Effect of Heparin Contaminated with Oversulfated Chondroitin Sulfate on the Collection and Analysis of Plasma

Tracey Bosworth[1], Yung Chan[2], Tan Nguyen[2], Steve Gutman[2], Alberto Gutierrez[3], Francis Kalush[3], Thomas Gwise[3], Steven Pollack[3], Ji Guo[3], Dinesh Patwardhan[3], Lucinda Buhse[4], John C. Reepmeyer[4] and Alan T. Remaley*[,1]

[1]*Department of Laboratory Medicine, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, MD 20892, USA*

[2]*Department of Pathology, Univ of Central Florida, FLA 32816, USA*

[3]*Center for Devices and Radiological Health, Food and Drug Administration, White Oak, Silver Spring, MD 20993, USA*

[4]*Division of Pharmaceutical Analysis, Center for Drug Evaluation and Research, Food and Drug Administration, St. Louis, MO 63101, USA*

Abstract: Oversulfated chrondroitin sulfate (OSCS) was recently identified as a contaminant of heparin and was associated with serious adverse events in patients treated with heparin. Because heparin is a common component of blood collection tubes, we tested the effect of OSCS on the laboratory analysis of plasma. Blood from healthy volunteers (N=50) was collected into tubes containing various mixtures of heparin and OSCS. Samples were inspected for microclots and were analyzed for a panel of 28 routine laboratory tests. No microclots were observed in tubes that contained only heparin but were detected in 18%, 88% and 76% of plasma samples containing 5%, 15%, 20% OSCS (%weight relative to heparin), respectively. OSCS at the highest dose (20%) caused a systematic bias for the following 6 tests: Lactate Dehydrogenase: 18% (12% to 24%); Triiodothyronine: -5.7% (-8.1% to -3.3%); Potassium: -2.8% (-4.2% to -1.4%); Total Protein: 2.5% (1.4% to 3.6%); Chloride: -1.4% (-1.8% to -1.0%) and Uric Acid: 1% (0.5% to 1.4%). In summary, OSCS contamination of heparin was found to potentially affect the anticoagulation of plasma and the analytical performance of several routine clinical laboratory tests.

Keywords: Heparin, blood collection tubes, pre-analytical variation, laboratory error.

INTRODUCTION

Heparin contaminated with oversulfated chrondroitin sulfate (OSCS) was recently identified [1] as the cause of severe adverse events, including lethal anaphylaxis, in patients anticoagulated with tainted heparin in over 12 countries [2, 3]. OSCS has been shown to activate both the kinin-kallikrein pathway and the complement system, producing an allergic type response in animal models, similar to the adverse events observed in patients treated with OSCS contaminated heparin [4]. As a consequence, regulatory agencies throughout the world have implemented measures to prevent this type of problem from reoccurring. The U.S. Food and Drug Administration (FDA) has issued recommendations for the monitoring of OSCS, not for only heparin used for the treatment of patients but also for its use for *in vitro* medical devices [5, 6]. Recently, the FDA has recalled a test for acetaminophen that uses heparin as a reagent, because of a negative bias from OSCS contamination [7]. Although no problems related to OSCS contamination of blood collection tubes have been reported, many previous problems arising from faulty blood collection tubes have been described [8]. Here we report the effect of OSCS contamination of heparin on the collection of plasma samples and the analysis of several routine clinical laboratory tests.

METHODS

10 mL of blood from 50 healthy volunteers (32 women, 18 men; age range 22-62; approx. 65% Caucasian, 20% Asian, and 20% African American) was collected from the antecubital vein, using a 21gauge butterfly needle into a 20 mL plastic syringe (Becton, Dickenson and Co., Franklin Lakes, NJ), which contained no anticoagulants. All samples were collected under an IRB approved protocol with patient consent. Immediately after collection, 2 mL of blood was transferred in random order to four borosilicate glass tubes (13 X 100 mm, Fisher Scientific, Inc.), containing 60 units of heparin lithium salt (Sigma-Aldrich, Inc.) and varying amounts of OSCS lithium salt so that the final concentration of OSCS relative to heparin by dry weight was 0%, 5%, 10% and 20%. The range of OSCS tested represents the possible level of OSCS contamination based on the previous analysis

*Address correspondence to this author at the National Inst. of Health, Building 10, Room 2C-433, Bethesda, MD 20892, USA; Tel: 301-402-9796; Fax: 301-402-0190; E-mail: aremaley1@cc.nih.gov

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of contaminated lots of heparin (1-3). OSCS was produced from Chondroitin Sulfate (Chondroitin Sulfate A, sodium salt, from bovine trachea, Sigma-Aldrich catalog no. C9819, lot 087K1416), as previously described [9] and was analyzed for the extent of sulfation and purity by elemental analysis, NMR, and capillary electrophoresis. After mild vortexing, the samples were centrifuged at 3200 x g for 10 minutes; plasma was removed and stored in 13 x 100 mm Synchron Microtube™ (Beckman Coulter Inc, Palo Alto, CA) sample cups. After 1 h at room temperature, the plasma samples were visually inspected for microclots by holding the samples up to a light and observing small opaque clots, which were removed when detected with a wooden applicator stick. The samples were analyzed in singlet on a Beckman Coulter Synchron LX® 20 general chemistry analyzer and a Siemens IMMULITE® 2500 immunoassay analyzer (Siemens, Berlin, Germany) for the standard 14 panel Comprehensive Metabolic Panel and the following immunoassay tests: thyroid stimulating hormone, free thyroxine, thyroxine, triiodothyronine, free triiodothyronine, total testosterone, dehydroepiandrosterone sulfate, cortisol, thyroxine-binding globulin, progesterone, estradiol, sex hormone binding globulin, C-peptide, and ferritin. The data were analyzed by a linear mixed effect model, treating subjects as random effects, thus controlling for random variation in test results from the different subjects. Model assumptions were checked, using residual plots. The Bonferroni method was used to conservatively adjust the significance level to $P < 0.001$ to account for multiple comparisons from the 28 tests analyzed.

RESULTS AND DISCUSSION

To test the effect of OSCS, blood was collected from patients with tubes containing heparin plus increasing amounts of OSCS to represent the possible range of contamination reported [1-3]. No microclots were observed in the 50 samples that contained only heparin but were detected in 18%, 88% and 76% of the aliquots (N=50) that contained 5%, 15%, 20% of OSCS, respectively. Although OSCS was recently identified to be a weak anticoagulant, when mixed with heparin, it was found to interfere with the ability of heparin to act as an anticoagulant [10]. Highly sulfated polysaccharides like OSCS can even promote *in vitro* clotting, by inducing contact activation [4]. Microclots can pose a significant problem, particularly in instrument without clot detectors, because they can result in spurious laboratory results by partially blocking the aspiration of samples by automated analyzers.

In addition to interfering with anti-coagulation, OSCS contamination was found at the highest dose but not at lower doses to cause either a statistically significant negative or positive bias for the 6 out of 28 laboratory tests evaluated (Table **1**). The mean biases introduced by 20% OSCS were relatively small and ranged from -5.7% to +18%, which were all smaller than the coefficient of variation for these assays except for lactate dehydrogenase (inter-assay CV 6-12%). The observed biases, although statistically significant, were below the medically allowable error for these tests, as defined by the Clinical Laboratory Improvement Act of 1988 [11]. It is important to note, however, that when compounded by other pre-analytical and analytical errors, small errors can still lead to differences in the interpretation of test results. The mechanism for the interference of OSCS on these tests is not known, but it could be due to a matrix effect and or from the fact that polyanionic polymers, such as OSCS, can bind a wide variety of substances and enzymes that are used as reagents in clinical laboratory assays [12]. A small subset of whole blood samples collected in OSCS containing tubes was also analyzed on a blood gas analyzer (ABL800Flex, Radiometer), and 20% OSCS had no perceptible effect on pH (7.40±.04 for control *vs*. 7.41±.05 for 20% OSCS; P=0.745; N=3), indicating that a non-specific change in pH from the OSCS did not contribute to the observed laboratory test changes.

In summary, OSCS contamination of heparin was found to affect the anticoagulation of plasma samples and had a small effect on the analytical performance of several clinical laboratory tests. The ongoing surveillance for OSCS contamination of heparin, however, should prevent future problems arising from this issue. Based on these results, it will be important, as per current FDA recommendations, for companies that produce blood collection tubes to also determine the level of OSCS contamination for those tubes that are still in use but were produced prior to the recent monitoring of heparin for OSCS. Several tube manufactures have completed such analyses and found less than 1% OSCS contamination in their older lots of heparinized blood collection tubes and in one study found no clotting or analytical test problems from this low level of contamination [13]. Finally, it is important that physicians and in particular clinical laboratory personnel to be aware of this potential problem and to

Table 1. Effect of 20% OSCS on the Measurement of Analytes

Assay	Control Mean	95% CI	OSCS Effect	95% CI	p-value	% OSCS Effect
LD (U/L)	144	134, 154	+26	18, 34	<0.0001	+18%
TP (G/dL)	6. 8	6.6, 7.0	+0.17	0.10, 0.25	0.0001	+2.5%
URIC (mg/dL)	5. 3	4.7, 5.9	+0.05	0.03, 0.08	<0.0001	+1.0%
CL (mmol/L)	111	110.1, 111.2	-1.6	-2.0, -1.1	<0.0001	-1.5%
K (mmol/L)	4.4	4.2, 4.5	-0.12	-0.18, -0.06	<0.0001	-2.8%
T3 (ng/dL)	127	118, 135	-7.2	-10.2, -4.1	<0.0001	-5.7%

Results were analyzed by a linear mixed effect model, treating subjects as random effects. OSCS Effect represents mean negative or positive bias from the control mean. % OSCS Effect is the mean bias as a percentage of the control mean. LD: lactate dehydrogenase, TP: total protein, URIC: uric acid, CL: chloride, K: potassium, T3: triiodothyronine.

always be vigilant about unexpected sources of laboratory error.

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