Analysis of Reaction Products of Morphine and Codeine with Hydrogen Peroxide by High-Performance Liquid Chromatography/Mass Spectrometry

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Changes in the chemical structures of morphine and codeine in the presence of hydrogen peroxide, a major component of hair dye and decolorant treatments, were examined with high-performance liquid chromatography/mass spectrometry (LC/MS). A mixture of morphine and hydrogen peroxide solution, after incubation at 39°C for 24 h, produced two reaction products (hydroxymorphines). When codeine was used in place of morphine, one reaction product (hydroxycodeine) was produced, in which the benzene ring was hydroxylated.

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Introduction

Hair analysis is an effective forensic tool for determining the use of abused drugs. However, a problem with such analyses is that cosmetic treatments, such as hair dye and decolorant treatments, can change concentrations of several drugs, including cocaine, morphine, codeine and tetrahydrocannabinol.¹⁻⁶

Recently, we found that methamphetamine (MA) was degraded by hair dye and decolorant treatments and that MA was unstable in the presence of hydrogen peroxide, the major component of hair dye and decolorant treatments.⁷ MA was changed to three compounds and two other intermediates in the presence of hydrogen peroxide.⁸

On the other hand, it has been reported that morphine, codeine and cocaine were also unstable in the presence of hydrogen peroxide and that the degradation was due to the effect of hydrogen peroxide.³ We found that cocaine was converted directly to six compounds in the presence of hydrogen peroxide.⁹ However, it was not clear whether hydrogen peroxide had similar effects on morphine and codeine.

In the present study, we used LC/MS to determine the structures of the reaction products that were obtained by mixing either morphine or codeine with hydrogen peroxide.

Experimental

Chemicals

Morphine hydrochloride and codeine phosphate were obtained from Tanabe Pharmaceuticals (Osaka, Japan) and Sankyo (Tokyo, Japan), respectively. Hydrogen peroxide was of ultrapure grade (30%, stabilizer free; Kanto Chemical, Tokyo,

Japan). Methanol was of HPLC grade (Wako Pure Chemical Industries, Tokyo, Japan). All other reagents used were of analytical reagent grade. Ultrapure water provided by Milli-RX12 α and Milli-Q SP systems (Millipore, Bedford, MA, USA) was used for all procedures.

High-performance liquid chromatography/mass spectrometry

The LC/MS system and its conditions were the same as those in our previous reports,^{8,9} except for the composition of the mixture of 10 mM ammonium acetate and methanol as the mobile phase (4:1 (v/v) for morphine analysis and 7:3 (v/v) for codeine analysis).

Treatment of morphine and codeine with hydrogen peroxide

The concentration of hydrogen peroxide in hair dye or decolorant treatments is usually *ca.* 6%. In this study, however, 30% hydrogen peroxide solution was used to increase its effect. A reaction mixture containing 0.5 mL each of 100 μ g/mL morphine or codeine solution with 30% hydrogen peroxide was incubated with gentle shaking for 24 h at 39°C (this temperature is commonly used when the hair is treated with hair dye or decolorant). Aliquots of the solution (test solution) were applied to the LC/MS system.

Results and Discussion

The total ion chromatogram (TIC, upper chromatogram in Fig. 1) and mass chromatograms (below the TIC) of the reaction mixture of morphine with hydrogen peroxide showed morphine (R_t 12.6 min) and two major products (1 with R_t 3.1 and 2 with R_t 3.6 min). The base peaks in the mass spectra of these three compounds were m/z 286, 302 and 302, respectively. The base peak of morphine (molecular weight, 285) was m/z 286, which is equivalent to $[M+H]^+$, the base peaks of products 1 and 2, whose m/z values were 302, thus seemed to be equivalent to $[M+H]^+$.

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Fig. 1 LC/MS and LC/MS/MS analysis of the reaction mixture of morphine with hydrogen peroxide.

Product Ion Mass Spectrum

Fig. 2 LC/MS and LC/MS/MS analysis of the reaction mixture of codeine with hydrogen peroxide.

For the reaction of codeine and hydrogen peroxide, the TIC (upper chromatogram in Fig. 2) and mass chromatograms (below the TIC) showed codeine (R_t 14.7 min) and a single major product (3, R_t 4.0 min). The base peaks in the mass spectra of these two compounds were m/z 300 and 316, respectively. The base peak of codeine (molecular weight, 299) was m/z 300, which is equivalent to $[M+H]^+$, so the base peak of the mass spectrum of product 3, whose m/z value was 316, seemed to be equivalent to $[M+H]^+$. In addition, two minor products (4 and 5, R_t 9.1 and 11.3 min, respectively) were observed in the mass chromatogram, and the m/z values of these products (316) seemed to be equivalent to $[M+H]^+$. However, because products 4 and 5 were detected at trace levels, the mass spectra of these products could not be determined.

The reaction products of morphine and codeine with hydrogen peroxide were further analyzed by LC/MS/MS to confirm their chemical structures. The CID analysis of the precursor ions of products 1 and 2 of morphine (both m/z 302) indicated that the main product ions had m/z values of 162 and 285 (middle part of Fig. 1). These ions were due to the cleavage of morphine at positions a and b, respectively (bottom part of Fig. 1). Namely, m/z 162 and 285 correspond to $[M-C_7H_7O_3]^+$ and $[M-OH]^+$, respectively. Therefore, products 1 and 2 might be hydroxymorphines.

On the other hand, the CID analysis of the precursor ion of product 3 of codeine (m/z 316) indicated that the main product

ions had m/z values of 162 and 299 (middle part of Fig. 2). These ions were due to the cleavage of codeine at positions a and b, respectively (bottom part of Fig. 2). Namely, m/z 162 and 299 correspond to $[M-C_8H_9O_3]^+$ and $[M-OH]^+$, respectively. Therefore, product 3 might be hydroxycodeine. This result agreed with the fragment pattern of the reaction products of morphine with hydrogen peroxide, suggesting that the proposed structures of the reaction products in Figs. 1 and 2 are correct.

Consequently, morphine and codeine were oxidized to hydroxymorphine and hydroxycodeine, respectively, in the presence of hydrogen peroxide.

The reaction products 1 and 2 of morphine with hydrogen peroxide and the reaction product 3 (but not 4 and 5) of codeine with hydrogen peroxide were all formed immediately after mixing the test solution (data not shown). After the incubation with hydrogen peroxide at 39°C for 24 h, the concentrations of morphine and codeine were 49% and 89%, respectively, of the initial concentrations. Yegles *et al.*³ found similar decreases in the concentrations of morphine and codeine as a result of incubation with hydrogen peroxide. We previously observed extremely low concentrations of MA and its main metabolite, amphetamine (AP) in MA abusers' hair samples (MA 13.7 ± 25.9 ng/mg ($n = 39$), AP 1.49 ± 1.88 ng/mg ($n = 31$))¹⁰ in spite of the high concentrations in urine (MA 68.1 ± 87.7 µg/mL ($n = 12$), AP 6.56 ± 5.90 µg/mL ($n = 12$)).¹¹ We reported that MA

was changed to MA *N*-oxide or *N*-hydroxy MA before changing to the *ortho*-, *meta*- and *para*-hydroxy MAs in the presence of hydrogen peroxide.⁸ This type of reaction, in which treatment with acid caused the rearrangement of aryl hydroxylamines, is known as the Bamberger Rearrangement.¹² Furthermore, cocaine was converted directly to six compounds (ecgonine methyl ester, benzoylecgonine, *ortho*-, *meta*- and *para*-hydroxycocaines and dihydroxycocaine) in the presence of hydrogen peroxide.⁹ The fact that no intermediates could be detected in the two reaction mixtures with hydrogen peroxide suggested that morphine and codeine were directly converted to hydroxymorphine and hydroxycodeine, respectively. However, because minor reaction products of codeine with hydrogen peroxide were observed, it would be worthwhile to determine whether they include codeine *N*-oxide or morphine *N*-oxide.

Thus, in the analysis of hair to determine morphine and codeine use, it is important to know whether the hair was treated with hair dye or decolorant. In addition, it is important to clarify whether the reaction products of morphine and codeine with hydrogen peroxide provided by hair dye and decolorant treatments remain in hair.

References

1. M. J. Welch, L. T. Sniegowski, C. C. Allgood, and M. Habram, *J. Anal. Toxicol.*, **1993**, *17*, 389.
2. V. Cirimele, P. Kintz, and P. Mangin, *J. Anal. Toxicol.*, **1995**, *19*, 331.
3. M. Yegles, Y. Marson, and R. Wenning, *Forensic Sci. Int.*, **2000**, *107*, 87.
4. C. Jurado, P. Kintz, M. Menendez, and M. Repetto, *Int. J. Legal Med.*, **1997**, *110*, 159.
5. L. Pötsch and G. Skopp, *Forensic Sci. Int.*, **1996**, *81*, 95.
6. G. Skopp, L. Pötsch, and M. R. Möller, *Forensic Sci. Int.*, **1997**, *84*, 43.
7. N. Takayama, S. Tanaka, R. Kizu, and K. Hayakawa, *Biomed. Chromatogr.*, **1999**, *13*, 257.
8. S. Tanaka, R. Iio, S. Chinaka, N. Takayama, and K. Hayakawa, *Biomed. Chromatogr.*, **2001**, *15*, 45.
9. S. Tanaka, R. Iio, S. Chinaka, N. Takayama, and K. Hayakawa, *Biomed. Chromatogr.*, **2002**, *16*, 390.
10. N. Takayama, S. Tanaka, R. Kizu, and K. Hayakawa, *Jpn. J. Sci. Tech. Iden.*, **1998**, *3*, 11.
11. K. Hayakawa, Y. Miyoshi, H. Kurimoto, Y. Matsushima, N. Takayama, S. Tanaka, and M. Miyazaki, *Biol. Pharm. Bull.*, **1993**, *16*, 817.
12. J. March, "Advanced Organic Chemistry: Reactions, Mechanisms and Structure", **1977**, McGraw-Hill, New York, 616.