SALICYLAMIDE IN URINE AFTER INTAKE OF ACETYLSALICYLIC ACID DUE TO DEGRADATION OF SALICYLIC ACID CONJUGATES

Kurt BESSERER[1], Detlef TIESS[2], Maria KAŁA[3]

[1]*Formerly Institute of Forensic Medicine, University in Tübingen, Tübingen, Germany*

[2]*Formerly Institute of Forensic Medicine, University in Rostock, Rostock, Germany*

[3]*Institute of Forensic Research, Cracow, Poland*

ABSTRACT: In addition to well-known acetylsalicylic acid (ASA) metabolites, salicylamide (SA) was detected in original or acidified urine samples taken from healthy persons after intake of ASA. SA was found in diethyl ether extracts of urine after samples alkalisation by ammonia solution. SA was identified by means of gas chromatography-mass spectrometry (GC/MS). Broader examinations showed that SA was detected in urine samples only after its alkalisation by ammonia solution. Using dimethyl-, diethyl- or dibutylamine for alkalisation in urine samples corresponding salicylic acid dialkylamides were detected. Analytical results lead to the conclusion that ester type salicylic acid glucuronide conjugates undergo degradation by hydrolytical amination to corresponding amides. This gives the possibility of a specific demonstration of the presence of ester type glucuronide conjugates. Further study will show if this reaction can be transferred to other active substances or other decomposition products with carboxyl groups.

KEY WORDS: Salicylamide; Salicylic acid; Degradation of glucuronide.

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INTRODUCTION

Acetylsalicylic acid (ASA) remains one of the most commonly used drugs. It is administered to adults for its analgesic and antipyretic effects. Recently low doses of ASA have frequently been given long term to selected patients, because of ASA's anticoagualant properties.

Virtually the whole dose of ASA is hydrolized by hydrochloric acid of the stomach and both liver and blood esterases to salicylic acid.

Salicylic acid is excreted in the urine unchanged and in the form of well-known free metabolites (Figure 1). The formation of the major metabolites includes corresponding conjugates (Figure 2).

Fig. 1. Major free metabolites of acetylsalicylic acid.

Fig. 2. Major conjugates of acetylsalicylic acid.

In addition to well-known ASA metabolites, salicylamide (SA) was detected in original and acid urine samples taken from healthy persons after oral intake of ASA.

A study was carried out to explain the reason of presence of SA in urine samples.

MATERIAL AND METHODS

Identification of AS was carried out using the GC/MS technique. A Hewlett-Packard model 6890 GC and a 5973 mass selective detector (MSD) and a QP 5050A Shimadzu System were used for analyses. The MSD was operated in Scan and SIM modes. Mass ions and typical fragments were the basis for identification (Table I).

TABLE I. COMPARISON OF [m/z] MOLECULAR (*) AND FRAGMENT IONS FOR SUBSTANCES OF INTEREST

Substance	[m/z]

Acetylsalicylic acid	43, 92, 64, 120, 138*
Salicylic acid	53, 64, 92, 120, 138*
Metoxybenzoic acid	53, 63, 152*
Metoxysalicylamide	52, 63, 92, 122, 151* (44, 77, 105, 134)
Salicylamide	53, 65, 92, 120, 137*
Salicylic acid dimethylamide	53, 65, 93, 121, 165*
Salicylic acid diethylamide	53, 65, 93, 121, 192* (58, 72)
Salicylic acid dibutylamide	53, 65, 93, 121, 248*

Urine samples were taken from healthy adults after intake of 300–1000 mg ASA in trade brand tablets. Samples were collected in the first day after 2–3, 6 and 12 hours after medicine administration and next in 12 h intervals up to third day. Following collection samples were analysed directly or frozen until time of analyses.

5 ml aliquot of original (pH 5–6) or acidified (by 1 M H_2SO_4 to pH 1) urine samples were extracted 3 times by ethyl ether (15 ml each). After acidic extraction the urine samples were alkalised by $NaHCO_3$ and Na_2CO_3 or 25% ammonia solution, dimethyl-, diethyl- and dibutylamine, respectively, to pH 11–12. Corresponding ether phases were evaporated at 30°C to dryness in vaccum concentrator. Dry residues were reconstituted in 50 µl of methanol or ethyl acetate and 1 or 2 µl aliquots were injected into GC/MS systems.

RESULTS

1. SA was found in diethyl ether extracts of urine (pre-extracted by diethyl ether at pH 1) after samples alkalisation by ammonia solution. SA was not detected in urine samples after alkalisation by $NaHCO_3$ and Na_2CO_3.
2. Using dimethyl-, diethyl- and dibutylamine for alkalisation of urine samples corresponding salicylic acid dialkylamides were detected in the extracts.
3. SA and salicylic acid dimethyl- and diethylamides were identified by means of gas chromatography-mass spectrometry (GC/MS). These substances are not commercially available and do not exist in any mass spectral libraries.
4. After carrying out a simulation study (by injection of mixtures of SAA standard substance with trimethylphenylammonium acetate (TMPAA) and dimethylamine ($(CH_3)_2NH$)), respectively, and SA with (TMPAA) the substances showed in Figure 3 and Figure 4 were identified.
5. On the basis of these we hypothesised that salicylic acid dialkylamides are formed by degradation of ester type salicylic acid glucuronide by hydrolytical amination to corresponding amides: salicylamide, salicylic acid dimethyl-, diethyl-, and dibuthylamide, as it is presented in Figures 5–7.

CONCLUSIONS

Analytical results lead to the conclusion that ester type salicylic acid glucuronide conjugates undergo degradation by hydrolytical amination to corresponding amides.

Fig. 3. Pathway mechanism of derivatization (with TMPAA – trimethylphenylammonium acetate and $(CH_3)_2NH$ – dimethylamine) of salicylic acid and salicylamide.

Fig. 4. TIM recording and mass fragmentation spectrum of methoxysalicylamide.

Fig. 5. Mechanism of formation of salicylamide, its TIM recording and mass spectrum.

Fig. 6. Mechanism of formation of salicylic acid dimethylamide, its TIM recording and mass spectrum.

Fig. 7. Mechanism of formation of salicylic acid diethylamide, its TIM recording and mass spectrum.

This gives the possibility of a specific demonstration of the presence of ester type glucuronide conjugates.

Further study will show if this reaction can be transferred to other active substances or other decomposition products with carboxyl groups.

However the use of other techniques (e.g. TLC, LC/MS) is required.

ADDENDUM

The authors intend to prepare a detailed publication with experimental data and references.