Argatroban as an Anticoagulant for Both Hematologic and Chemical Tests

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Argatroban possesses strong antithrombin-like activity. We evaluated the usefulness of argatroban in anticoagulating specimens for routine laboratory tests. Results using blood anticoagulated with argatroban corresponded well with the results of blood treated with ethylenediamine tetra-acetic acid (EDTA) in the complete blood count (CBC), including the WBC differential count and morphol-

ogy of blood cells, when the CBC was performed within 2 hr of blood collection. Clinical chemistry results from argatroban-treated samples were similar to results obtained with serum specimens. Thus, argatroban may be a useful anticoagulant for emergency laboratory medicine. *J. Clin. Lab. Anal.* 14:136–140, 2000. © 2000 Wiley-Liss, Inc.

Key words: argatroban; anticoagulant; ethylenediamine tetra-acetic acid (EDTA); hematologic tests; chemical tests

INTRODUCTION

Blood tests are used widely for clinical monitoring and diagnosis. Relatively high volumes of blood and multiple sampling tubes usually are required: 2 ml of blood in an EDTA-containing tube for hematologic tests, at least 5 ml in an anticoagulant-free tube for most chemical tests, and more blood in special tubes for other tests such as those of blood sugar and coagulation. These tubes of blood are loaded into different automated analyzers and the entire sample usually is not used. Patients often feel that too much blood is drawn for clinical tests. To decrease specimen waste, universal anticoagulants have been sought for clinical laboratory use.

EDTA currently is used for hematologic tests requiring whole blood. A chelator of divalent cations, EDTA interferes with blood coagulation by removing calcium ions from the sample. While plasma obtained from whole blood containing EDTA is used for chemical tests in some emergency laboratories, results of some chemical tests are distorted

both by chelation and by the sodium or potassium ions associated with EDTA. An alternative anticoagulant to EDTA is argatroban (Fig. 1), known to strongly inhibit blood coagulation by blocking the action of thrombin (1–3). However, no trials have assessed its use as a laboratory anticoagulant. We studied the use of argatroban in specimens for hematologic and chemical tests.

MATERIALS AND METHODS

Reagents

After argatroban (Mitsubishi Chemistry, Tokyo, Japan) was dissolved in distilled water (10 mg/ml), quantities of this so-

lution were placed in blood specimen collection tubes at a ratio of 10 µl of argatroban per 2 ml of blood. Tubes containing 2.4 mg of dipotassium ethylenediamine tetra-acetic acid (EDTA-2K) per 2 ml of blood (Terumo, Tokyo, Japan) were used as controls for hematologic tests.

Venous Blood

Venipuncture-derived samples from 50 healthy normal subjects (25 males and 25 females) were divided into argatroban-containing tubes for hematologic and chemical tests, EDTA-containing tubes for hematologic control samples, and anticoagulant-free tubes as serum controls for chemical tests. Tubes were allowed to stand for at least 15 min at room temperature to stabilize samples before testing. All tests were completed within 2 hr of blood collection.

Hematologic Studies

A CBC and WBC differential count were performed with a hematologic analyzer (NE-8000, Toa Medical Electronics, Kobe, Japan). Parameters evaluated were the WBC, RBC, Hb, hematocrit (Hct), MCV, MCH, MCHC, platelet count (Plt), and percentages of the WBC representing neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

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Fig. 1. Structural formula of argatroban. The chemical name of the compound is (2R,4R-4-methyl-L-[N²-((RS)-3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-arginyl]-2-piperidinecarboxylic acid hydrate. The molecular formula is C₂₃H₃₆N₆O₅·H₂O. The molecular weight is 526.66. The tripod structure formed by the arginine frame, piperidine frame, and guanine frame of argatroban combines stereoscopically with the active site of thrombin, selectively inhibiting the action of thrombin.

Morphology of Blood Cells

Blood samples taken from tubes within 2 hr after collection were smeared on slides, fixed, and stained by the Giemsa method for microscopic observation of 1,000 red cells, 200 white cells, and 100 platelets.

Stability of Hematologic Specimens

CBC and WBC differential counts were taken immediately and at 1, 2, and 3 hr after blood collection using the NE-8000 analyzer.

Chemical Tests

Chemical tests were performed using a fully automated analyzer (7170 Automatic Analyzer, Hitachi, Tokyo, Japan) for determinations of aspartate aminotransferase (AST, IU/l), alanine aminotransferase (ALT, IU/l), total bilirubin (T-Bil, mg/dl), lactate dehydrogenase (LDH, IU/l), blood urea nitrogen (BUN, mg/dl), creatinine (Cre, mg/dl), sodium (Na, mEq/l), potassium (K, mEq/l), chloride (Cl, mEq/l), total protein (TP, g/dl), albumin (Alb, g/dl), total cholesterol (T-Chol, mg/dl), triglyceride (TG, mg/dl), alkaline phosphatase (ALP, IU/l), leucine aminopeptidase (LAP, IU/l), cholinesterase (Ch-E, IU/l), gamma glutamyl transpeptidase (γ -GTP, IU/l), and uric acid (UA, mg/dl).

Reproducibility of Hematologic and Chemical Parameters

CBC, WBC differential counts and chemical components were repeated five times with the same instrument, and the coefficient of variation (CV) was calculated.

Statistics

Statistical analysis was carried out using the Stat View—4.5J software package (Abacus Concepts, Berkeley, CA). A paired *t*-test was used for comparison analyses; *P* values less than 0.01 were considered significant.

RESULTS

Hematologic Tests

No significant differences were noted for any item between EDTA-treated and argatroban-treated blood (Table 1). Any possible effects of argatroban on blood cell morphology were meticulously sought. No effects were seen on white cells, red cells, or platelets.

Stability of Hematologic Specimens

For 2 hr after blood collection, no significant difference was seen in CBC or differential WBC count between EDTA-treated blood and argatroban-treated blood. However, when the measurement was performed 3 hr after collection, WBC, RBC, and platelet counts showed decreases due to coagulation in argatroban-treated blood (Fig. 2). Four hours later, the specimen had completely coagulated, and measurements could not be performed.

Chemical Tests

Significant differences were seen for K (*P* < 0.0001) and TP (*P* < 0.0001) between argatroban-treated plasma and serum (Table 2). However, very good correlation was noted between the treated plasma and untreated serum for K (*r* = 0.893) and TP (*r* = 0.971, Fig. 3).

TABLE 1. CBC parameters and WBC differential count with EDTA and argatroban anticoagulation^a

Item	EDTA	Argatroban	<i>P</i> ^b
WBC ($\times 10^9$ /l)	5.19 $\pm$ 0.77	5.03 $\pm$ 0.76	NS
RBC ($\times 10^{12}$ /l)	4.21 $\pm$ 0.54	4.24 $\pm$ 0.43	NS
Hb (g/dl)	13.2 $\pm$ 1.5	13.2 $\pm$ 1.6	NS
Hct (%)	38.5 $\pm$ 4.1	38.1 $\pm$ 3.8	NS
MCV (fl)	90.8 $\pm$ 6.9	89.5 $\pm$ 6.8	NS
MCH (pg)	31.2 $\pm$ 1.8	31.2 $\pm$ 1.9	NS
MCHC (g/dl)	33.5 $\pm$ 1.7	34.1 $\pm$ 1.4	NS
Plt ($\times 10^9$ /l)	246 $\pm$ 65	233 $\pm$ 57	NS
Neutrophils (%)	56.8 $\pm$ 10.7	58.1 $\pm$ 9.7	NS
Lymphocytes (%)	31.3 $\pm$ 9.9	29.9 $\pm$ 10.8	NS
Basophils (%)	0.6 $\pm$ 0.4	0.6 $\pm$ 0.3	NS
Eosinophils (%)	2.1 $\pm$ 1.5	2.0 $\pm$ 1.3	NS
Monocytes (%)	5.8 $\pm$ 2.2	6.3 $\pm$ 1.9	NS

^aValues are mean $\pm$ S.D.

^bBy paired *t*-test; NS, not significant; *n* = 50.

Fig. 2. Serial changes of WBC, RBC, and platelet count (Plt) with EDTA and argatroban anticoagulation. ○, EDTA-treated blood; ●, argatroban-treated blood. Data are mean $\pm$ SD ($n = 5$). * $P < 0.01$ vs. corresponding EDTA-treated blood by paired t -test.

Reproducibility of Hematologic and Chemical Parameters

Good reproducibility was demonstrated for each item (Tables 3 and 4). The results indicated that the reliability of determinations performed by the hematologic and chemical analyzer was not affected by argatroban.

DISCUSSION

Most physicians order multiple laboratory tests to evaluate patient complaints, requiring substantial volumes of blood (4). Tubes for hematologic tests have 2- or 5-ml capacities, while tubes for chemical tests have from 5- to 10-ml capacities, exceeding volumes required for the tests. A single col-

TABLE 2. Commonly measured chemical components in serum and argatroban-treated plasma^a

Item	Serum	Argatroban-treated plasma	P^b
AST (IU/l)	18 $\pm$ 7	20 $\pm$ 7	NS
ALT (IU/l)	13 $\pm$ 7	13 $\pm$ 7	NS
T-Bil (mg/dl)	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2	NS
LDH (IU/l)	322 $\pm$ 76	335 $\pm$ 57	NS
BUN (mg/dl)	14.2 $\pm$ 4.8	14.3 $\pm$ 4.9	NS
Cre (mg/dl)	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	NS
Na (mEq/l)	140 $\pm$ 4	142 $\pm$ 4	NS
K (mEq/l)	4.6 $\pm$ 0.4	4.1 $\pm$ 0.3	< 0.0001
Cl (mEq/l)	102 $\pm$ 3	105 $\pm$ 3	NS
TP (g/dl)	6.7 $\pm$ 0.5	7.1 $\pm$ 0.5	< 0.0001
Alb (g/dl)	3.5 $\pm$ 0.4	3.6 $\pm$ 0.4	NS
T-Chol (mg/dl)	168 $\pm$ 39	166 $\pm$ 38	NS
TG (mg/dl)	84 $\pm$ 24	81 $\pm$ 25	NS
ALP (IU/l)	172 $\pm$ 51	170 $\pm$ 50	NS
LAP (IU/l)	99 $\pm$ 22	96 $\pm$ 22	NS
Ch-E (IU/l)	161 $\pm$ 54	159 $\pm$ 54	NS
γ -GTP (IU/l)	23 $\pm$ 11	22 $\pm$ 11	NS
UA (g/dl)	3.7 $\pm$ 1.1	3.8 $\pm$ 1.1	NS

^aValues are mean $\pm$ S.D.

^bBy paired t -test; NS, not significant; $n = 50$.

lection tube for all tests would be desirable. Argatroban was studied in this context as an anticoagulant.

Argatroban has an extremely high affinity for thrombin, making it a potent anticoagulant. This agent inhibits platelet aggregation induced by thrombin, and efficiently inhibits thrombin when bound to a fibrin clot (5–7). In clinical trials argatroban has been confirmed to be a highly selective and potent inhibitor of thrombin (8–11).

EDTA, a potent calcium ion chelator, is used widely for hematologic tests because it assures stable measurements for at least 24 hr after blood collection. Stability and reliability of CBC and automated WBC differential count results using EDTA have worked against the development of new reagents. Nevertheless, our results showed that argatroban-treated and EDTA-treated blood gave similar values for CBC and WBC differential counts, and no morphologic changes due to argatroban could be observed within 2 hr of blood collection.

EDTA-treated plasma is used for chemical tests in some emergency laboratories. However, measurements of calcium, iron, alkaline phosphatase, leucine aminopeptidase, and sodium and potassium are distorted by chelation and by sodium or potassium ions associated with EDTA. Chelation artifacts did not occur with argatroban and correspondence between results using serum and argatroban-treated plasma was found to be high. Significant differences between argatroban-treated plasma and serum were seen only for K ($P < 0.0001$) and TP ($P < 0.0001$) concentrations. The difference for K may have resulted from a release of platelet-derived K in serum during blood coagulation which does not occur in argatroban-treated plasma. The TP value was higher in argatroban-treated plasma than in serum because argatroban-treated plasma con-

Fig. 3. Correlations for potassium (K) and total protein (TP) concentrations between serum and argatroban-treated plasma.

TABLE 3. Coefficient of variation (CV) for CBC parameters and WBC differential count with argatroban anticoagulation^a

Item	CV (%)
WBC	1.78 ± 0.39
RBC	1.42 ± 0.48
Hb	1.12 ± 0.42
Hct	1.15 ± 0.33
MCV	0.42 ± 0.18
MCH	0.78 ± 0.22
MCHC	0.43 ± 0.15
Plt	4.8 ± 1.23
Neutrophils	2.64 ± 1.21
Lymphocytes	4.76 ± 1.33
Basophils	19.5 ± 10.3
Eosinophils	16.5 ± 10.1
Monocytes	11.3 ± 4.6

^aValues are mean ± SD; n = 5.

TABLE 4. Coefficient of variation (CV) for chemical components in argatroban-treated plasma^a

Item	CV (%)
AST	1.25 ± 0.19
ALT	1.22 ± 0.21
T-Bil	0.92 ± 0.18
LDH	1.67 ± 0.33
BUN	1.12 ± 0.18
Cre	0.89 ± 0.22
Na	1.43 ± 0.24
K	1.58 ± 0.39
Cl	1.52 ± 0.41
TP	0.88 ± 0.22
Alb	0.96 ± 0.16
T-Cho	1.21 ± 0.18
TG	1.33 ± 0.22
ALP	1.56 ± 0.32
LAP	1.62 ± 0.18
Ch-E	1.56 ± 0.32
γ-GTP	1.33 ± 0.27
UA	0.79 ± 0.15

^aValues are mean ± SD; n = 5.

tains fibrinogen. Because differences between such plasma and serum were limited and predictable, argatroban may be useful for emergency laboratory use as well as for maximizing diagnostic yield from small blood samples.

The number of samples evaluated in the present small pilot study was not large, and the ranges of values compared were limited, but our data indicate suitability of argatroban anticoagulation for hematologic and chemical test specimens. While these data suggest that argatroban is an attractive candidate for use as a laboratory anticoagulant, further clinical trials are necessary.

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