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N-acetylcysteine interference of Trinder-based assays

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ABSTRACT

Objectives: The primary objective of this study was to evaluate potential interference of Trinder-based chemistry assays by *N*-acetylcysteine (NAC). A secondary objective was to look for evidence of interference in patients treated with NAC for acetaminophen (APAP) overdose.

Design and methods: Dilutions of NAC in plasma were tested for interference using the following Roche Diagnostics Trinder-based assays on a cobas 8000 system: enzymatic creatinine (Cr), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), triglycerides (TRIG), and uric acid (UA). Two non-Trinder Roche assays — urea nitrogen (BUN) and glucose (GLUC) — were tested as controls. Sekisui N-geneous® low-density lipoprotein cholesterol (LDL-C) reagent was also evaluated. Retrospective chart review of APAP overdose cases over 49 months was conducted to look for differences in plasma Cr before and after intravenous (IV) NAC administration.

Results: NAC concentrations (shown in parentheses) that caused ≥10% inhibition for individual assays were (in order of sensitivity to interference): TRIG (570 mg/L) > CHOL (740 mg/L) ≈ Cr (790 mg/L) > UA (1100 mg/L) > HDL-C (1760 mg/L) > LDL-C (2900 mg/L). Neither BUN nor GLUC achieved significant inhibition up to 10,000 mg/L NAC. Evidence for relatively minor inhibition of Cr was observed in patients after NAC administration.

Conclusions: NAC inhibition of the assays investigated typically occurs at concentrations higher than expected during IV and oral NAC therapy.

1. Introduction

The term *Trinder reaction* refers to two chemical reactions described by Dr. Paul Trinder (b.1919–d.2015). Its first use (a ferric chloride-based rapid urine test for the detection of salicylates) will not be discussed further in this manuscript [1]. His subsequent work in identifying non-carcinogenic alternatives in oxidase–peroxidase detection systems led to the development of a second Trinder reaction [2,3]. This use refers to the reaction of 4-aminophenazone (plus a phenol derivative) with hydrogen peroxide (H_2O_2) to form a detectable color product when peroxidase is present [2]:

$$H_2O_2 + 4\text{—aminophenazone} + \text{phenol derivative} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + H_2O.$$

While the original method used glucose oxidase for the generation of H_2O_2, the remarkable utility of the Trinder reaction is that any source of H_2O_2 can theoretically be monitored. As such, oxidase reactions are commonly included in many clinical laboratory assays. Inhibitors and/or antioxidants, however, may interfere with these reactions. Dr. Trinder noted that the amount of reaction product may be reduced by the anti-oxidant ascorbic acid, albeit at concentrations higher than expected in whole blood [2]. Interferences with Trinder reactions (involving potassium ferrocyanide and/or ceruloplasmin) have also been observed [4]. Additionally, the hemostatic drug ethamsylate (not available in the U.S.) has also been shown to interfere with Trinder reactions [5,6]. NAC interference of acetaminophen (paracetamol; APAP) APAP assays has previously been described [7,8].

Additional Trinder reaction interferences have recently been reported, particularly in regards to NAC. NAC is a medication used intravenously (IV) and/or orally for the prevention of hepatotoxicity with APAP overdose. It is also used in lower doses for a variety of cardiovascular, pulmonary, renal, and/or neurologic disorders and for protection against contrast dye-induced nephropathy [9]. In December 2014, Beckman Coulter distributed an Urgent Medical Device Recall in the U.S. indicating potential NAC interference with cholesterol (CHOL), lactate (LAC), lipase (LIP), and uric acid (UA) AU-series reagents [10]. A subsequent Urgent Field Safety Notice from Beckman Coulter in Ireland included enzymatic creatinine (Cr) on this list [11]. In May 2015, Roche Diagnostics issued an Urgent Medical Device Correction noting Trinder interferences due to NAC, *N*-acetyl-*p*-benzoquinone imine (NAPQI, an APAP metabolite), and metamizole (an analgesic, unavailable in the U.S.). Roche assays

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affected include CHOL, enzymatic Cr, high-density lipoprotein cholesterol (HDL-C), LAC, low-density lipoprotein cholesterol (LDL-C), triglycerides (TRIG), and UA [12,13].

Limited quantitative information for NAC-related interferences is available. The Beckman recall states that NAC may interfere at "therapeutic concentrations (for the treatment of [APAP] overdose)" [10]. Sekisui (supplier of Abbott APAP reagents) provided a Field Correction in May 2015 lowering their stated concentration of NAC that does not cause >10% variance in APAP results from 800 mg/L to 200 mg/L [14]. In a Questions and Answers document provided to customers, Roche provided concentrations of NAC that start to affect assays results, but did not provide detailed information on the magnitude of interference with increasing concentrations [12].

At what concentration(s) does NAC interfere with specific Trinder-based assays? What is the magnitude of such interference, and is it "clinically relevant"? To address these questions, the present report provides concentration–response relationships for five Roche assays expected to show interference based on vendor notifications [12,13]: CHOL, enzymatic Cr, HDL-C, TRIG, and UA. Two Roche assays not expected to demonstrate NAC interference (urea nitrogen, BUN; glucose, GLUC), and one open channel assay (N-geneous® LDL cholesterol reagent; Sekisui Diagnostics, Lexington, MA), were also evaluated. Retrospective chart review was then conducted to evaluate potential inhibition of one Trinder-based assay (enzymatic Cr) in the context of IV NAC administration for APAP overdose management.

2. Materials and methods

2.1. NAC interference experiments

Previously collected lithium heparin plasma specimens (Plasma Separator Tubes™; BD, Franklin Lakes, NJ) were obtained from refrigerated storage (4 °C) and de-identified in accordance with an Institutional Review Board (IRB) approved protocol (University of Utah IRB Protocol #0007275). In order to effectively detect assay inhibition, specimens were selected to target the following concentrations in the final pool of plasma used for all subsequent studies: enzymatic Cr, 1–3 mg/dL; TRIG, 100–300 mg/dL, GLUC, 90–180 mg/dL.

NAC was obtained from Sigma-Aldrich (St. Louis, MO). NAC stock solutions were created (n = 5; 10,000 mg/L) using pooled plasma. Serial dilutions from each stock solution were prepared with pooled plasma and then incubated for 2 h at room temperature prior to testing. Most assays were performed on a Roche cobas c502 chemistry instrument using the following Roche reagents: *cholesterol gen.2*, *creatinine plus ver.2* (enzymatic), *glucose HK*, *HDL-cholesterol plus gen.3*, *triglycerides*, *urea/BUN*, and *uric acid ver.2*. LDL testing was conducted using *N-geneous® LDL cholesterol reagent* (Sekisui Diagnostics, Lexington, MA) on a Roche cobas c702 instrument. The Roche *LDL-C plus gen.2* and *lactate* reagents were not evaluated, as they were not used for clinical testing at ARUP.

Results were plotted in SigmaPlot 11 (Systat Software Inc., San Jose, CA), with data normalized to baseline analyte concentrations in the absence of NAC. Curve fits were performed in SigmaPlot using 4-parameter logistic regression. Results are presented as mean ± standard deviation (SD) unless otherwise indicated.

2.2. Retrospective analysis — University of Iowa

Using an IRB-approved protocol (University of Iowa IRB-01 Committee Protocol # 201506810), the University of Iowa Hospitals and Clinics (UIHC) electronic health record (EHR) (Epic; Epic Systems, Inc., Madison, WI) was searched for all orders of IV administration of NAC used for suspected or proven APAP toxicity. This review covered patients seen at UIHC from May 1, 2011 to June 19, 2015 (49 months; start date based on when the UIHC clinical laboratory switched to an enzymatic Roche method for Cr determination in plasma). Patient results (BUN, Cr, GLUC), as well as information on additional IV hydration, were included if both baseline and post-NAC initiation (within 8 h) testing was performed. During the time period of retrospective analysis, the same protocol for IV NAC treatment of APAP toxicity was used throughout the institution, namely 150 mg/kg loading dose over 1 h followed by maintenance doses of 50 mg/kg over 4 h and 100 mg/kg over 16 h. This is a common regimen for management of APAP toxicity [15].

Results from chart review were plotted in SigmaPlot 11 as Box Plots, with patient results normalized to a baseline (pre-NAC) initiation analyte measurement. Post-NAC time periods were binned into 0.0–3.9 h and 4.0–8.0 h intervals; no patients had laboratory test results within both intervals. Statistical analysis was conducted using Student's or paired t-tests, as appropriate.

3. Results

3.1. NAC inhibition of Trinder-based assays — concentration–response relationships

Baseline analyte results (in the absence of NAC) in our plasma pool were as follows (in mg/dL, n = 5): BUN, 36.3 ± 0.5; CHOL, 140.8 ± 3.3; enzymatic Cr, 2.6 ± 0.1; GLUC, 122 ± 1.9; HDL-C, 35 ± 0.7; LDL-C, 71.6 ± 0.5; TRIG, 180.2 ± 2.9; UA, 6.2 ± 0.1. Data presented in Fig. 1 are normalized to these baseline values, and show the concentration–response relationship for NAC potential interference with each assay.

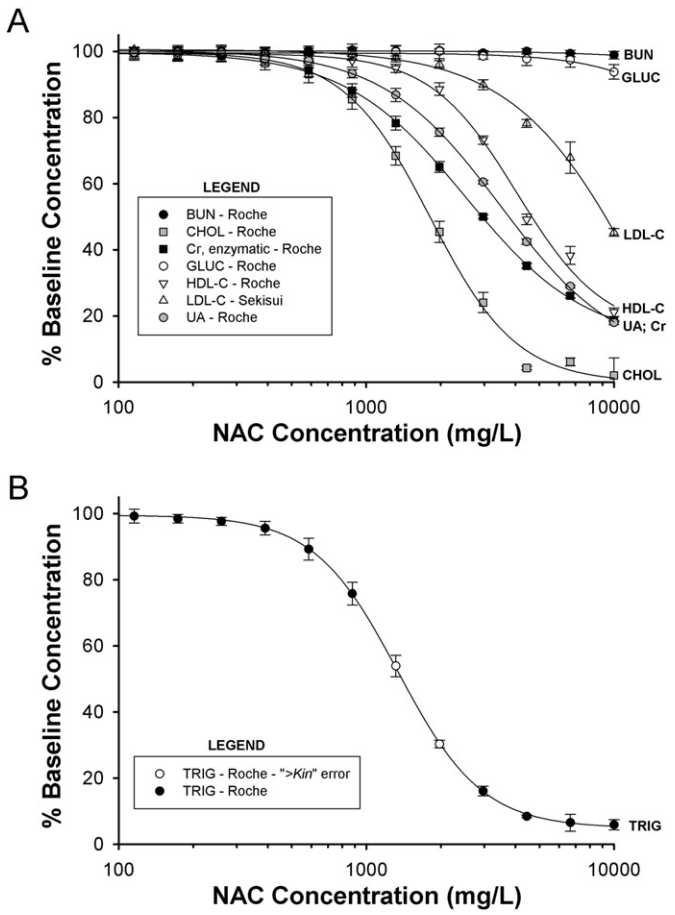

Fig. 1. NAC interference of Trinder-based assays. A. Concentration–response relationships showing potential NAC effect on the following assays: BUN, CHOL, Cr, GLUC, HDL-C, LDL-C, UA (see Legend, insert). Data is expressed as % baseline concentration for the individual analyte (mean ± SD). B. Concentration–response relationship showing NAC effect on the TRIG assay. Black circles show results (mean ± SD) reported with no initial "*>Kin error*". At two NAC concentration points (1317 mg/L NAC and 1975 mg/L NAC) an initial "*>Kin error*" flag was reported by the instrument, prompting auto-dilution on the c502. Results before auto-dilution are shown as open circles (auto-dilutions not shown).