiliary gas (N_2) flow rate 5 (a.u.), sweep gas flow rate 5 (a.u.), and capillary temperature 275 °C; profile full scan mass spectra were acquired in the Orbitrap in the *m/z* range 120–700 with a target mass resolution of 100.000 (FWHM as defined at *m/z* 400) and a scan time of 0.65 s.

HPLC-HRMS

For the determination of drug concentration in hair, 10 μL of the hair extracts were injected in an Atlantis T3 (150 × 1.0 mm, 3 μm) column (Waters Corporation, Milford MA, USA). HPLC separation was achieved by gradient elution at a constant flow rate of 300 μL/min. HPLC conditions: A (water, 0.1% formic acid HCOOH) and B (methanol, 0.1% HCOOH); initial conditions 10% B, linear gradient to 25% B in 4 min, 25% B hold from 4th to 7th min, then ramped to 40% B in 5 min, to 60% B in 4 min and to 90% B in 2 min; 90% B hold from 18th to 26th min. The column temperature was 40 °C. MS conditions were: positive ion ESI; capillary voltage 10 V, sheath gas flow rate 50 (arbitrary units, a.u.), auxiliary gas (N2) flow rate 5 (a.u.), sweep gas flow rate 5 (a.u.), and capillary temperature 275 °C; profile full scan mass spectra were acquired in the Orbitrap in the *m/z* range 120–700 with a target mass resolution of 60.000 (FWHM as defined at *m/z* 400) and a scan time of 0.45 s. Detection of the analytes and the IS was based on retention time, accurate mass measurements of MH^+ ions, and correspondence of the observed isotopic pattern to the calculated one.

Drug concentrations were determined from peak area ratios of analyte to its IS compared to calibrator curves of peak area ratios to concentrations. The method is routinely used in the laboratory and was fully validated exhibiting a linear range from 0.1 to 50 ng/mg (determined from regression with $1/x^2$ weighting utilizing six calibration points), lower limits of quantification of 0.1 ng/mg and limits of detection of 0.05 ng/mg for all the target analytes, intra-day imprecision and inaccuracy always lower than 18% and 20% and inter-day imprecision and inaccuracy always lower than 20% and 23%, respectively.

Results and discussion

Photodegradation in methanol solutions exposed to UVB light

In Supplementary Figures S1A–S1F, the UV absorption spectra of all the analytes irradiated at increasing UVB doses up to 100 J/cm^2 are displayed. As can be observed in Supplementary Figure S1A, methadone is characterized by a band at around 220 nm with a small shoulder between 290 and 320 nm. By increasing UVB irradiation, a small decrease in spectral intensity was detected at 220 nm, followed by the appearance of a new absorption band with a maximum at 250 nm, thus indicating that some changes in the molecule take place. Spectral changes can also be seen for EDDP under the same irradiation, with the appearance of a new band between 300 and 350 nm, as evidenced in supplementary Figure S1B. Under increasing irradiation 6-MAM gradually loses the absorption band between 270 and 300 nm, and, at the highest doses, an isosbestic point is observed. This indicates the formation of one or more species that are present in the solution together with the intact compound; the concentration of these species rises with increasing UVB doses (supplementary Figure S1C). Regarding morphine, the same photomodification as 6-MAM is observed, with the loss of the absorption band at 270–300 nm; during irradiation, depending on the light dose, new compounds are reasonably formed. Moreover, morphine shows a blue shift between 210 and 250 nm during irradiation, indicating changes in its structure (supplementary Figure S1D). For cocaine, its spectrum shows the typical UV absorption of benzoic acid, with bands at 272 and 282 nm. Cocaine proved to be quite photostable and, at the highest UVB doses (100 J/cm^2), rather low spectral changes can be observed (supplementary Figure S1E). BZE behaves similarly to cocaine, but its spectral modifications are slightly more evident (supplementary Figure S1F).

Yield of photodegradation in methanol solutions

The photodegradation of target analytes in methanol was measured by direct flow ESI-HRMS analysis: pure standard solutions at 10^{-6} M were kept in the dark and were then analyzed by full scan HRMS, and the absolute abundance of the protonated molecules was considered as 'zero degradation'. Solutions exposed to UVB light at increasing doses were analyzed under the very same ESI-HRMS conditions, and relative abundances were calculated and expressed as 'percent degradation'.

The maximum light dose was chosen to be 100 J/cm^2 depending on the spectrophotometric changes of the compounds observed during irradiation, as outlined above.

In Figure 1, the yields of photodegradation obtained by analysing solutions after 20, 40, and 100 J/cm^2 of UVB irradiation are shown for all the investigated analytes. As may be observed, the trend is similar for all compounds, but 6-MAM and morphine show the highest photolability (up to 90%) followed by methadone (70%) and BZE (67%), whereas cocaine and EDDP are the most 'resistant' to photolysis. However, as Petrovich *et al.*[21] found, cocaine turned out to be rather unstable under irradiation in water solutions, undergoing extensive hydrolysis/oxidation reactions, suggesting the importance of the role of solvents and the environment when photodegradation is being studied.

Photodegradation in hair exposed to UVB light

Eight positive samples of hair for each class (methadone, opiates, and cocaine) were collected; their characteristics are outlined in Table 1.

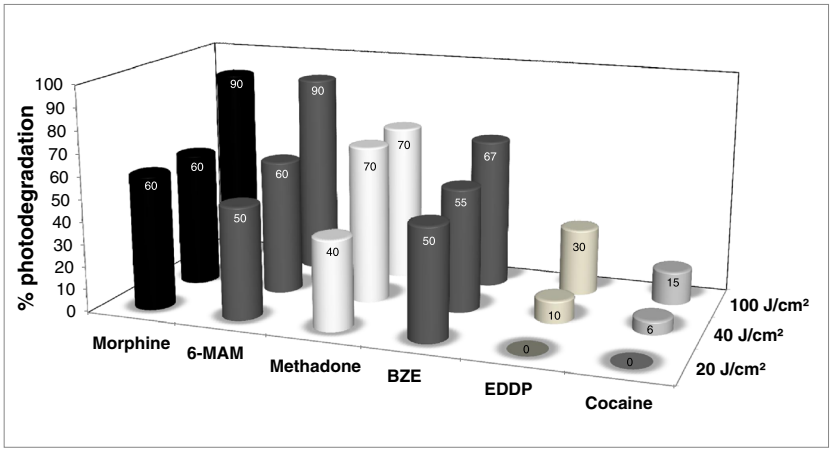

Figure 1. Relative degradation of target drugs in methanol solutions with increasing doses of UVB light (% photodegradation calculated using the absolute abundance of protonated molecules in HRMS spectra of solutions kept in the dark as controls for 0% degradation).

Table 1. Hair samples, physical characteristics and drug concentrations determined by HPLC-HRMS in aliquots kept in the dark

Sample	Color	Thickness	Straight	Curl	Methadone ng/mg	EDDP ng/mg	6-MAM ng/mg	Morphine ng/mg	Cocaine ng/mg	BZE ng/mg
#1 Fair	grey and white	very thin	x		—	—	0.23	0.25	—	—
#2 Fair	light brown	thin	x		15.29	0.56	0.58	0.28	—	—
#3 Fair	dark blond	thin	x		1.90	0.18	—	—	—	—
#4 Fair	dark blond	thin	x		6.20	1.20	—	—	2.26	0.48
#5 Dark	black	thick			3.25	1.57	0.30	0.50	2.23	0.08
#6 Dark	brown	thick	x		4.30	_	0.30	0.30	1.45	0.18
#7 Dark	dark brown	thin		x	5.50	0.79	—	—	0.24	0.12
#8 Dark	dark brown	thick	x		4.50	1.29	—	—	—	—
#9 Dark	brown	thick	x		—	—	—	—	11.98	2.33
#10 Dark	dark brown	thin	x		—	—	—	—	31.26	1.82
#11 Dark	brown	thin	x		—	—	—	—	8.26	4.04
#12 Dark	black	thick	x		—	—	—	—	5.64	0.51
#13 Dark	brown	thin	x		2.50	0.90	0.50	0.30	—	—
#14 Dark	brown	thin	x		—	—	0.70	0.30	—	—
#15 Dark	brown	thin	x		—	—	0.60	0.28	—	—
#16 Dark	brown	thin	x		—	—	0.50	0.35	—	—

In Table 2, the percentage of photodegradation yields of methadone and EDDP, 6-MAM and morphine, cocaine and BZE are outlined. These yields were determined for hair exposed to UVB light for a period of 32 h, corresponding to about 300 J/cm^2, and calculated using the concentration of the sample kept in the dark as control:

$$\%\ \text{photodegradation} = (\text{drug conc.}_{\text{dark}} - \text{drug conc.}_{\text{irradiatied}})/\text{drug conc.}_{\text{dark}} * 100$$

The UVB light dose was selected to be three times higher than that used for irradiating the pure solutions of the analytes; this is because other chromophores, which are present in the hair matrix, might shield the incoming irradiation. For each hair sample, whether it was kept in the dark or irradiated, three 10 mg aliquots were analyzed and the average concentrations were used for the above calculations.

The same table 2 also outlines the yields of degradation for each drug class and for all eight hair samples. These yields are reported as range, average, and standard deviation.

A clearly different behaviour of methadone is evidenced when compared to all the other drugs/metabolites. Indeed, the average degradation of methadone is 55%, much higher than was found for all the other compounds (averages from 10 to 21%). Interestingly, a colour effect seems to be present when considering the photodegradation of methadone: in the three fair-hair samples (#2, #3, and #4) the photodegradation is 85, 82, and 80%, respectively (Table 2); for the same samples, EDDP also exhibits a strong 'colour' effect, its degradation in fair hair being about three times higher than in dark hair. The colour of hair depends on the relative amounts of pheomelanin (red) and eumelanin (black) that are thought to represent the first defence against UV in human hair and skin.[22–24] Generally, a part of the light is absorbed by the hair matrix itself without any photochemical effect. In dark hair, the eumelanin could protect the drugs/metabolites to a higher degree than in fair hair. However, melanin may also react with

Table 2. Hair samples and relative degradation of drugs in aliquots irradiated at 300 J/cm^2 with UVB light

				% *photodegradation*					*Average (n=8)*	*Range*	*SD*
Hair sample	*#2*	*#3*	*#4*	*#5*	*#6*	*#7*	*#8*	*#13*			
Methadone	85	82	80	53	38	28	34	36	55	28–85	24
EDDP	32	33	32	0	—	10	5	7	17	0 –33	15
Hair sample	*#1*	*#2*	*#5*	*#6*	*#13*	*#14*	*#15*	*#16*			
6-MAM	0	29	7	43	22	33	26	11	21	0–43	14
Morphine	0	25	0	43	19	28	15	9	17	0–43	15
Hair sample	*#4*	*#5*	*#6*	*#8*	*#9*	*#10*	*#11*	*#12*			
Cocaine	0	4	26	16	15	0	22	0	10	0–26	11
Benzoylecgonine	24	0	63	0	0	27	17	30	20	0–63	21

% photodegradation = (drug conc.$_{dark}$ - drug conc.$_{irradiatied}$)/ drug conc.$_{dark}$ * 100.

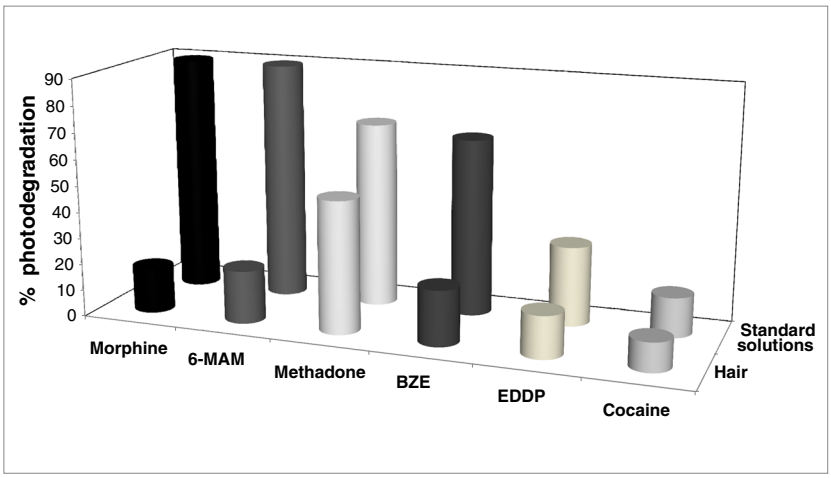

Figure 2. Average relative photodegradation of methadone, EDDP, 6.MAM, morphine, cocaine and BZE in authentic positive hair irradiated by UVB light (n = 8 for each drug class) and comparison with the maximum relative degradation observed for the methanol solutions of the same drugs.

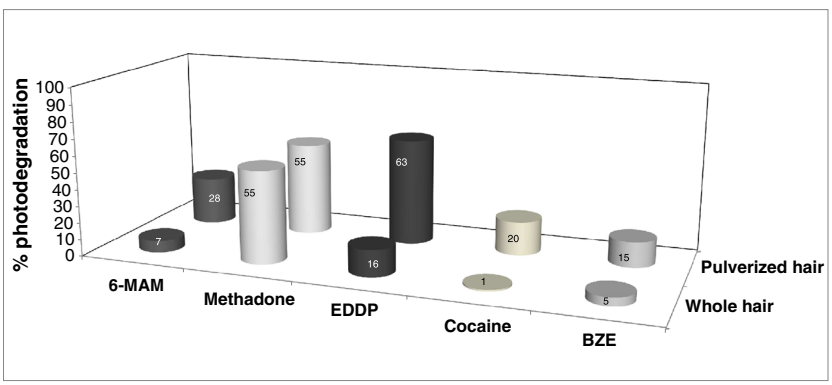

Figure 3. Comparison of the relative photodegradation of drugs in authentic positive hair samples as integral hair strands or powder when irradiated by UVB light.

oxygen under irradiation, producing particularly reactive species such as superoxide anions that can induce photolysis of melanin itself,[25] thus weakening the photoprotective effect of the melanin.

Regarding opiates, on the basis of the *in vitro* experiments (Figure 1), they were expected to photodegrade more readily than methadone and EDDP. Unexpectedly, the *in vivo* experiments exhibited an average degradation of 21 and 17% for 6-MAM and morphine, respectively, with a range of 0–43% for both molecules. In the only study available in the literature,[8] experiments performed to study changes brought about by exposure to daily light found concentrations of 6-MAM and morphine in irradiated samples in the range of 17–40% (6-MAM) and 33–69% (morphine) of

non-irradiated samples. The present results, obtained under UVB conditions, seem to confirm a large inter-individual variability in the degradation yields.

Figure 2 reports, to visually compare, the average photodegradation yields of the target compounds when irradiated in hair and in methanol solution. In our experiments, cocaine was the most resistant molecule, both *in vitro* and *in vivo* (average photodegradation of 10% in hair, range 0–26), whereas BZE degrades more efficiently than its precursor in both experiments (average photodegradation of 20% in hair, range 0–63). Interestingly, higher degradation yields were observed for cocaine (average of 45%) and BZE (average of 55%) by Skopp,[8] when hairs were exposed to natural light (direct and diffuse sunlight) for three months.

To further investigate the role of hair structure, i.e. thickness and the integrity of the keratin/melanin organization within the shaft, we thought it would be of interest to compare results for hair irradiated as a whole hair and after pulverization. Pulverization is known to facilitate drug extraction, enhancing the surface exposed to solvents and the same physical process of disaggregation could reasonably reduce hair thickness and its protective effect, thereby leaving the drugs more exposed to the light. If such a hypothesis is true, a more abundant degradation of drugs should be expected for pulverized, irradiated hair. In Figure 3, the degradation yields of the supplementary samples that tested positive for the target molecules are presented. The samples were powdered in a ball mill and put between two optical glasses to be irradiated for 32 h. In the same figure, percentages for photodegradation are compared to those obtained for the corresponding whole hair irradiated for the same time period; the concentration of drugs determined in the aliquots kept in the dark again represented the control. As a general trend, even though the number of pulverized samples examined is lower when compared to those presented in Table 1, drugs in pulverized hair exhibited higher percent degradations than intact hair (15–63% *vs* 1–55%), demonstrating that the structure of the hair shaft plays an important role in protecting drugs from UVB degradation. Interestingly, methadone, which is the most labile drug in the whole hair tests, does not degrade further in the powdered hair analysis (55% in both cases).

Therefore, the significantly different yields observed for the same drugs in different hair samples (see e.g. 6-MAM and morphine in samples #1 and #5 *vs* all the other opiate positive samples) could be ascribed to even slight individual differences in the hair's structure (melanin, keratin, fibres, etc.) or its ultrastructure.[26]

Conclusions

The photodegradation studies, undertaken to elucidate how light exposure may decrease concentrations of the most frequent drugs of abuse in keratin matrices, revealed that UVB irradiation modifies the levels of methadone and EDDP incorporated in the hair. In true positive hair samples, methadone was affected by irradiation more than the other drugs of abuse (28–85%) and opiates turned out to be much less susceptible (0–43%). In comparing fair hair and dark hair, a much larger photodegradation of methadone and EDDP was observed in the former. This supports the hypothesis of the protective effect of eumelanin for these molecules. Drugs and their metabolites were revealed to be more sensitive to UVB when irradiation was performed on pulverized hair, suggesting an important role of the whole hair structure in protecting drugs from light.

The narrow number of samples for each drug/metabolite and their limited variability in colour and in concentrations may represent limitations in the present study and suggest the need for further investigation. In particular, the structural identity of transient and/or stable species arising from drug photolysis *in vitro* will be the aim of a further study aimed at understanding if the same degradation products can be found *in vitro* and in hair as new markers of drug consumption, incorporation and degradation.

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