

The Effect of the Use of Mouthwash on Ethylglucuronide Concentrations in Urine

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Abstract

Two studies were performed to evaluate the effect of alcohol containing mouthwash on the appearance of ethyl glucuronide (EtG) in urine. In the first study, 9 volunteers were given a 4-oz bottle of mouthwash, which contained 12% ethanol. They gargled with all 4 oz. of the mouthwash at intervals over a 15-min period. All urine samples were collected over the next 24 h. Of 39 provided urine samples, there were 20 > 50 ng/mL, 12 > 100 ng/mL, 5 > 200 ng/mL, 3 > 250 ng/mL, and 1 > 300 ng/mL. The peak concentrations were all within 12 h after the exposure. In the second study, 11 participants gargled 3 times daily for 5 days. The first morning void was collected. Sixteen of the 55 submitted samples contained EtG concentrations of greater than 50 ng/mL. All of them were less than 120 ng/mL. These studies show that incidental exposure to mouthwash containing 12% ethanol, when gargling according to the manufacturer's instructions, can result in urinary EtG values greater than 50 ng/mL. All specimens were negative for ethanol. The limits of detection and quantitation for the EtG testing were 50 ng/mL.

Introduction

Traditionally, recovering alcohol addicts have been monitored for compliance to an abstinence program through testing for ethanol in randomly collected urine samples. (1) Interpretation of a positive result may be problematic because ethanol may be present in an unpreserved urine specimen because of fermentation (2). This can occur when glucose and yeast are both present in the urine, especially if a urine specimen is stored or shipped without refrigeration in the warm weather.

An ideal marker for monitoring an individual for the ingestion of ethanol would be a direct metabolite that is formed only in vivo. Ethyl glucuronide (EtG) has proven to be useful in the investigation of positive urine ethanol tests.

EtG is a minor metabolite of ethanol. It is only formed in vivo as a consequence of ethanol consumption. A few oxidation processes (via alcohol dehydrogenase, catalase, and the microsomal ethanol-oxidizing system) metabolize approximately 95% of ingested ethanol to acetaldehyde; this is further oxidized to acetic acid (acetate). Most of the remainder of ethanol is excreted unchanged in urine, breath, and sweat. However, a very small amount [0.02% in human tests) of a dose of ethanol is conjugated with uridine 5'-diphospho-glucuronic acid (UDPGA) to form EtG (3)]. EtG is excreted into the urine. Because EtG can be detected in the urine for up to 5 days after heavy ethanol consumption, this compound is regarded as a biomarker of excessive ethanol consumption and, potentially, relapses (4). A positive finding that EtG is present in the urine of an individual provides a strong indication that the person was recently drinking an alcoholic beverage, even if the ethanol itself is no longer detectable. The presence of EtG in urine is a specific and relatively long-lasting marker of ethanol ingestion. (5) Ethylglucuronide (EtG) is routinely analyzed in urine samples for the purpose of monitoring recovering addicts who are in an alcohol recovery program.

EtG is eliminated into the urine. Urinary concentrations of the compound up to 1.3 million ng/mL have been measured. An examination of the EtG concentrations in 252 clinical urine samples selected at random from outpatients undergoing treatment of alcohol and drug dependence ranged from 0 to 1.2 million ng/mL with 159 (63%) being positive for EtG (6). Of the positive urine samples, 32% showed EtG values below 100 mg/L and 32% were below 10,000 ng/mL. Although the liquid chromatography-mass spectrometry (LC-MS) analysis used was sensitive to 100 ng/mL, the authors suggested "a higher reference limit than the 100 ng/mL currently used should be considered for routine use".

As the utilization of this test has become more common, it has become necessary to determine if exposure to sources of ethanol other than consumption of an alcoholic beverage can be responsible for the presence of EtG in an individual's urine sample.

The purpose of the studies presented here was to determine the effect of the use of alcohol containing mouthwash on the appearance of EtG in urine samples.

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Experimental

Study 1

This study consisted of nine participants. All participants were to abstain from alcohol in any form for a minimum of 5 days prior to the start of the study. All participants provided a urine specimen prior to the first mouthwash exposure. This sample was required to be negative for alcohol (detection limit 0.01 g/dL) and EtG (detection limit 50 ng/mL). Each participant was given one 4-oz. bottle of commercially available mouthwash (Cepacol®) that had an alcohol concentration of 12%. The alcohol content was determined by headspace gas chromatography (GC). The 4-oz. bottles of mouthwash were purchased at a local pharmacy. The participants were instructed to take a mouthful of the mouthwash and to gargle for 30 s and then expectorate. This was to be repeated until the entire 4 oz. had been consumed and was to be completed over a 15-min period. All urinary voids were self-collected over the following 24-h period. The time of the void was noted. The pre-study specimens and any specimens collected at the laboratory

were placed into refrigeration within an hour of collection. Specimens collected outside of the laboratory setting were kept a room temperature until they were brought to the laboratory the day after the gargling took place. The specimens were kept refrigerated until aliquoted for analysis. Once all of the specimens were provided the analysis was completed within 24 h. Analyses included the measurement of EtG, ethanol, creatinine, nitrite, and pH.

Study 2

In this study, the 11 participants were subjected to the same qualifying criteria listed in Study 1. Each subject was given a bottle of commercially available mouthwash (Cepacol), which contained 12% alcohol. The participants were instructed to gargle with the mouthwash according to the manufacturer's instructions after each meal. In this study all participants gargled 3 times each day for 5 days. The first morning urinary void was collected each day and delivered to the laboratory. The specimens remained refrigerated until the time of analysis, which was performed daily. Analyses included the measure-

Table I. Distribution of EtG Results and Time Intervals of Sample Collection from Individuals Who Gargled and Expecterated 4 oz. of Mouthwash (12% ethanol) over a 15-min Period*

Donor Id	Time	Elapsed Time	EtG (ng/mL)	Creat (mg/dL)	Donor Id	Time	Elapsed Time	EtG (ng/mL)	Creat (mg/dL)
1: ELH-Pre Study Void	5:42 PM		ND	67	5: LML-Pre Study Void	12:00 PM		ND	108
Initial Gargle	5:50 PM	0:00			Initial Gargle	12:20 PM	0:00		
1-A: ELH-1	6:30 PM	0:40	ND	128	5-A: LML-1	1:50 PM	1:30	345	152
1-B: ELH-2	7:04 PM	1:14	237	30	5-B: LML-2	5:45 PM	5:25	251	130
1-C: ELH-3	9:53 PM	4:03	72	14	5-C: LML-3	8:15 PM	7:55	102	75
1-D: ELH-4	1:40 AM	7:50	ND	20	5-D: LML-4	9:12 PM	8:52	103	112
1-E: ELH-5	7:00 AM	13:10	ND	208	5-E: LML-5	6:45 AM	18:25	70	136
2: DS-Pre Study Void	11:30 AM		ND	87	6: EMA-Pre Study Void	5:00 PM		ND	236
Initial Gargle	11:33 AM	0:00			Initial Gargle	6:10 PM	0:00		
2-A: DS1	1:50 PM	2:17	261	133	6-A: EMA-1	6:52 PM	0:42	ND	228
2-B: DS2	4:05 PM	4:32	94	96	6-B: EMA-2	10:10 PM	4:00	ND	159
2-C: DS3	9:15 PM	9:42	0	140	6-C: EMA-3	6:08 AM	11:58	ND	160
2-D: DS4	3:30 AM	15:57	0	102	7: SON-Pre Study Void	10:49 PM		ND	205
2-E: DS5	6:45 AM	19:12	82	86	Initial Gargle	11:00 PM	0:00		
2-F: DS6	10:14 AM	22:41	0	66	7-A: SON-1	3:25 AM	4:25	157	207
2-G: DS7	11:20 AM	23:47	0	83	7-B: SON-2	7:07 AM	8:07	72	234
3: RM-Pre Study Void	3:19 PM		0	24	7-C: SON-3	8:50 AM	9:50	244	163
Initial Gargle	6:20 PM	0:00			7-D: SON-4	10:50 AM	11:50	117	96
3-A: RM-1	10:12 PM	3:52	73	137	8: AMC-Pre Study Void	12:20 PM		ND	207
3-B: RM-2	12:02 AM	5:42	0	62	Initial Gargle	12:55 PM	0:00		
3-C: RM-3	6:35 AM	12:15	0	84	8-A: AMC-1	4:45 PM	3:50	154	202
4: LEV-Pre Study Void	4:45 PM		ND	12	8-B: AMC-2	8:35 PM	7:40	63	180
Initial Gargle	4:55 PM	0:00			8-C: AMC-3	10:55 PM	10:00	ND	128
4-A: LEV-1	6:10 PM	1:15	ND	32	9: NR-Pre Study Void	3:12 PM		ND	190
4-B: LEV-2	7:05 PM	2:10	51	41	Initial Gargle	3:26 PM	0:00		
4-C: LEV-3	8:11 PM	3:16	ND	25	9-A: NR-1	8:32 PM	5:06	122	177
4-D: LEV-4	9:40 PM	4:45	91	225	9-B: NR-2	9:19 AM	17:53	ND	138
4-E: LEV-5	11:00 PM	6:05	0	62					
4-F: LEV-6	3:40 AM	10:45	90	208					
4-G: LEV-7	5:10 AM	12:15	102	226					

* All urine voids over the 24 h following the 15-min study period were collected and analyzed for EtG.

ment of EtG, ethanol, creatinine, nitrite, and pH. None of the participants in Study 1 participated in Study 2.

Analytical

Aliquots (50 μ L) of the urine samples were fortified with internal standard (EtG-d₅, Medichem, Germany), acidified with 25 μ L 0.5N HCl, extracted with 1.0 mL of acetonitrile, centrifuged, and concentrated under a stream of nitrogen. The dried extracts were reconstituted in acetone (250 μ L) and injected into a Micromass Quattro Micro LC–tandem MS (Waters Corp., Milford, MA) with negative ion electrospray. Separation was accomplished with a Betasil Silica column (50 mm \times 2.1-mm i.d. 5 micron, Thermo Electron Corp., Waltham, MA). The elution was isocratic, and the mobile phase was a mixture of ammonium acetate buffer (10%) and acetonitrile (90%). This is a modification of previously reported methods (6). Both the limit of detection and the limit of quantitation was 50 ng/mL. Results \geq 50 ng/mL were quantitated and reported. Any result that had the appearance of EtG but quantitated at < 50 ng/mL was reported as negative.

The measurement for creatinine was made with the SYVA cR Perfect reagent kit (Syva Corp., Dade Behring, Cupertino, CA). Nitrite was measured using SYVA nT Perfect and the pH was value was determined with SYVA pH Perfect. All of these urine chemistry measurements were made on an Olympus 400.

Ethanol determination of both the mouthwash and the urine samples was performed by headspace GC.

Results

Study 1

A total of 39 postexposure urine samples were provided. Of these there were 22 that were greater than 50 ng/mL, 12 of 22 were greater than 100 ng/mL, 5 of 22 were greater than 200 ng/mL, 3 of 22 were greater than 250 ng/mL and one sample was greater than 300 ng/mL. All of the EtG results of this study along with the time of the gargling and the timing of each sample collection are provided in Table I. The first appearance of EtG in a urine sample was at 1 h, 30 min. All peak concentrations were achieved within 12 h, 15 min after the exposure. One donor (MW6A) did not show the presence of EtG in any of the three provided urine samples (Figure 1). An interview of this donor did not reveal any medical or procedural

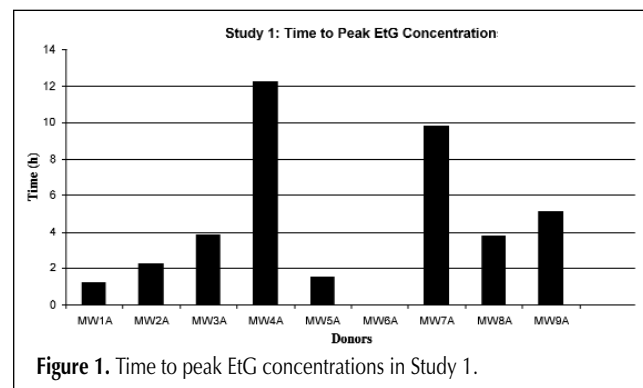


Figure 1. Time to peak EtG concentrations in Study 1.

information that would have made these results predictable. All specimens were negative for ethanol at the detection limit of 0.01 G/dL.

Study 2

Neither alcohol nor EtG were detected in any of the pre-dose samples. EtG was not detected in any samples on Day 1. Sixteen of the 55 submitted samples contained EtG greater than 50 ng/mL. All of them were less than 120 ng/mL (Table II).

The mouthwash was also analyzed for EtG content and found to be negative at the detection limit of 50 ng/mL.

Discussion

In addition to the possibility of ethanol being present in urine specimens because of fermentation the issue of incidental exposure via usage of commercially available food, medicinal, or personal hygiene products have been offered as reasons for the presence of ethanol in a urine specimen.

Mouthwash has been suggested as a legitimate reason for the appearance of ethanol or EtG in a urine sample. In both of the studies presented here, a commercially available brand of mouthwash that contained 12% ethanol and verified to not contain EtG was used.

In order for EtG to be present in the urine specimens of the study participants, alcohol must have entered the circulatory system. It is reasonable to believe that small amounts of the mouthwash may have been ingested and some may have been absorbed through the buccal cavity.

EtG can only be present in a urine specimen because of in vivo metabolism; therefore, the source of the ingested alcohol must be determined. These studies have shown that routine use of mouthwash which contains 12% ethanol is more likely to not cause the presence of EtG in a urine specimen. In study

Table II. Results of EtG Analysis in Samples Collected from Study 2: Gargling with Mouthwash Three Times Daily Following Manufacturer's Instructions*

ID#	Pre-Dose EtG (ng/mL)	Day 1 EtG (ng/mL)	Day 2 EtG (ng/mL)	Day 3 EtG (ng/mL)	Day 4 EtG (ng/mL)	Day 5 EtG (ng/mL)
MW1	ND [†]	ND	ND	ND	71	91
MW2	ND	ND	ND	60	ND	ND
MW3	ND	ND	90	54	ND	ND
MW4	ND	ND	ND	ND	52	ND
MW5	ND	ND	79	117	ND	ND
MW6	ND	ND	ND	ND	ND	ND
MW7	ND	ND	ND	ND	ND	62
MW8	ND	ND	ND	ND	ND	ND
MW9	ND	ND	99	63	70	108
MW10	ND	ND	ND	ND	ND	ND
MW11	ND	ND	93	ND	93	53

* Detection limit = 50 ng/mL.

[†] ND, none detected.

Table III. All Positive Routine Samples Submitted for EtG Testing*

EtG (ng/mL)	Number of Samples	Percent of Total
250–499	1119	23
500–999	855	18
1000–9999	1530	31
10,000–100,000	816	16
> 100,000	289	6
Totals	4609	100

* The applied laboratory cutoff was 250 ng/mL; 77% of these results were greater than or equal to 500 ng/mL.

2, only 16 of 55 submitted samples contained EtG above 50 ng/mL. Fourteen of these were < 100 ng/mL, and none of these were above 120 ng/mL.

In an intense exposure to mouthwash as was assimilated in Study 1, EtG can be present in the urine in higher concentrations but the peak concentrations were achieved in 1.5 to 12.25 h.

A survey of patient data from the routinely submitted population of recovering healthcare professionals in monitoring programs showed that most EtG concentrations in urine are greater than 500 ng/mL (Table III). This information was taken from analyses performed at National Medical Services, Inc. The routine reporting limit that was applied is 250 ng/mL. The number of specimens which would show the presence of EtG between 50 and 100 ng/mL is not known.

Conclusions

Alcohol may be absorbed into the body from the use of alcohol-containing mouthwash. EtG formed from the alcohol may be present in the urine samples of individuals whose only

source of alcohol exposure was the use of such mouthwash. Findings of EtG in urine specimens must be investigated in order to determine the origin of the alcohol exposure. We have neither established nor recommended a particular cutoff. We do, however, conclude that the presence of EtG in an individual's urine specimen is only possible if ethanol was metabolized in the body. We recommend that all positive EtG results should be reviewed by a qualified individual such as a Medical Review Officer, who can conduct an investigation in order to determine the source of the ethanol ingestion.

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