Detecting Pharmaceuticals in the Red Blood Cell Inventory of a Hospital Blood Bank

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We tested 220 red blood cell units for the presence of pharmaceuticals; 15 units (6.8%) were confirmed to contain low concentrations of opiates, benzodiazepines, stimulants, or barbiturates. Further study is needed to determine whether these drugs, which are not prohibited in donated blood by current Food and Drug Administration standards, could mediate adverse reactions in children. *(J Pediatr 2017;189:227-31).*

Prior to donating blood, all voluntary, nonremunerated donors complete a universal donor health questionnaire, undergo a vital sign assessment, and submit to a limited physical examination, all intended to identify evidence of active intravenous drug use.[1,2] If the potential donor meets qualifying criteria, blood is collected and undergoes infectious disease testing for hepatitis B and C, human immunodeficiency virus, West Nile virus, and syphilis. Additional testing for *Trypanosoma cruzi*, Zika virus, Babesia, and antibodies to human leukocyte antigens may be performed, depending on the patient's medical and donation history, the product being collected, and the geographic region of the blood collection center. Donations that meet Food and Drug Administration (FDA) standards are sent to hospitals for clinical use. Although the donor health questionnaire asks screening questions relating to illicit drug use and exposure to potentially teratogenic pharmaceuticals (**Table I**), donor blood is not directly tested for the presence of illicit or legal pharmaceuticals, nor are parallel specimens (eg, hair or urine) collected from the donor as an alternative means of toxicology testing.[2]

Regulatory standards governing blood donation and screening are subject to ongoing revision, based on emerging areas of risk. Recent initiatives have focused on reducing further the infectious risk of donated blood, as reflected by the implementation of additional donor deferrals based on potential travel-related exposure to emerging pathogens (such as Ebola and Zika viruses).[3,4] In contrast, there have been no recent regulatory developments addressing the use of prescription or illicit drugs among blood donors, despite recent population trends toward an aging of the blood donor population.[5,6] As older individuals are more likely to take at least 1 prescription medication,[7] and as older adults account for a progressively larger percentage of the blood donor pool,[5,6] the question as to whether unidentified prescription medications and their metabolites are present in the blood supply has the potential to become more clinically relevant.[8,9]

Therefore, the purpose of this exploratory study was to determine whether pharmaceuticals and their metabolites could be detected at specific minimum concentrations in red blood cell (RBC) units stocked at a large university hospital blood bank in the US. If pharmaceuticals can be detected, then further studies are warranted to determine whether the concentrations are high enough to mediate adverse events in vulnerable populations, such as neonates and small children.

FDA	Food and Drug Administration
RBC	Red blood cell
TOF	Time of flight

Methods

The study protocol was approved by the Institutional Review Board at Vanderbilt University Medical Center.

The RBC units tested in this study were obtained from qualified, volunteer, nonremunerated blood donors and were available for transfusion from the hospital blood bank.

Segments from 220 units of RBCs (approximately 4 days' worth of inventory) of blood group A (n = 88), B (n = 15), AB (n = 7), and O (n = 110) were randomly selected from the Vanderbilt University Medical Center blood bank. These sealed, sterile tubing segments were sent in a 4°C shipment container to the ARUP Laboratories (Salt Lake City, Utah). The tubing segments were stored at 4°C at the ARUP facility prior to analysis.

Consistent with general testing protocols in clinical and forensic toxicology, a 2-step testing methodology was chosen to determine the presence of pharmaceuticals in the specimens. A highly sensitive target screen was first carried out by time of flight (TOF) mass spectrometry, followed by orthogonal confirmation testing by tandem quadrupole mass spectrometry.

The entire contents of the residual RBC segments (250-400 μL each) were transferred into individual storage tubes. Samples were centrifuged at 3000 × g, and the supernatant was used for analysis. Samples were prepared according to the protocol previously published by Marin et al.[10] Briefly, 250 μL of sample was deproteinated using 750 μL of acetonitrile containing 100 ng/mL of 11-nor-9-carboxytetrahydrocannabinol-d3, morphine-d3, and diazepam-d5 as representative internal

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Supported in part by Vanderbilt University Medical Center CTSA Grant # UL1 RR024975-01 from NCRR/NIH. The authors declare no conflicts of interest.

http://dx.doi.org10.1016/j.jpeds.2017.06.010

Table I. Allogeneic donor medication deferral list*

Generic names	Trade names	Deferral period
Finasteride	Proscar, Propecia	1 mo from last dose
Isotretinoin	Absorica, Accutane, Amnesteem, Claravis, Myorisan, Sotret, Zenatane	1 mo from last use
Dutasteride	Avodart, Jaylyn	6 mo from last dose
Acitretin	Soriatane	3 y from last dose
Etretinate	Tegison	Permanent
Bovine insulin from UK		Indefinite
Aspirin, aspiring containing medications	Feldene	2 full d after last dose
Prasugrel, ticagrelor	Effient, Brilinta	7 d
Clopidogrel, Ticlopidine, Vorapaxar	Plavix, Ticlid, Zontivity	14 d after last dose
Warfarin	Coumadin	For plasma products-7 d after last dose
Heparin and derivatives		For plasma products-7 d after last dose†
Dabigatran	Pradaxa	2 d after last dose†
Rivaroxaban	Xarelto	2 d after last dose†

UK, United Kingdom.
*Adapted from AABB Standards for Blood Banks and Transfusion Services.[1]
†Or as defined by the facility's medical director.

standards to monitor assay performance. After vortexing, the protein-depleted samples were centrifuged at 14 000 rpm at 0°C for 5 minutes, transferred to a 1.5 mL autosampler vial, and evaporated under nitrogen at 40°C. Samples were then reconstituted in 100 uL of a 10:90 methanol:water mixture and 10 µL was injected onto an Agilent Poroshell 2.7 µm, 100 × 2.1 mm column (Agilent Technologies, Santa Clara, California) using an Agilent 1260 HPLC and an Agilent 6230 TOF mass spectrometer (liquid chromatography-TOF).

Individual drugs and metabolites were then identified based upon a combination of chromatographic retention time, chromatographic peak shape, accurate mass, compound score (including isotope pattern and spacing), and area counts. Consistent with Clinical Laboratory Improvement Amendments of 1988 and College of American Pathologists requirements for clinical testing, each analytical batch included positive and negative controls for each analyte; these controls were placed at the beginning and end of the injection sequence at the established laboratory cut-offs to ensure that instrument sensitivity changes did not occur during the analyses. Established cut-off values for included drugs and metabolites are provided (**Table II**; available at www.jpeds.com).

An orthogonal mass spectrometry method was used to confirm the presence of identified drugs from the TOF screen, consistent with forensic toxicology and routine clinical toxicology testing work flows. Residual RBCs were lysed by repeated freeze-thaw cycles and 250 µL of lysate was prepared as described for targeted screening. Analysis was conducted using an Agilent 1260 HPLC, Agilent Poroshell 2.7 µm, 100 × 2.1 mm column, and an Agilent 6460 triple quadrupole mass spectrometer (liquid chromatography-tandem mass spectrometry). Individual drug/metabolite identification was based upon chromatographic retention time, peak shape, area counts, and 2 validated mass transitions with empirically established ion ratios.

Results

Of the 220 RBC units tested, 21 (9.5%) had positive screening results for at least 1 pharmaceutical or metabolite (**Table III**). Seventeen units (7.7%) tested positive for a single drug or metabolite, while 4 (1.8%) tested positive for more than 1 drug or metabolite. Two of the units that tested positive for more than 1 drug (0.9%) were positive for drugs in different pharmaceutical classes (eg, hydrocodone plus tramadol; oxazepam plus fentanyl), indicating polypharmacy. Detected substances were fentanyl (n = 6), phentermine (n = 2), phenobarbital (n = 1), codeine (n = 2), alprazolam (n = 3), hydrocodone (n = 2), zolpidem (n = 1), butalbital (n = 2), temazepam (n = 2), tramadol (n = 1), oxazepam (n = 1), tramadol metabolites (n = 2), fentanyl metabolites (n = 1), and clonazepam metabolites (n = 1).

Of the 21 initially positive units, 15 (6.8% of all tested units) remained positive for at least 1 pharmaceutical agent when retested using the orthogonal confirmatory assay, which is known to be more specific, but differs in sensitivity when compared with the screening assay. The most common substance detected by the screening test, but not detected by the confirmatory test, was fentanyl (n = 6). The other drugs identified by the screening test but not the confirmatory assay were butalbital (n = 1) and a metabolite of tramadol, N-desmethyltramadol (n = 1). There were no instances in which a class of drug was detected by the confirmatory assay that had not been previously detected by the screening assay.

The assays used in this analysis were qualitative rather than quantitative, with positive results for each pharmacologic agent determined by comparison to an established cut-off consistent with clinical laboratory guidelines. Of the drugs tested, the minimum concentration required to produce a positive result for both the screening and confirmatory assay ranged from 1 ng/mL for fentanyl metabolites up to 500 ng/mL for phenobarbital (**Tables II** and **III**).

Discussion

Volunteer blood intended for transfusion is tested for multiple blood-borne pathogens but does not include testing for pharmaceuticals or illicit drugs. This exploratory study demonstrates that it is possible to detect pharmaceutical agents and