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The Effect of Yogurt on Acetaminophen Absorption by Activated Charcoal and Burnt Toast

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Although acetaminophen overdose can be treated with N-acetylcysteine, activated charcoal is useful in preventing absorption of acetaminophen from the gut. Mixing activated charcoal with yogurt may make the dose more palatable. We investigated effects of yogurt on absorption of acetaminophen by burnt toast or activated charcoal in intestinal fluid using an in vitro model. The aliquots of phosphate buffer saline (PBS) were supplemented with high concentrations of acetaminophen after adjusting the pH to 7.2 (to mimic intestinal fluid). Then specimens were treated with various dosages (15 mg/mL, 25 mg/mL, or 50 mg/mL) of activated charcoal or burnt toast. A small amount of fluid was withdrawn at 0, 5, 10, 20, and 30 min and acetaminophen concentrations were measured by the fluorescence polarization immunoassay (FPIA). We also treated other aliquots of PBS buffer containing acetaminophen with activated charcoal and yogurt or burnt toast and yogurt. Then small aliquots were withdrawn at specific time intervals to determine concentrations of acetaminophen. Activated charcoal was very effective in removing acetaminophen from intestinal fluids and the presence of yogurt insignificantly affected such absorptions. In contrast, burnt toast had a modest effect on removing acetaminophen from fluids but yogurt significantly increased the capability of burnt toast to absorb acetaminophen. However, the activated charcoal/yogurt combination is more effective than the burnt toast/yogurt combination for absorbing acetaminophen. J. Clin. Lab. Anal. 21:393–397, 2007. © 2007 Wiley-Liss, Inc.

Key words: acetaminophen; burnt toast; simulated intestinal fluid; immunoassay

INTRODUCTION

Acetaminophen (N-acetyl-p-aminophenol) is an analgesic and antipyretic agent. This drug is available alone or in combination with other medicines for oral use. Although acetaminophen is a safe drug, accidental overdose may even cause death due to acute hepatic necrosis. In 2004, the American Association of Poison Control Centers received reports of 150 deaths due to acetaminophen poisoning. Hepatic damage is related to the metabolism of acetaminophen to N-acetyl-p-benzoquinoneimine. In the case of overdose excessive production of N-acetyl-p-benzoquinoneimine may saturate the glutathione deactivation system of the liver causing hepatotoxicity (1). Kapur et al. (2) reported that acetaminophen (paracetamol) was one of the most common drugs used by individuals for self poisoning. The authors commented that although administration of antidote, N-acetylcysteine significantly reduced mortality, other interventions such as prompt administration of activated charcoal, better airway management, and improved prehospital care may also improve the outcome (2). Graham et al. (3) recommended routine testing for acetaminophen in all overdosed patients who deny ingestion of that substance due to widespread use of this drug for self poisoning.

Because hepatotoxicity is the major toxic manifestation of acetaminophen overdose, patients with hepatic impairment are very susceptible to acetaminophen toxicity. Children consistently do well after acute acetaminophen poisoning probably due to increased metabolism of acetaminophen by sulfation. Children

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may also have greater glutathione concentration and liver regenerative capacity (4). Usually, toxicity occurs in adults after ingestion of at least 10 to 15 gm of acetaminophen and serum concentration usually exceeds 200 μg/mL. In one report, a woman who died of acetaminophen toxicity showed a serum level of only 51 μg/mL because hepatotoxicity of acetaminophen in alcoholic patients may occur with much lower serum concentrations of acetaminophen (5). The effectiveness of N-acetylcysteine in treating acetaminophen overdose is well established and is recommended to all patients with a risk of developing hepatotoxicity. Usually N-acetylcysteine is administered if serum acetaminophen concentrations in patients 4 hr after ingestion exceed 200 μg/mL using Rumack-Matthew nomogram, but use of this antidote for patients with serum acetaminophen concentration over 150 μg/mL is also common. However, patients with no risk factor may not require this antidote (6). Dosage of the antidote administered is crucial for successful management of patients overdosed with acetaminophen (7,8).

Despite the role of N-acetylcysteine in treating acetaminophen poisoning, activated charcoal is still used in treating patients overdosed with acetaminophen. Although it is usually recommended that activated charcoal should be given within 1 or 2 hr after ingestion of acetaminophen (9,10), Spiller et al. (11) recently reported that administration of activated charcoal may be effective even 4 hr after ingestion of acetaminophen. Buckley et al. (12) reported that activated charcoal reduces the need for N-acetylcysteine in treating patients overdosed with acetaminophen. Another study indicated that patients who received both activated charcoal and N-acetylcysteine had better outcome compared to those patients who only received N-acetylcysteine (13).

Toast is made of wheat and burnt toast is mostly composed of carbon (charcoal). If burnt toast is crushed into small particles and given orally it may prevent some absorption of acetaminophen from the gut. Moreover, it may be more palatable to mix burnt toast with yogurt. We tested the capability of a burnt toast and yogurt mix to absorb acetaminophen by using an in vitro model. Absorption of acetaminophen by activated charcoal as well as activated charcoal and yogurt mix was also studied for comparison.

MATERIALS AND METHODS

White bread was toasted in a toaster until it was completely burnt and turned black. Then it was crushed by hand to produce very small black particles. Activated charcoal, phosphate buffer saline (PBS), 2N hydrochloric acid, and acetaminophen were purchased from Sigma Chemical Company (St. Louis, MO). Acetaminophen concentrations were determined using the fluorescence polarization immunoassay (FPIA) and the AxSYM analyzer (both from Abbott Laboratories, Abbott Park, IL).

The FPIA assay for acetaminophen uses a polyclonal rabbit antibody against acetaminophen. The assay is linear up to an acetaminophen concentration of 200 μg/mL. Therefore, any specimen with expected acetaminophen concentration exceeding 200 μg/mL was diluted with drug-free serum prior to analysis. The limit of detection of the acetaminophen assay is 1.0 μg/mL and any acetaminophen concentration below 1 μg/mL was considered as "none detected."

The PBS buffer (10 ×) was diluted with 0.9% saline to achieve a PBS buffer concentration of 1 × (pH: 7.4). Then the pH of this buffer was further adjusted with 2N hydrochloric acid to obtain a pH of 7.2. This fluid was considered as simulated intestinal fluid as described earlier (14) and was used for further experiments. A stock solution of acetaminophen was prepared by dissolving 100 mg of acetaminophen in 10 mL of absolute ethanol. In the first series of experiments, 100 mL of fluid was supplemented with 4 mL of acetaminophen stock solution to target an acetaminophen concentration of 400 μg/mL. Then 10 mL aliquots of fluid were treated with 150, 250, or 500 mg of activated charcoal. The desired dosages of charcoal were 15 mg/mL, 25 mg/mL, or 50 mg/mL. We also used the same dosages of burnt toast, as 10 mL aliquots of the fluid were also treated with 150 mg, 250 mg, or 500 mg of burnt toast. After adding fluids to tubes already containing activated charcoal or burnt toast, a very small amount of specimen (150 μL) was withdrawn to measure acetaminophen concentration at 0 min. Then, fluid was allowed to mix with the activated charcoal or burn toast by gentle shaking using a mechanical shaker (Model 4651; Ames Company, Elkhart, IN). Another tube containing only fluid but no activated charcoal or burnt toast was also subjected to shaking (control).

Other than 0 min specimens, we also withdrew 150 μL of fluid from each tube at 5 min, 10 min, 15 min, 20 min, and 30 min intervals. Activated charcoal or burnt toast particles were separated from the fluid by centrifugation at the high speed (15,000 *g*) using a TDx centrifuge (Abbott Laboratories) and clear supernatant was analyzed for acetaminophen concentration. When expected concentration of acetaminophen in fluid exceeded the linearity of acetaminophen immunoassay, 1:1 dilution of specimen was necessary to produce a valid result. We used drug-free serum instead of dilution buffer for this purpose. Each acetaminophen measurement was performed in triplicate and values were expressed as the mean ± standard deviation (SD).

To further validate our initial results, in another set of experiments, 100 mL of fluid (after pH adjustment) was supplemented with 2.5 mL of stock acetaminophen solution to achieve a target concentration of 250 μg/mL. Then, 10 mL aliquots were treated with activated charcoal or burnt toast using the same protocol.

To ensure that 1:1 dilution of fluid with drug-free serum has no effect on validity of determination of acetaminophen using the FPIA, we prepared five fluid specimens containing known amounts of acetaminophen and then measured acetaminophen concentrations using the FPIA. We compared target acetaminophen concentrations with observed acetaminophen concentrations to evaluate any potential matrix effect.

In another set of experiments, another 100 mL of fluid (PBS buffer 1 × after adjusting pH to 7.2) was also supplemented with 4 mL of stock acetaminophen solution (target acetaminophen concentration: 400 μg/mL). Then 10-mL aliquots of supplemented fluid were added to tubes containing 150 mg, 250 mg, or 500 mg of activated charcoal along with yogurt. Although we varied the amount of activated charcoal, we did not vary the amount of yogurt. In each tube 0.2 mL of plain low fat yogurt was added, followed by mixing with activated charcoal using a vortex mixture. After that, 10 mL of fluid containing acetaminophen was added to each tube. The same combination of burnt toast and yogurt was also used for comparison. Again a small amount of specimen was withdrawn at 0 min, 5 min, 10 min, 20 min, and 30 min intervals for determination of acetaminophen concentration.

To further validate these results, we supplemented another 100-mL aliquot of the simulated intestinal fluid with 2.5 mL of stock acetaminophen solution to achieve a target concentration of 250 μg/mL. Then, 10-mL aliquots were treated with a combination of activated charcoal and yogurt or burnt toast and yogurt.

Finally, we treated a 10-mL aliquot of simulated fluid containing 400 μg/mL of acetaminophen with 50 mg/mL of burnt toast and 0.2 mL of yogurt for 30 min. Then, the acetaminophen concentration was measured and the fluid was further treated with activated charcoal (15 mg/mL) for 5 min and the concentration of acetaminophen was measured again.

Statistical analyses were done using independent *t*-test, two-tailed. A difference was considered statistically significant only at 95% confidence interval or higher ($P < 0.05$).

RESULTS

No matrix effect was observed when aliquots of simulated intestinal fluid were supplemented with various concentrations of acetaminophen and concentrations were measured by the FPIA assay. We observed good agreement between the target acetaminophen concentrations and the observed acetaminophen concentrations, indicating that a 1:1 dilution of fluid specimen with drug-free serum provides a valid matrix for the determination of acetaminophen concentration using the FPIA (data not shown).

Activated charcoal is very effective in removing acetaminophen from the simulated intestinal fluid. Complete removal of acetaminophen can be achieved within 5 min of treatment even with the lowest dosage of activated charcoal (15 mg/mL) (Table 1). Burnt toast only demonstrated moderate effects in absorbing acetaminophen from simulated intestinal fluid. For example, in the aliquot of fluid treated with 15 mg/mL of burnt toast, the observed concentration of acetaminophen was reduced from 381.2 μg/mL to 339.4 μg/mL after 5 min of treatment (Table 2).

Activated charcoal is also effective in removing acetaminophen when given in combination with yogurt. For example, the concentrations of acetaminophen were 374.2 μg/mL, 7.5 μg/mL, 3.1 μg/mL, and "none detected" at 0 min, 5 min, 10 min, 15 min, and 30 min, respectively, following treatment of fluid specimen with a combination of 15 mg/mL of activated charcoal and yogurt (Table 3). Surprisingly, the presence of yogurt significantly increased the absorption of acetaminophen by the burnt toast. For example, the concentration of acetaminophen was reduced from 374.2 μg/mL to 241.4 μg/mL after treatment of a fluid specimen with a combination of 50 mg/mL of burnt toast and yogurt for 5 min. Eventually, the concentration of acetamino-

TABLE 1. Removal of acetaminophen from the simulated intestinal fluid by activated charcoal†

Specimen	Control	Activated charcoal 15 mg/mL	Activated charcoal 25 mg/mL	Activated charcoal 50 mg/mL
	Acetaminophen concentration, μg/mL, mean (SD), n = 3			
Experiment 1: simulated intestinal fluid (pH 7.2) + acetaminophen				
0 min	384.4 (8.6)	378.4 (12.1)	386.6 (6.9)	376.2 (8.6)
5 min	379.2 (4.8)	ND*	ND*	ND*
10 min	384.4 (11.2)	ND*	ND*	ND*
20 min	380.2 (7.8)	ND*	ND*	ND*
30 min	378.4 (4.2)	ND*	ND*	ND*
Experiment 2: simulated intestinal fluid (pH 7.2) + acetaminophen				
0 min	245.4 (4.6)	247.7 (3.8)	243.8 (8.9)	250.2 (8.6)
5 min	241.6 (4.8)	ND*	ND*	ND*
10 min	249.8 (10.2)	ND*	ND*	ND*
20 min	244.2 (5.8)	ND*	ND*	ND*
30 min	253.1 (4.8)	ND*	ND*	ND*

*Significantly less than the corresponding control value by independent *t*-test, two tailed ($P < 0.05$).
ND,—.

TABLE 2. Removal of acetaminophen from the simulated intestinal fluid by burnt toast†

Specimen	Control	Burnt toast 15 mg/mL	Burnt toast 25 mg/mL	Burnt toast 50 mg/mL
	Acetaminophen concentration, μg/mL, mean (SD), n = 3			
Experiment 1: simulated intestinal fluid (pH 7.2) + acetaminophen				
0 min	384.4 (8.6)	381.2 (11.2)	386.6 (6.9)	386.2 (8.6)
5 min	379.2 (4.8)	339.4 (8.9)*	323 (6.6)*	324.7 (9.8)*
10 min	384.4 (11.2)	348.6 (6.6)*	329.2 (8.8)*	317.7 (4.2)*
20 min	380.2 (7.8)	351.6 (7.8)*	327.6 (8.4)*	330.2 (6.4)*
30 min	378.4 (4.2)	356.8 (5.2)*	338.2 (10.2)*	340.2 (7.8)*
Experiment 2: simulated intestinal fluid (pH 7.2) + acetaminophen				
0 min	243.6 (4.8)	241.0 (12.1)	244.6 (6.7)	240.2 (6.6)
5 min	240.1 (5.2)	214.6 (3.1)*	205.5 (9.2)*	197.8 (4.8)*
10 min	238.7 (9.2)	216.1 (4.6)*	206.5 (2.3)*	194.5 (4.6)*
20 min	244.8 (6.8)	213.0 (8.8)*	199.8 (4.6)*	189.4 (2.4)*
30 min	236.5 (5.2)	207.4 (7.2)*	196.8 (4.4)*	187.2 (6.1)*

*Significantly less than the corresponding control value by independent *t*-test, two tailed ($P<0.05$).

TABLE 3. Removal of acetaminophen from the simulated intestinal fluid by activated charcoal in the presence of yogurt†

Specimen	Control	Activated charcoal+yogurt (0.2 mL/mL) 15 mg/mL	Activated charcoal+yogurt (0.2 mL/mL) 25 mg/mL	Activated charcoal+yogurt (0.2 mL/mL) 50 mg/mL
	Acetaminophen concentration, μg/mL, mean (SD), n = 3			
Experiment 3: simulated intestinal fluid (pH 7.2)+acetaminophen				
0 min	369.4 (9.6)	374.2 (11.2)	376.2 (4.6)	376.4 (8.2)
5 min	364.2 (4.8)	7.5 (0.8)*	ND*	ND*
10 min	376.2 (10.2)	3.1 (0.6)*	ND*	ND*
20 min	370.8 (4.8)	ND*	ND*	ND*
30 min	378.2 (8.6)	ND*	ND*	ND*
Experiment 4: simulated intestinal fluid (pH 7.2)+acetaminophen				
0 min	234.7 (2.7)	235.9 (6.8)	232.0 (8.4)	243.6 (5.2)
5 min	239.8 (3.9)	ND*	ND*	ND*
10 min	234.5 (4.5)	ND*	ND*	ND*
20 min	236.0 (7.3)	ND*	ND*	ND*
30 min	241.2 (5.2)	ND*	ND*	ND*

*Significantly less than the corresponding control value by independent *t*-test, two tailed ($P<0.05$).
ND,—.

phen after 30 min of treatment with a combination of burnt toast and yogurt was 266.6 μg/mL. We observed a similar effect when another simulated fluid containing 250 μg/mL was treated with a combination of burnt toast and yogurt (Table 4).

The presence of burnt toast and yogurt does not have any effect on further absorption of acetaminophen from simulated intestinal fluid by activated charcoal. When an aliquot of fluid containing356.92 ± 13.2 (n = 3) μg/mL of acetaminophen was treated with a combination of burnt toast (50 mg/mL) and yogurt (0. m/mL), a significant reduction of acetaminophen level

TABLE 4. Removal of acetaminophen from the simulated intestinal fluid by burnt toast in the presence of yogurt†

Specimen	Control	Burnt toast+ yogurt (0.2 mL/mL) 15 mg/mL	Burnt toast+ yogurt (0.2 mL/mL) 25 mg/mL	Burnt toast+ yogurt (0.2 mL/mL) 50 mg/mL
	Acetaminophen concentration, μg/ml, Mean (SD), n = 3			
Experiment 3: simulated intestinal fluid (pH 7.2)+acetaminophen				
0 min	380.6 (10.2)	381.2 (11.2)	386.6 (6.9)	374.2 (6.4)
5 min	379.3 (8.6)	339.4 (8.9)*	323.4 (6.6)*	241.4 (11.2)*
10 min	383.6 (11.2)	348.6 (6.6)*	329.2 (8.8)*	226.2 (9.8)*
20 min	369.4 (12.2)	351.6 (7.8)*	327.6 (8.4)*	244.6 (8.6)*
30 min	384.2 (6.9)	356.8 (5.2)*	338.2 (10.2)*	256.6 (4.8)*
Experiment 4: simulated intestinal fluid (pH 7.2)+acetaminophen				
0 min	236.8 (1.7)	235.1 (4.8)	237.0 (5.4)	237.8 (6.2)
5 min	235.8 (4.9)	204.6 (2.9)*	196.4 (4.6)*	181.4 (2.1)*
10 min	239.0 (2.5)	201.5 (5.6)*	192.6 (3.8)*	172.6 (4.7)*
20 min	236.9 (6.3)	202.1 (4.6)*	177.9 (4.4)*	170.1 (2.5)*
30 min	240.1 (4.2)	198.6 (3.1)*	180.3 (3.6)*	167.2 (4.8)*

*Significantly less than the corresponding control value by independent *t*-test, two-tailed ($P<0.05$).

to 223.7 ± 4.4 (n = 3) μg/mL was observed. When we added activated charcoal (15 mg/mL) to this mixture, the concentration of acetaminophen was dramatically reduced to3.6 ± 0.8 (n = 3) μg/mL with an additional 5 min of treatment.

DISCUSSION

Acetaminophen poisoning is common and activated charcoal is effective in reducing the absorption of acetaminophen. Burnt toast can be easily made and may be used as a homemade remedy for acetaminophen poisoning before the patient can be transferred to the hospital or paramedic personnel arrive. Our initial in vitro model is the preliminary first step to study such a possibility. Hoegberg et al. (15) recently reported that the presence of yogurt reduced the acetaminophen absorption capacity of activated charcoal in vitro by 9–13%. We observed a slight reduction in absorption capacity of activated charcoal in the presence of yogurt. More interestingly, yogurt significantly increased the capacity of burnt toast to absorb acetaminophen. The mechanism of such effects is unclear at this point.

Our in vitro results indicate that burnt toast is capable of reducing the concentration of acetaminophen in intestinal fluid and in the presence of yogurt such reductions are more significant. If these results can be validated with case studies then one can speculate that giving a patient a mixture of burnt toast and yogurt may prevent some absorption of acetaminophen during the time period when the patient is being transferred to the hospital. In addition, our in vitro study also indicated that when activated charcoal is added to the simulated

intestinal fluid already treated with burnt toast and yogurt, further reductions of acetaminophen concentrations were observed due to absorption of acetaminophen by activated charcoal. Therefore, neither burnt toast nor yogurt interferes with absorption of acetaminophen by activated charcoal in our in vitro model. Provided this phenomenon is also valid in vivo, a patient already given burnt toast and yogurt can be further treated with activated charcoal to further prevent absorption of acetaminophen.

We conclude that a combination of burnt toast and yogurt is partially effective in removing acetaminophen in vitro in simulated intestinal fluid compared to activated charcoal. However, such therapy can only be a first-line defense before proper medical treatment can be initiated and is not a replacement for treatment of acetaminophen poisoning using activated charcoal and antidote.

REFERENCES

1. White ML, Liebelt EL. Update on antidotes for pediatric poisoning. Pediatr Emerg Care 2006;22:740–746.
2. Kapur N, Turnbull P, Hawton K, Simkin S, et al. The hospital management of fatal self-poisoning in industrialized countries: an opportunity for suicide prevention? Suicide Life Threat Behav 2006;36:302–312.
3. Graham CA, Irons AJ, Munro PT. Paracetamol and salicylate testing: routinely required for all overdosed patients? Eur J Emerg Med 2006;13:26–28.
4. Johnston SC, Pelletier LL. Enhanced hepatotoxicity of acetaminophen in the alcoholic patients. Two case reports and a review of literature. Medicine (Baltimore) 1997;76:185–191.
5. Bryant S, Bellamy L, Paloucek F, Wahl M. Acute acetaminophen poisoning in children: kids aren't just little adults. J Emerg Med 2003;24:472–473.
6. Brandwene EL, Williams SR, Tunget-Johnson C, et al. Refining the level for anticipated hepatotoxicity in acetaminophen poisoning. J Emerg Med 1996;14:691–695.
7. Little M, Murray L, McCoubrie D, Daly FF. A potentially fatal prescribing error in the treatment of paracetamol poisoning. Med J Aust 2005;21:535–536.
8. Kanter MZ. Comparison of oral and IV acetylcysteine in the treatment of acetaminophen poisoning. Am J Health Syst Pharm 2006;63:1821–1827.
9. Chamberlain JM, Gorman RL, Oderda GM, et al. Use of activated charcoal in a poisoning with acetaminophen: a new dose for N-acetylcysteine? Ann Emerg Med 1993;22:1398–1402.
10. Christophersen AB, Levin D, Hoegberg LC, et al. Activated charcoal alone or after gastric lavage: a simulated larger paracetamol intoxication. Br J Clin Pharmacol 2002;53:312–317.
11. Spiller HA, Winter ML, Klein-Schwartz W, Bangh SA. Efficacy of activated charcoal administration more than four hours after acetaminophen overdose. J Emerg Med 2006;30:1–5.
12. Buckley NA, Whyte IM, O'Connell DL, Dawson AH. Activated charcoal reduces the need for N-acetylcysteine treatment after acetaminophen (paracetamol) overdose. J Toxicol Clin Toxicol 1999;37:753–757.
13. Spiller HA, Krenzelok EP, Grande GA, et al. A prospective evaluation of the effect of activated charcoal before oral administration of N-acetylcysteine in acetaminophen overdose. Ann Emerg Med 1994;23:519–523.
14. Dasgupta A, Veras E. Effectiveness of activated charcoal and equilibrium dialysis in removing Asian, American, Siberian and Indian ginseng from human serum. Clin Chim Acta 2006;367;144–149.
15. Hoegberg LC, Christophersen AB, Christensen HR, Angelo HR. Comparison of the absorption capacities of an activated charcoal-yogurt mixture versus activated charcoal water slurry in vivo and in vitro. Clin Toxicol (Phila) 2005;43:269–275.