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Inhalation but not transdermal resorption of hand sanitizer ethanol causes positive ethyl glucuronide findings in urine

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ABSTRACT

Background: Ethyl glucuronide (EtG) in urine is considered a specific marker of recent ethanol consumption. There is an ongoing debate about whether inhalation or transdermal resorption of sanitizer ethanol is the underlying cause for positive EtG findings after hand disinfection.
Methods: Desderman® pure (Schülke & Mayr GmbH, Norderstedt) with 78.2 g 96% (v/v) ethanol/100 g and approx. 10% 2-propanol was used for multiple hand disinfection without and under an exhauster. Simulating a common working day in a clinic, 5 co-workers of our lab used the sanitizer 32 fold within 8 h and 2 persons were merely exposed to the sanitizer vapor but without any dermal sanitizer contact. Any additional ethanol intake or exposition was reliably excluded. Spot urine was collected at baseline, after 1, 2, 4, 6 . . . 14, and finally 24 h after the first sanitizer use. A validated LC-MS/MS was used for MRM and MS^3 of EtG and qualitative analyses of ethyl sulfate and 2-propyl glucuronide.
Results: Multiple hand disinfection caused positive EtG findings of up to 2.1 mg/L or 1.7 mg/g creatinine in 4 out of 5 test persons and even of 0.6 mg/L or 0.8 mg/g for 2 controls which were merely exposed to the sanitizer vapor but without any sanitizer contact. EtG results between the clinical (0.5 mg/g) and the forensic (0.1 mg/g) cut-off were obtained even 6 h after the last sanitizer exposition. An exhauster prevented the sanitizer vapor inhalation and reduced the EtG excretion to mostly below the detection limit of 0.02 mg/g. The maximum value was 0.09 mg/g. Ethyl sulfate and 2-propyl glucuronide (2-PpG) were detectable only in the EtG positive samples. 2-PpG is a metabolite of 2-propanol, which is quite frequently used in disinfectants. Thus, the detection of this substance can be used in cases of odd EtG results as an indicator of (unintended) sanitizer exposition.
Conclusion: Ethanol from hand sanitizers is predominantly incorporated by the respiratory tract but not via the skin. It can cause a distinct ethyl glucuronide excretion and thus analytically true-positive but forensically false-positive EtG findings in the urine of ethanol abstaining persons. Since accidental ethanol inhalation can occur quite frequently in the working place or even private household, such a situation should always be considered when EtG is used as a marker of recent ethanol consumption.

1. Introduction

Ethyl glucuronide (EtG) in urine is considered a specific marker of recent ethanol (alcohol) intake (review in [1]). Positive EtG findings despite abstinence have been reported after use of ethanol-containing hand sanitizers [2–5]. There is an ongoing debate about whether the sanitizer alcohols are incorporated via skin or the respiratory tract [2–8]. For propyl alcohols, the respiratory pathway has been proven to be the predominating route [6]. For ethanol, the exact pathway is still unknown. The aim of this study was to assess whether inhalative and/or transdermal resorption is the underlying cause for positive EtG analyses in urine after ethanolic hand disinfection.

2. Materials and methods

2.1. Experimental protocol

Desderman® pure (Schülke & Mayr GmbH, Norderstedt) with 78.2 g 96% (v/v) ethanol/100 g and approx. 10% 2-propanol was used for multiple hand disinfection according to the DIN EN 1500:2011-05 standard [9]. Simulating a common working day in a clinic, 5 co-workers of our laboratory used 3 mL of the sanitizer 4 fold per hour. Altogether, 32 hand sanitations were done within 8 h. The sanitizer was dissipated on the palm, back and fingers of the hands

Abbreviations: EtG, Ethyl glucuronide.

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hold in front of the body 30–40 cm distant from the nose. The hands were rubbed until dryness which occurred after 40–45 s. This procedure was done in a room of 5 m × 2.3 m × 3 m. The test persons using the sanitizer and 2 additional persons merely exposed to the sanitizer vapor but without any dermal sanitizer contact were standing in a semicircle having small talk and thus normal breathing.

The same protocol was applied to hand sanitation under an exhauster with the following alterations: 2 persons were using 1 exhauster in parallel. The front of the exhauster was closed leaving a gap of approx. 25 cm for the forearm. Hands were rubbed under the exhauster until dryness. This took 40–45 s and thus essentially the same time as for hand disinfection without an exhauster.

Spot urine samples were collected into Urine Z Sarstedt Monovettes (Numbrecht, Germany) at baseline and 1, 2, 4, 6, 8, 10, 12, 14, and 24 h after the initial disinfection. Urines were stored at 4–8 °C for a maximum of 1 week until LC-MS/MS analysis.

2.2. LC-MS/MS

2.2.1. Instrumentation

MRM and MS^3 experiments were done with a 1260 Infinity LC (Agilent, Waldbronn, Germany) coupled to a MistraSwitch column oven (Maylab Analytical Instruments, Vienna, Austria), a QTrap 5500 mass spectrometer (AB Sciex, Darmstadt, Germany) and a PAL HTC-xt autosampler (CTC Analytics, Zwingen, Switzerland). Details regarding sample preparation and chromatography are described in [6,10].

2.2.2. Mass spectrometry

General settings for the MRM and MS^3 experiments were: ESI negative, curtain gas (CUR) 50 psi nitrogen, ion spray (IS) −3500 V, temperature (TEM) 600 °C, nebulizer gas (GS 1) 50 psi zero air, turbo gas (GS 2) 50 psi nitrogen.

2.2.3. MRM experiments

Ethyl glucuronide 221–75 (collision energy (CE) −20 V, declustering potential (DP) −40 V), 221–85 (CE −22 V, DP −30 V), ethyl glucuronide-D5 226–85 (CE −22 V, DP −25 V), ethyl sulfate 125–97 (CE −22 V, DP −25 V), 125–80 (CE −40 V, DP −35 V), ethyl sulfate-D5 130–98 (CE −24 V, DP −20 V), propyl glucuronides 235–75 (CE −20 V, DP −110 V), 235–85 (CE −22 V, DP −105 V), dwell time 50 ms per MRM. The detection limit (LoD) for EtG in the MRM-mode was 0.02 mg/L. EtG and ethyl sulfate analysis was validated according to the guidelines of the GTFCh [11].

2.2.4. MS^3 experiments for qualitative EtG confirmation

A time period 1 from 0 to 4.5 min was used for the MRM experiments for ethyl glucuronide, ethyl glucuronide-D5 and the propyl glucuronides with a dwell time of 35 ms per MRM (the same transitions as described above). Parallel we used the trap-functionality of the Sciex QTrap 5500 to obtain MS^3-spectra for qualitative EtG confirmation. To achieve that, Q1 was used for separation of the 1st precursor ion of m/z 221. Q2 was used as a collision cell with a collision energy of −16 V, CAD set at high, declustering potential −85 V. Q3 was used for separation of the 2nd precursor ion of m/z 203 (corresponding to a loss of H_2O between Q1 and Q3, 221–203) and as linear ion trap with a fixed fill time of 200 ms, excitation time 20 ms, excitation energy 45 mV, scan range m/z 70–170. Time period 2 (4.5–15 min) was used only for MRM of ethyl sulfate and ethyl sulfate-D5 (dwell time 100 ms per MRM).

Creatinine was determined on an Olympus AU680 analyzer (Beckman Coulter, Krefeld, Germany), using a modification of the Jaffe method (test kit from Thermo Fisher Scientific Microgenics, Passau, Germany).

3. Results

Multiple hand disinfection with desderman® pure according to the EU standard [9] caused distinctly elevated EtG concentrations of up to 2.1 mg/L or 1.7 mg/g creatinine in the urine of 4 out of 5 sanitizer users. Even 2 controls which were merely exposed to the sanitizer vapor but without any dermal sanitizer contact showed maximum EtG concentrations of 0.6 mg/L or 0.8 mg/g creatinine (Fig. 1).

EtG results above the forensic cut-off of 0.1 mg/L were obtained even 6 h after the last sanitizer use or after the last passive exposition to the sanitizer vapor (Fig. 1).

Ethyl sulfate, considered an even more specific marker of ethanol consumption, was detectable in every EtG positive urine sample. Data are not shown here because EtS is not directly related to the aim of the present study, being the question whether ethanol is incorporated via skin or the respiratory tract during hand disinfection.

Qualitative LC-MS/MS analysis of 2-propyl glucuronide (a metabolite of 2-propanol which is another component of desderman® pure) revealed distinct signals for EtG positive urines (Fig. 2) but not for EtG negative samples.

Hand disinfection under an exhauster prevented the inhalation of ethanol vapor from the sanitizer. Following from this, the EtG excretion was distinctly reduced when compared to that after hand disinfection without an exhauster (Fig. 1B and C). Maximum EtG/creatinine ratios were 1.7 mg/g after hand disinfection without an exhauster (Fig. 1B) and 0.09 mg/g when using an exhauster (Fig. 1C). The latter was obtained after 24 fold use of the sanitizer (Fig. 1C). The corresponding value from the same person and 24 fold sanitizer use without an exhauster was 0.6 mg/g instead (Fig. 1B).

4. Discussion

Our experiments were solely performed by employees of our lab on a common working day. Sources of ethanol intake or unintended ethanol exposition in addition to the sanitizer experiments were reliably excluded and thus cannot explain the positive EtG findings described here.

EtG excretion after ethanolic hand disinfection has been described already e.g. in [3]. However, the pathway of the ethanol incorporation is since then under discussion [2–8]. Commenting their own results from [3] in [5], the authors "... do not believe that ethanol vapor contributed significantly to the ethanol exposure or urinary EtG concentrations".

This conclusion is not supported by our data, pointing clearly to inhalation but not to significant transdermal resorption of the sanitizer ethanol during hand disinfection. Transdermal resorption of ethanol and propyl alcohols from disinfectants has been studied also in [12,13]. After applying 20 mL of different alcohol-containing disinfectants with a 200 cm^2 gauze swab on the skin for 10 min, the authors did not find a statistically significant increase of the ethanol and/or propanol blood concentrations within 1 h after the application, regardless of whether the alcohols were used as single preparation or in combination [12,13]. Similar results were obtained after multiple use of a propyl alcohol-based hand sanitizer and analysis of propyl alcohol glucuronides in spot urine samples obtained during the experiments and until 16 h after the last sanitizer use [6]. Distinct amounts of propyl glucuronides were found in urine after regular multiple sanitizer use but not after using the sanitizer under an exhauster, preventing any propyl alcohol inhalation [6].

Our present study, using an ethanolic hand disinfectant, confirms the findings from [6] and again clearly points to an inhalative resorption of the sanitizer vapor. We consider this the