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Preliminary Results of Excretion Studies with Salbutamol  
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Preliminary results of excretion studies with Salbutamol

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1. Introduction

Salbutamol (fig. 1) is a very useful and widely used sympathomimetic agent. But due to their anabolic side effects sympathomimetic agents are prohibited in sports.

It is important to treat also sportsmen in case of asthma or an exercise induced asthma with adequate medications. For this reason salbutamol as well as terbutaline and salmeterol is permitted as inhaled application only.

In dope control samples salbutamol is detected often and in those cases it is mostly declared as inhaled application on the corresponding control forms. But up to now there is no accepted method to distinguish between permitted (inhaled) and prohibited (oral) application.

Salbutamol is excreted mainly unchanged and as 4’-O-sulphate (1). A different ratio of unchanged and conjugated salbutamol after oral and inhaled application has been described (1).

To find differences in the excretion of salbutamol depending on its application which are suitable for dope control purposes in sport excretion studies were performed and analysed.

![Structure formula of salbutamol](image)

Figure 1: Structure formula of salbutamol
2. Experimental

Two excretion studies were performed with salbutamol:

a. orally - 10 volunteers were treated with a single dose of 8 mg salbutamol (Volmac®, Glaxo Wellcome)

b. inhaled - 8 volunteers were treated with a single dose of 0.2 mg salbutamol (Sultanol®, Glaxo Wellcome).

and the urine was collected for 48 hours.

Enzymes used: β-glucuronidase from e.coli (Boehringer Mannheim) and β-glucuronidase/aryl-sulphatase from helix pomatia (Boehringer Mannheim).

Sample preparation: see fig. 2, in case of enzymatic hydrolysis the adequate pH values were adjusted.

Terbutaline was used as internal standard.

The ion 86 amu/z as common ion of the TMS-derivatives of salbutamol (fig. 3) and terbutaline (fig. 4) was used for the quantitative determination by GC/MS.

GC/MS conditions:

GC (HP 5890)
- column: HP Ultra1, 17m, 0.2mm i.d., 0.11μm film thickness
- temperature program: 160°C, 5°C/min - 200°C, 30°C/min - 320°C, 1min
- carrier: 13 psi He
- split: 10 ml He/min

MS (HP 5971)
- SIM-Ions (dwell time: 30ms): 86, 356, 369 amu/z and additionally 2 ions
- source temperature: about 200°C

Urinary concentrations were calculated for a specific gravity of 1.020 g/ml by the following equation:

\[
\text{Concentration}_{\text{Corrected}} = \frac{1.020 - 0.998}{\text{S.g.}_{\text{measured}} - 0.998} \times \text{Concentration}_{\text{detected}}
\]
2ml urine

\[ \downarrow \]

hydrolysis or no hydrolysis

\[ \downarrow \]

add 0.2g K₂CO₃/NaHCO₃ buffer and
1-2 drops 5M KOH to achieve pH 10-11

\[ \downarrow \]

add 20μl terbutaline (50ppm solution), 1 ml t.-butanol,
5ml t.-butyl-methyl-ether and 1.5-2g NaCl

\[ \downarrow \]

shake for 20 min

\[ \downarrow \]

centrifuge and transfer the organic phase to a fresh tube

\[ \downarrow \]

dry the etherlayer and dry completely while dipping the tube
into water (60°C) and rotating slowly for 10 min

\[ \downarrow \]

add 100μl MSTFA for derivatisation at 80°C (20 min)

\[ \downarrow \]

after cooling transfer the sample to a closed vial

*in the case of acidic hydrolysis:*

\[ \begin{align*}
\text{add 0.4ml 3M HCl} \\
\downarrow \\
\text{mix and heat up (80°C) for 50 min} \\
\downarrow \\
\text{add quickly 250μl 5M KOH to stop the reaction}
\end{align*} \]

Figure 2: Sample preparation for quantitative determination of salbutamol in human urine
Figure 3: mass spectrum of salbutamol-tris-O-TMS, $M^+ = 455$

Figure 4: mass spectrum of terbutaline-tris-O-TMS, $M^+ = 441$
3. Results and Discussion

The enzymatic hydrolysis experiments using β-glucuronidase from e.coli and β-glucuronidase/aryl-sulphatase from helix pomatia had no or only little effects on the cleavage of the conjugated salbutamol. Acidic hydrolysis showed a cleavage of the conjugate but also a decomposition of salbutamol depending on pH, temperature and time. The best results were obtained under the following conditions: hydrolysis for 45min at 80°C in 0.5M HCl (decomposition of about 15%).

The excretion profile of unconjugated and total salbutamol is similar after oral (fig. 5) and inhaled application (fig. 6). But the ratio of unconjugated and total salbutamol is not constant. The amount of unconjugated varies in a range of 10 to 90% of the total amount. A differentiation by this parameter is not possible.

In the inhaled experiment the maximum concentrations of the unconjugated salbutamol did not exceed 174 ng/ml (the total excreted salbutamol did not exceed 410 ng/ml) (fig. 7). Whereas the corresponding concentrations after oral application are significantly higher. Figure 8 shows the concentrations of the unconjugated salbutamol in urine 24 hours after the oral application. All values are higher then the maximum value obtained from the inhaled application.

Ten suspicious dope control samples were quantitatively analysed. The results are tabulated in table 1. One sample has a salbutamol concentration of 2790ng/ml - far above the concentration observed in the inhaled experiment.

The concentration of the excreted salbutamol may be a parameter to detect the misuse of salbutamol in sports - especially in the first 24 hours after application. Further experiments have to follow.

4. References

Figure 5: Concentrations (corrected) of unconjugated and total salbutamol in the urine of V4 after a single oral dose.

Figure 6: Concentrations (corrected) of unconjugated and total salbutamol in the urine of V6 after a single inhaled dose.
Figure 7: Maximal concentrations (corrected) of unconjugated and total salbutamol in the urine 8 volunteers after a single inhaled dose.

Figure 8: Concentrations (corrected) of unconjugated salbutamol in urine 24h after a single oral dose of 8mg.
<table>
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<th>no.</th>
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<tr>
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<tr>
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Table 1: Concentrations (corrected) of unconjugated salbutamol in 10 routine urine samples