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INSTRUCTIONAL DESIGN AND ASSESSMENT

An Interactive Lesson in Acid/Base and Pro-Drug Chemistry Using Sodium Gamma-Hydroxybutyrate and Commercial Test Coasters

Nathaniel A. Page,* Meaghan Paganelli,* Kathleen M.K. Boje, and Ho-Leung Fung, PhD

School of Pharmacy and Pharmaceutical Sciences, University at Buffalo

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Objective. To develop a classroom activity that applied pertinent pharmaceutical concepts to examine the use and limitations of a commercially available test drink coaster in detecting the presence of a date-rape drug, sodium γ-hydroxybutyrate (NaGHB), in beverages.
Design. An activity exercise involving a combination of self-study, hands on participation, and classroom discussion was developed. Topics incorporated into the activity were drug-assisted rape, the concepts of false positives and negatives, and prodrug and pH chemistry.
Assessment. Based on questionnaires completed by the students, the intended concepts were reinforced and students demonstrated an increased awareness of the potential shortcomings of the commercial test devices. The activity was well received by the majority of students.
Conclusion. The developed activity stimulated student awareness and interest in several principles relevant in pharamceutical education, including drug-assisted rape, consumer-based drug testing of NaGHB, and the chemical basis for its limitations. The activity requires no special equipment other than the drink coasters and can be easily completed in one 2-hour classroom session.

Keywords: GHB, drug of abuse, sodium hydroxybutyrate, laboratory tests

INTRODUCTION

Sodium γ-hydroxybutyrate (GHB, Figure 1) is used by humans for various purposes. Xyrem, a commercial brand of GHB that has been approved by the Food and Drug Administration, is used for the treatment of "a small population of patients with narcolepsy who experience episodes of cataplexy, a condition characterized by weak or paralyzed muscles."[1] More frequently, the drug is used as a "club" or "party" drug, and for its various purported benefits such as muscle-building.[2] A much more insidious use of GHB is in drug-assisted rape, wherein the drug is slipped unknowingly into the beverage of a victim to induce unconsciousness.

There are many street names for GHB, including "Grievous Bodily Harm," "Liquid Ecstasy," and "Liquid X." The symptoms and severity of GHB intoxication, which resemble those from ethanol, are dose-dependent. After ingesting a low dose (10 mg/kg of body weight), the most common symptoms are amnesia and hypotonia. At a dose of 20-30 mg/kg of body weight, there is an induction of normal rapid-eye-movement (REM) and non-REM sleep patterns. Marked anesthesia can occur at a dose of about 50 mg/kg of body weight. At doses greater than 50 mg/kg, the drug becomes life threatening and symptoms may include decreased cardiac output, severe respiratory depression, coma, apparent seizure, and vomiting. After ingestion, 2 compounds, the lactone and alcohol analogs γ-butyrolactone (GBL) and 1, 4-butanediol (BD; Figure 1), are rapidly converted to GHB in the body. For this reason, these compounds are classified as prodrugs of GHB.[3,4] These analogs produce the same pharmacological and toxicological effects in humans, and heavy abuse of them, like that of GHB itself, has led to sustained coma and death.[5,6]

These 2 compounds are also much easier to obtain than GHB itself, because being solvents used in industrial applications, they are less strictly regulated by the US Drug Enforcement Agency. While explicit marketing of these analogs is illegal, they are often sold ostensibly as "dietary supplements" with the analog's name disguised, even though warning labels are affixed stating that they are not intended to be ingested.[7] Because of the use of these compounds in date rape and abuse, the availability of simple detection devices (in contrast to methods requiring sophisticated instruments[8]) would obviously be

Corresponding Author: Ho-Leung Fung, PhD. Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, Buffalo, NY 14260. Tel: 716-645-2842 x 222. Fax: 716-645-3693. E-mail: hlfung@buffalo.edu
*Mr. Page was a PharmD student at the University at Buffalo, and Ms. Paganelli was a high school student at the time they contributed to this work.

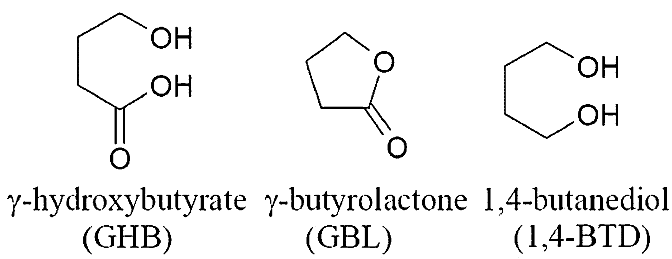

Figure 1. Structures of γ-hydroxybutyrate and its analogs.

helpful to potential victims, parents, and possibly the police. Such a device is indeed available commercially. Drink Safe Coaster (Drink Safe Technology, Anderson, Ala), is a drink coaster that contains "chemical" spots on which tests for the presence of GHB and ketamine (another date-rape drug) can be carried out. To conduct the test for GHB, a drop of the suspicious drink is placed on the appropriate spot on the coaster. The presence of GHB is indicated if the color of the spot changes from green to blue upon drying.

Several obvious questions can be raised about this test:

(1) What is the chemical basis for this test?
(2) How specific is this test for GHB, ie, when might one see false positives or false negatives? If these false outcomes are present, how can they be interpreted in light of the chemical basis for the test?
(3) Does this test work for the prodrugs of GHB, ie, GBL and BD? Again, why or why not, in light of the chemical basis for this test?

A series of simple tests to determine the answers to these questions is described here. A classroom activity involving these tests was designed, and its effectiveness in teaching or reinforcing several pharmaceutical concepts including consumer-based drug testing, false positive/negative results, prodrug, and pH chemistry was evaluated.

The learning objectives for this activity were as follows:

(1) To use the biomedical literature to identify and read articles on date rape drugs;
(2) To explore the utility and limitation of existing consumer devices to detect these drugs;
(3) To apply the principles of acid/base chemistry and pH indicators;
(4) To describe the principles of false positives and negatives in testing; and
(5) To discuss the concept of prodrugs, particularly those related to GHB.

DESIGN

The experimental design involved the testing of GHB, GBL, and BD by themselves, and when GHB was mixed with 0.1N HCl, 0.1N NaOH, wine, beer, cola or orange juice. Control experiments without GHB were also performed with different sodium and potassium salts of weak acids (bicarbonate or citrate), HCl and NaOH alone. The concentration of NaGHB in these "simulated" drinks was estimated based on its possible use in date rape. For a young adult female with a body weight of 50 kg (approximately 110 pounds), an acute dose of 10 mg/kg body weight (a low dose) would be equivalent to about 0.5 gram. If this dose is added to an 8-ounce drink, the concentration of GHB would be 0.5g/240 ml (1 US fl oz $\approx$30 ml), approximately equal to 0.2% w/v, or 0.0165 molar (the molecular weight of NaGHB is 127).

Two sessions were conducted to evaluate this classroom activity in different student populations. One 2-hour session was presented in the course *Apothecarial Adventures*, with an enrollment of 31 lower-division honors students (primarily freshmen and sophomores). Another 2-hour session was presented in the course *Case Studies in Pharmaceutical Sciences*, with 38 upper-division students (primarily juniors and seniors in the pharmaceutical sciences and pharmacy majors). Student demographics are presented in Table 1.

In preparation for the activity, students were required to use their literature search skills to identify, retrieve, and read an article on drug-assisted rape and/or consumer drug test kits from the biomedical literature. Students then wrote a 4-5 sentence synopsis of their paper and posted it,

Table 1. Demographics of Students Participating in an Interactive Lesson in Acid/Base and Pro-Drug Chemistry

	Students in PHC250, n = 31	Students in PHC331/431, n = 37
Student level		
Freshman	20	0
Sophomore	9	0
Junior	2	10
Senior	0	27
Gender		
Male	9	15
Female	22	22
Ethnicity		
Caucasian	27	14
Black	0	7
Asian	4	14
Hispanic	0	0
Other	0	2
Age, mean (SD)	18.1 (0.8)	22 (2.0)

PHC250: *Apothecarial Adventures*; PHC331/431: *Case Studies in Pharmaceutical Sciences*

along with the paper citation, in the discussion board area of *Blackboard*, an online class management system. In preparation for this class, the instructional staff prepared the chemical solutions listed in Table 2.

At the start of the session, students were given approximately 10 minutes to complete a pretest, which was designed to gather demographics concerning students' possible illicit drug use and their familiarity and confidence of commercial drug testing devices available for consumer use. Additionally, students' conceptual knowledge concerning the definitions of *a prodrug, false positive, and false negative* was determined. The instructor then gave a short presentation with classroom discussion on date rape drugs, GHB, prodrugs, criteria for a validated drug assay, and consumer drug test kits. The simulated drinks, listed in Table 2, were available at the front of the classroom. Each student received the laboratory handout, 1 Drink Safe Coaster, 2 strips of pH paper, and 2 assigned drinks to test. The students recorded the pH of their assigned drinks, the color observed with the Drink Safe Coaster, and their interpretation of the results (Table 3). After all students tested their drinks and recorded their results, the class convened to discuss their findings, as well as the underlying chemical principles. Students were then given 10 minutes to complete a posttest. The aim of the posttest was to assess the effectiness of the activity in reinforcing the learning objectives and to re-examine the students' confidence in the accuracy and reliability of commercial drug testing devices. The following are examples of the questions that were used to initiate and drive the classroom discussion (acceptable answers are in italics):

(1) What is the pH range observed from solutions that give either a true positive or a false positive? *Solutions with a pH of 7.5 or higher appear to give a positive result, whether or not GHB is present.*

(2) What is the pH range observed from solutions that give either a true negative or a false negative? *Solutions with a pH of 7 or lower appear to give a negative result, even if GHB is present.*

(3) What is the relationship between the solution pH and the test results? *Regardless of the presence or absence of GHB, basic solutions will test positive, while neutral or acidic solutions will test negative.*

(4) State your conclusions about the use and limitations of the drink coasters in their ability to truly detect the presence and absence of NaGHB. *The coaster is essentially a pH indicator rather than a GHB indicator. Substances that produce 8 an alkaline pH in solution will produce a false positive, while GHB cannot be detected in an acidic environment. Beverages that have an intrinsic color (eg, wine, cola, orange juice, etc) will produce inconclusive or false negative results because of color interference. The presence of GBL and BD cannot be detected by the GHB drink coaster.*

Table 2. Solutions Available to Students Participating in an Interactive Lesson in Acid/Base and Pro-Drug Chemistry Detection of Sodium γ-Hydroxybutyrate Using Commercial Test Coasters

Code	Solution/Mixture
A	Water
B	0.0165M NaGHB in water
C	0.0165M $KHCO_3$ in water
D	0.0165M Na Citrate in water
E	0.05N HCL
F	0.05N NaOH
G	1:1 mixture of 0.033M NaGHB and 0.1N HCl
H	1:1 mixture of 0.033M NaGHB and 0.1N NaOH
I	0.0165M NaGHB in Red Wine
J	0.0165M NaGHB in Beer
K	0.0165M NaGHB in Cola
L	0.0165M NaGHB in Orange Juice
M	1:1 GBL:water
N	1:1 BD:water

ASSESSMENT

Sixty-eight students participated in the activity and responded to the survey. One student asked to be excused from the activity for unidentified personal reasons. The survey revealed that over half of the students in each class knew of someone who had taken drugs at a rave party, and 3%-15% of the students had self-administered rave drugs (Table 4). Less than 10% of each class had any substantial knowledge of consumer drug test kits.

As a result of the session, students changed their perceptions of the accuracy and reliability of drink test coasters (Table 5). Prior to the activity, there was a general lack of understanding of what defined a prodrug, while after completion, nearly all students correctly defined a prodrug. Few changes were observed in the percentage of students correctly identifying false negative and false positive test results. After the lecture and discussion, but prior to the students testing of the spiked drinks, an

Table 3. Summary of Anticipated Results of an Experiment Involving Acid/Base and Pro-Drug Chemistry Detection of Sodium γ-Hydroxybutyrate Using Commercial Test Coasters

Mixture	Description	Color	Approx. pH	Test Result
A	Water alone	Green	6	True Negative
B	GHB in water	Blue	7.5	True Positive
C	KHCO3 in water	Blue	8	False Positive
D	Na citrate in water	Blue	8	False Positive
E	0.05N HCL	Yellow	2	True Negative
F	0.05N NaOH	Blue	11	False Positive
G	GHB + 0.1N HCl	Yellow	3	False Negative
H	GHB +0.1N NaOH	Blue	11	True Positive
I	GHB + red wine	Red	5	Inconclusive/False Negative
J	GHB + beer	Dark Green	6	Inconclusive/False Negative
K	GHB + cola	Dark Green	5	Inconclusive/False Negative
L	GHB + orange juice	Green	6	False Negative
M	GBL + water	Green	7	True Negative
N	BD + water	Green	6	True Negative

informal survey (show of hands) of student confidence in the drink coasters revealed that most students were neutral about the coasters. A change in student level of confidence was evident after the activity, as the posttest revealed that the majority of students had low or no confidence in the drink coaster as a tool to detect drugs in spiked drinks.

DISCUSSION

A review of the patent literature revealed that the active substance contained in the spot test for GHB is bromocresol purple, an acid/base indicator dye, which changes color from yellow to purple within the transition pH range of 4.8 to 6.8.[9] Although it is likely that the manufacturer added other substances to alter either the pH range or the colors themselves, the principle that the GHB spot test on the coaster is essentially a pH indicator is consistent with the observations obtained from the experiments described.

To understand the design of the drink coaster, it is first necessary to understand the solution properties of NaGHB. The pH of a solution containing 0.0165 molar NaGHB when dissolved in water can be estimated by the formula $pH = ½ (\log C + pKa + pKw)$, where C is the concentration, pKa is the −log of the dissociation constant of γ-hydroxybutyric acid (4.72[10]), and pKw is the self-dissociation constant of water (with a value of 14). At a concentration of 0.0165 molar NaGHB, the theoretical pH is estimated to be 8.46. The measured value is about 7.5 (less basic because the water used is slightly acidic with dissolved carbon dioxide from air, and a measured pH of about 6). Thus, when NaGHB is dissolved in a drink containing water or alcohol, it will impart a weakly alkaline pH to the solution. The coaster's spot test takes advantage of this fact to provide a test for NaGHB.

Accordingly, any alkaline solution with a similar or higher pH will produce a positive test, as exemplified by solutions containing NaOH, $KHCO_3$ and sodium citrate, even though no GHB is present. On the other hand, when the GHB solution is placed in an acidic pH environment, ie, when it is mixed with 0.1N HCl, the presence of

Table 4. Pretest Survey Responses of Students Participating in an Interactive Lesson in Acid/Base and Pro-Drug Chemistry Detection of Sodium γ-Hydroxybutyrate Using Commercial Test Coasters

	Students in PHC 250, %	Students in PHC 331/431, %
Do you know of someone (other than yourself) who has taken drugs at a party/rave? (alcohol & cigarettes don't count).	74 (yes)	46 (yes)
Have you taken drugs at a party/rave? (alcohol & cigarettes don't count.)	15 (yes)	3 (yes)
How familiar are you with commercial drug test kits for consumer use?		
No knowledge or familiarity	55	74
A little knowledge or familiarity	39	26
Some knowledge or familiarity	6	0
Lots of knowledge or familiarity	0	0

PHC250: *Apothecarial Adventures*; PHC331/431: *Case Studies in Pharmaceutical Sciences*

Table 5. Comparison of Students' Test Responses Before and After Participating in an Interactive Lesson in Acid/Base and Pro-Drug Chemistry Detection of Sodium γ-Hydroxybutyrate Using Commercial Test Coasters

	PHC 250		PHC 331/431	
	Pretest	**Posttest**	**Pretest**	**Posttest**
Commercial test kits for consumer use are accurate.	52 (agree)	0 (agree)	22 (agree)	3 (agree)
Commercial test kits for consumer use are reliable.	39 (agree)	0 (agree)	14	0 (agree)
Percent of the class that could correctly define a prodrug	0	93	68	100
Percent of the class that could correctly define a false negative test result	90	93	61	64
Percent of the class that could correctly define a false positive test result	74	77	42	62
I would use a commercial drug kit for consumer use.	NA	6	NA	8
My level of confidence in the Drink Safe Coaster is				
High	NA	0	NA	0
Somewhat high	NA	0	NA	0
Neutral	NA	0	NA	3
Somewhat low	NA	13	NA	8
Low	NA	87	NA	89

NA – Not applicable. The students were not asked this question

NaGHB could not be detected. Since the detection mechanism for the coaster involves a color change, any drinks that contain a deep color (eg, red wine and cola) will mask the true results of the test.[11]

The inability of the test coaster to detect prodrugs of GHB (GBL and BD) is understandable based on the chemistry of these compounds. Both GBL and BD are neutral compounds, being a lactone and an alcohol, respectively. When dissolved in an aqueous environment, conversion to GHB is not sufficiently rapid or extensive to produce a positive test. The conversion of these prodrugs to GHB depends on the availability of bioactivating enzymes in the body. BD is metabolized by hepatic alcohol dehydrogenase to the aldehyde and then by acetaldehyde dehydrogenase to GHB. GBL is rapidly hydrolyzed by liver and plasma lactonases to the active acid.[3,12]

Students may be further encouraged to learn more about the biological aspects of GHB (for a review, refer to the article by Snead and Gibson[13]). Briefly, GHB is an endogenous carboxylic acid and an analog of the neurotransmitter, γ-aminobutyric acid (GABA). Its sedative effects are believed to derive from its ability to act at GABA receptors in the central nervous system. At concentrations near the endogenous level, GHB is almost exclusively metabolized via GHB dehydrogenase to succinic semialdehyde, which in turn is converted to succinic acid by succinic semialdehyde dehydrogenase. Succinic acid then enters the tricaboxclic acid cycle (Kreb cycle) where it is metabolized to carbon dioxide and water.[12] The systemic elimination of GHB is nonlinear and saturable.[4] At endogenous GHB levels, little or no GHB is found in the urine. However, after acute intoxication, the metabolic pathway becomes saturated, and an increasing concentration of GHB is found in the urine, due to capacity-limited reabsorptive transport of GHB in the renal tubules.[14] Transport of GHB into the brain is also saturable, and is mediated by a monocarboxylate transporter.[15]

Overall, students were keenly interested in and highly enthused about this instructional activity. They thoroughly enjoyed the idea that they were testing beverages for illegal drugs and the activity was a welcomed break from lectures. By comparing pretest and posttest results, we were able to identify the instructional strengths and weakness of this classroom activity. The marginal improvement in the understanding of false positives and false negatives at the conclusion of the activity appeared to be one weakness of the current design. The exact reason for this failure is unknown at present. It is therefore recommended that these concepts can be explained in greater detail during the discussion to foster a better understanding. Additional examples of false positives and negatives should also be incorporated.

A barrier to accepting this activity and incorporating it into the college curriculum is the potential concern in permitting young individuals to handle GHB directly. Obtaining and disposing of GHB may also be prohibitively bureaucratic. However, as is evident from the chemistry, any weak acid salts of sodium or potassium, eg, acetate, citrate, or benzoate, can be used as a substitute for NaGHB, if the instructor has any hesitation about using the controlled substance. In our classes, sodium acetate was substituted for GHB. The results obtained from this salt are identical to those obtained from GHB. Aside from the GHB, the other materials for this activity are easily obtained or readily available and pose little risk to students. The coasters are relatively inexpensive (approximately $1 per coaster) and the remaining materials are available in any adequately stocked laboratory.

CONCLUSION

This simple classroom activity can be used to teach beginning pharmacy practice and pharmaceutical science students several important concepts: drugs used in date-rape; the utility and limitation of existing consumer devices to detect these drugs; the principles concerning pH and pH indicators; the principles of false positives and negatives in testing; and the concept of pro-drugs, particularly those related to GHB.

The activity was well received by the students and could easily be completed in one 2-hour classroom session with inexpensive readily available materials. In a broader perspective, this activity also teaches the students the need to understand the basic mechanisms of how things work, before they can interpret the myriad of (sometimes seemingly unconnected) results in a meaningful manner. This classroom activity illustrates the use and limitations of chemistry to answer questions which might affect the future life of a potential victim of date rape: Should I take this drink based on the results of a commercial coaster test?

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