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GC-MS Detection of Dihydroergotamine Artifact - Proof of Ingestion of Dihydroergotamine ?

Denis S. Theobald, Frank T. Peters, Jochen Beyer, Andreas H. Ewald, and Hans H. Maurer

Department of Experimental and Clinical Toxicology, University of Saarland, D-66421 Homburg (Saar), Germany, hans.maurer@uniklinikum-saarland.de

Summary

GC-MS screening analysis of samples from several clinical toxicology and postmortem cases using the PMW_tox3 GC-MS library led to detection of a dihydroergotamine artifact, although ingestion of dihydroergotamine by the respective individuals was very unlikely. The same compound was also detected in enzymatically cleaved rat urine samples from metabolism studies where definitely no dihydroergotamine had been administered. Therefore, it was investigated whether this compound can also derive form other sources. Chemically, it can be seen as a cyclo dipeptide of phenylalanine and proline also called cyclo (Phe-Pro), which is known to be a product of roast processes and hence contained in several foodstuffs. Therefore, extracts of cocoa powder, of urine samples from volunteers who had ingested high amounts of cocoa powder, and of water samples incubated with glucuronidase/arylsulfatase from *Helix pomatia* were analyzed by GC-MS. Cyclo (Phe-Pro) (= dihydroergotamine artifact) was detected in all these types of samples. This study shows that cyclo (Phe-Pro) is not only formed from dihydroergotamine, but it is also contained in cocoa powder and that it may be excreted unchanged in urine after ingestion of cocoa powder. It further shows that cyclo (Phe-Pro) is present in or formed from ingredients of a glucuronidase/arylsulfatase enzyme peparation. Based on these findings it could be concluded that the detection of dihydroergotamine artifact in human urine is not a reliable proof for ingestion of dihydroergotamine.

Introduction

Dihydroergotamine, a semi-synthetic ergot alkaloid, is a partial agonist at α-adrenergic and several serotonin receptors. It is used in the treatment of acute vascular headache and orthostatic hypotension. After injection of a methanolic solution of dihydroergotamine into a GC-MS, the mass spectrum of an artifact can be recorded. After ingestion of dihydroergotamine, this artifact can be detected in urine after acid hydrolysis, liquid-liquid extraction and acetylation (standard systematic toxicological analysis procedure, STA [3-5, 17]). Figure 1 shows the reaction of the formation of this artifact (upper part) and the electron ionization (EI) mass spectrum, taken from our PMW_tox3 GC-MS library [7-14] (lower part).

Surprisingly, we had detected the artifact also in several urine samples during our routine work in recent years, even though the respective patients most likely had not taken dihydroergotamine. In addition, we had detected it during our metabolism studies in rat urine samples treated with glucuronidase/arylsulfatase from *Helix pomatia*, which were definitely free of dihydroergotamine. Recently, our laboratory was contacted by a colleague who had used our PMW_tox3 GC-MS library [7-14] for screening of urine samples from several post-mortem cases, which had been treated with glucuronidase/arylsulfatase for conjugate cleavage prior to extraction. He had detected the above-mentioned dihydroergotamine artifact in these samples. As he considered it very unlikely that all of the respective individuals had taken dihydroergotamine, he inquired if we knew of any another explanation for the presence of this artifact in the samples. Since all these findings suggested, that there should be another source of this dihydroergotamine artifact besides dihydroergotamine, we decided to further investigate this problem.

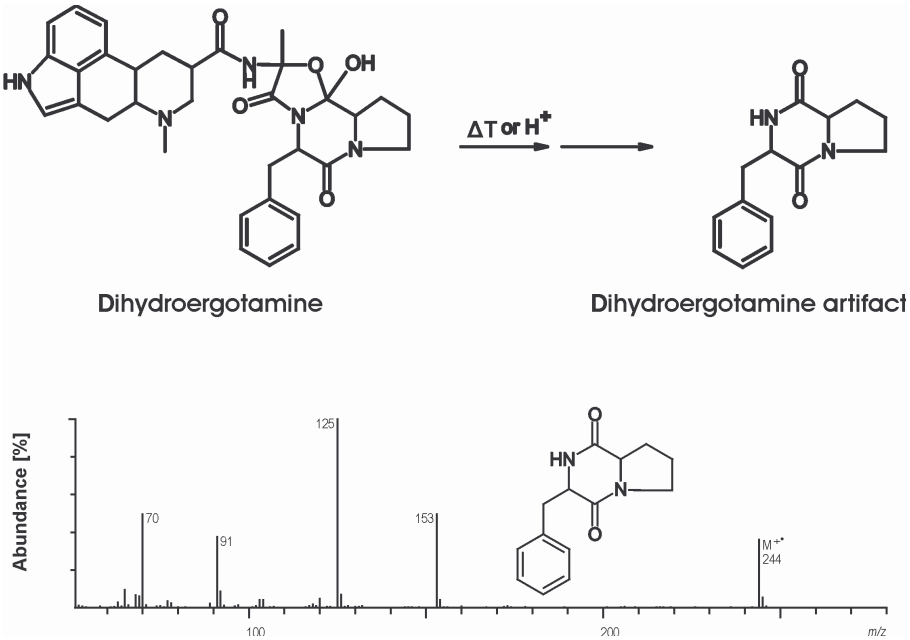

Figure 1: Artifact formation from dihydroergotamine (upper part) and structure and EI mass spectrum of dihydroergotamine artifact (lower part).

A closer look at the chemical structure of the artifact revealed that it can be seen as a cyclo dipeptide of phenylalanine and proline also called cyclo (Phe-Pro). A search of the literature yielded several publications describing that this cyclic product can be formed during roast processes by cyclocondensation as depicted in Figure 2. For example it was detected in cocoa, roasted coffee, chicken essence or roasted malt [1, 2, 6, 15, 16, 18]. However, it has never been studied, whether cyclo (Phe-Pro) is detectable in human urine after ingestion of such cyclo (Phe-Pro) containing foodstuffs.

Therefore, the first aim of our study was to check the detectability of cyclo (Phe-Pro)/ dihydroergotamine artifact in urine after ingestion of cyclo (Phe-Pro) containing foodstuffs. The second aim was to investigate a possible connection between detection of dihydroergotamine artifact and sample treatment with glucuronidase/arylsulfatase, since both the postmortem and rat urine samples in which dihydroergotamine artifact had been found had been treated in this way.

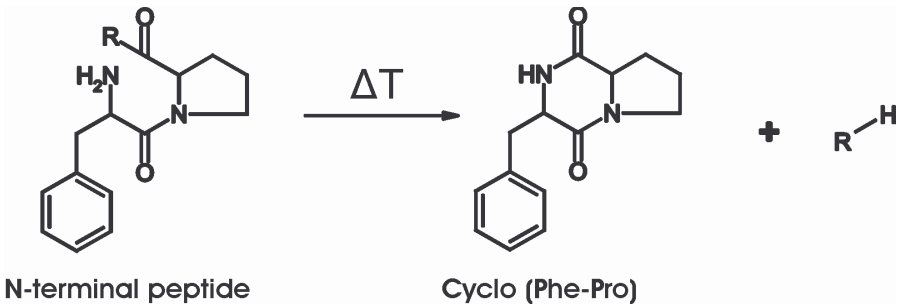

Figure 2: Formation of cyclo (Phe-Pro) from cyclocondensation of a protein containing phenylalanine and proline.

Methods

Analysis of cocoa powder

2.5 ml of an aqueous suspension of cocoa powder (1:10; w/v) were extracted with 5 ml of a dichloromethane-isopropanol-ethyl acetate mixture (1:1:3; v/v/v). After evaporation of the organic layer to dryness, the residue was dissolved in 100 µl of methanol. 2 µl of the extract were injected into the GC-MS.

Analysis of urine samples from volunteers after ingestion of cocoa

Two volunteers each drank suspensions of approximately 40 g of cocoa powder in milk. Single urine samples of these individuals were collected on the day before the experiment, on the day of the experiment (the first urine after ingestion of cocoa), and on the day after the experiment. The urine samples were analyzed according to our STA [3-5,17] with acid hydrolysis, liquid-liquid extraction, acetylation and full-scan GC-MS.

Analysis of glucuronidase/arylsulfatase solution

A 2-ml portion of water was adjusted to pH 5.2 with acetic acid (1 mol/l) and incubated at 50°C for 1.5 h with 50 µl of a mixture (100 000 Fishman units per ml) of glucuronidase (EC no. 3.2.1.31) and arylsulfatase (EC no. 3.1.6.1) from *Helix pomatia*, then adjusted to pH 8-9 and extracted with 5 ml of a dichloromethane-isopropanol-ethyl acetate mixture (1:1:3; v/v/v). After phase separation by centrifugation, the organic layer was carefully evaporated to dryness at 56°C under a stream of nitrogen. The residue was dissolved in 100 µl of methanol. As negative control, 2 ml of water without glucuronidase/arylsulfatase were treated in the same way. Aliquots (2µl) of the extracts were injected into the GC-MS (for apparatus details see in Ref. [17])

Results

As expected, cyclo (Phe-Pro) was detected in the cocoa powder and identified by library search as dihydroergotamine artifact. It was also detected in the urine samples of the volunteers, but only in those urine samples taken on the day they had ingested the cocoa powder. In the samples taken on the days before and after the experiment, cyclo (Phe-Pro)/dihydro-ergotamine artifact could not be detected. Finally, cyclo (Phe-Pro)/dihydroergotamine artifact was detected in the water sample treated with glucuronidase/arylsulfatase, but not in the sample without glucuronidase/arylsulfatase.

Discussion

Findings from the literature and the detection of cyclo (Phe-Pro)/dihydroergotamine artifact in the cocoa powder analyzed in the present study clearly show that cyclo (Phe-Pro) is contained in many common foodstuffs. In order to check if cyclo (Phe-Pro) contained in such foodstuffs may pass the human body and may be excreted unchanged in urine, the volunteer experiment was performed. Cocoa powder was chosen as a source of cyclo (Phe-Pro), because rather large doses can be ingested without expecting relevant side effects, in contrast to e.g. coffee. In fact, the ingested doses of cocoa powder in the described experiment were much higher than cocoa doses commonly ingested in form of e.g. drinks or chocolate. Nevertheless, the fact that the cyclo (Phe-Pro)/dihydroergotamine artifact could be detected shortly after, but not on the days before and after the experiments, suggests that cyclo (Phe-Pro) ingested with foodstuffs may indeed be excreted unchanged in urine to a certain extent. Furthermore, more common doses of other foodstuffs might contain the same amount of cyclo (Phe-Pro) as ingested in the described experiment in form of cocoa powder.

The detection of cyclo (Phe-Pro)/dihydroergotamine artifact in the water sample treated with glucuronidase/arylsulfatase from *Helix pomatia* demonstrates that this enzyme preparation can also be a source of cyclo (Phe-Pro)/dihydroergotamine artifact. Of course, this is of utmost importance and must be kept in mind when interpreting toxicological results obtained with sample workup procedures involving enzymatic cleavage of conjugates.

Conclusion

Detection of the chemical entity cyclo (Phe-Pro) which is listed in the PMW_tox3 GC-MS library [7-14] as dihydroergotamine artifact is not a proof of dihydroergotamine ingestion. The presence of this compound in toxicological samples may also be explained by consumption of cyclo (Phe-Pro) containing foodstuffs or by the use of glucuronidase/arylsulfatase from *Helix pomatia* for enzymatic conjugate cleavage during sample workup.

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