Anticoagulant Effect of Sugammadex

Just an In Vitro *Artifact*

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ABSTRACT

Background: Sugammadex prolongs activated partial thromboplastin time (aPTT) and prothrombin time (PT) suggestive of anticoagulant effects. To pinpoint its presumed anticoagulant site of action, the authors assessed Sugammadex's impact on a panel of coagulation assays.

Methods: Sugammadex, Rocuronium, Sugammadex and Rocuronium combined, or saline were added to blood samples from healthy volunteers and analyzed using plasmatic (*i.e.*, aPTT, thrombin time, and fibrinogen concentration) (n = 8 each), PT (quick), activities of plasmatic coagulation factors, and whole blood (extrinsically and intrinsically activated thromboelastometry) assays (n = 18 each). Furthermore, dose-dependent effects of Sugammadex were also assessed (n = 18 each) in diluted Russel viper venom time (DRVVT) assays with low (DRVVT1) and high (DRVVT2) phospholipid concentrations and in a highly phospholipid-sensitive aPTT assay.

Results: Sugammadex increased PT (+9.1%; $P < 0.0001$), aPTT (+13.1%; $P = 0.0002$), and clotting time in extrinsically (+33.1%; $P = 0.0021$) and intrinsically (+22.4%; $P < 0.0001$) activated thromboelastometric assays. Furthermore, activities of factors VIII, IX, XI, and XII decreased (–7%, $P = 0.009$; –7.8%, $P < 0.0001$; –6.9%, $P < 0.0001$; and –4.3%, $P = 0.011$, respectively). Sugammadex dose-dependently prolonged both DRVVT1 and the highly phospholipid-sensitive aPTT assays, but additional phospholipids in the DRVVT2 assay almost abolished these prolongations. Thrombin time, a thromboelastometric thrombin generation assay, clot firmness, clot lysis, fibrinogen concentration, and activities of other coagulation factors were unaltered. Rocuronium, Sugammadex and Rocuronium combined, and saline exerted no effects.

Conclusion: Sugammadex significantly affects various coagulation assays, but this is explainable by an apparent phospholipid-binding effect, suggesting that Sugammadex`s anticoagulant effects are likely an *in vitro* artifact. **(ANESTHESIOLOGY 2016; 124:1277-85)**

SUGAMMADEX (Bridion®; MSD, The Netherlands) is considered the first representative of a drug class called "selective relaxant binding agents."[1] It has been reported to be biologically inactive and highly selective for steroidal neuromuscular blocking agents without significantly binding to plasma proteins.[2,3] Furthermore, isothermal titration calorimetric analyses suggested that only few substances commonly used during or shortly after anesthesia have the potential for displacement interactions (*i.e.*, the binding of other molecules to Sugammadex instead of Rocuronium or Vecuronium).[4] Nevertheless, concerns regarding Sugammadex's selectivity have been raised by studies in healthy volunteers and surgical patients that demonstrate effects of Sugammadex on coagulation as suggested by prolonged activated partial thromboplastin time (aPTT) and prothrombin time (PT) assays.[5,6] These presumed anticoagulant effects of Sugammadex and concerns regarding potential allergic reactions have hitherto prevented approval by the U.S. Food and Drug Administration.

What We Already Know about This Topic

- Sugammadex prolongs activated partial thromboplastin time and potentially other laboratory coagulation tests, although there is no bleeding associated with this *in vitro* finding, and the mechanism of this effect is not well understood

What This Article Tells Us That Is New

- Sugammadex affects various coagulation assays by the binding of phospholipids by the cyclodextrin molecules, and this represents an *in vitro* artifact observed in commercial phospholipid-dependent assays such as the activated partial thromboplastin time

Data on Sugammadex's impact on coagulation are limited to few plasmatic coagulation assays (*i.e.*, aPTT and PT, and anti-factor Xa assays)[5–7] and platelet function testing,[8] limiting the understanding of this drug.

Accordingly, to elucidate this issue and to pinpoint Sugammadex`s potential site of anticoagulant action, we

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assessed Sugammadex's effects on various plasmatic and activated whole blood thromboelastometric coagulation assays as well as on activities of plasmatic coagulation factors. Furthermore, to verify that potential effects are mediated by Sugammadex but not Rocuronium or the Sugammadex-Rocuronium complex, all assays were conducted with addition of either drug alone, addition of both Sugammadex and Rocuronium in equimolar concentrations, and addition of sodium chloride, the latter to serve as a control, respectively. Since the results suggested that Sugammadex's effects could be explainable by a phospholipid-binding effect, we subsequently assessed the effects of increasing concentrations of Sugammadex on diluted Russel viper venom time (DRVVT) assays with low (DRVVT1) and high (DRVVT2) phospholipid concentrations, as well as a highly phospholipid-sensitive aPTT assay, respectively.

Materials and Methods

After ethics committee approval (Ethik-Komission der Universität Duisburg Essen, Essen, Germany) and written informed consent, the presumed anticoagulant effects of Sugammadex, Rocuronium, and the Sugammadex-Rocuronium complex were sequentially investigated in two experimental series (table 1). For plasmatic and whole blood coagulation assays, venous blood (60 ml) was drawn from healthy volunteers. All subjects had a negative history for bleeding and prothrombotic diathesis and denied the intake of any medication that might have influenced coagulation. Women who could not rule out pregnancy were not included. After venous puncture, the tourniquet was removed in order to avoid venous stasis and blood was drawn into tubes containing sodium citrate (capacity 3,000 μl; Monovette, Germany). After blood sampling, tubes were stored at room temperature and processed within 30 min.

Measurements

Standard coagulation assays, assessment of coagulation factor activities (factors II, V, VII, VIII, IX, X, XI, XII, and XIII, respectively), and DRVVT assays were performed using a BCS® XP or BCS® Analyzer (both from Siemens Healthcare Diagnostics Products GmbH, Germany) and commercially available assays, as appropriate. Specifically, Dade® BC Thrombin, Dade® Inulin®, Dade® Actin®, and Multifibren® U assays (all from Siemens Healthcare Diagnostics Products GmbH) were used for thrombin time, PT, aPTT, and fibrinogen measurements, respectively. Activities of coagulation factors II, V, VII, and X were derived from PT measurements using the Dade® Inovin® assay, after dilution of the plasma samples with commercially available standard human plasma deficient of the respective coagulation factor (Siemens Healthcare Diagnostics Products GmbH). Similarly, activities of coagulation factors VIII, IX, XI, and XII were derived from aPTT measurements using Dade® Actin® assays and standard human plasma deficient of the respective coagulation factor (Siemens Healthcare Diagnostics Products GmbH). Factor XIII activity was assessed using the chromogenic Berichrom F XIII® assay (Siemens Healthcare Diagnostics Products GmbH). LA1 Screening Reagent and LA2 Confirmation Reagent (Siemens Healthcare Diagnostics Products GmbH) were used for the DRVVT1 and DRVVT2 measurements, respectively. The TriniCLOT HS aPTT (Tcoag, Ireland) was used as a second phospholipid-sensitive assay in series 2, as recommended by the International Society on Thrombosis and Hemostasis guidelines for the detection of phospholipid-binding antibodies.[9]

Thromboelastometric measurements were performed according to the manufacturer's instructions using three rotational thromboelastometry (ROTEM®) delta devices (TEM® International GmbH, Germany) and commercially available thromboelastometric assay activated using tissue factor, phospholipids, and calcium chloride (EXTEM®; TEM® International GmbH) and thromboelastometric assay activated using ellagic acid, phospholipids, and calcium chloride (INTEM®; TEM® International GmbH). Assays were run for at least 60 min (until clot lysis index 60 could be determined) after initial clotting in order to assess potential

Table 1. Outline of Sequential Experimental Series Performed

	Series 1	Series 2
No. of healthy volunteers (n) (male/female)	18 (8/10)	18 (16/2)
Age (yr) (mean ± SD)	33.1 ± 7.1	34.4 ± 7.9
Investigated drugs and their targeted blood concentration	1. Sugammadex (130 μg/ml) 2. Rocuronium (32.5 μg/ml) 3. Sugammadex (130 μg/ml) + Rocuronium (32.5 μg/ml) 4. Saline (control)	1. Sugammadex (130 μg/ml) 2. Sugammadex (65 μg/ml) 3. Sugammadex (32.5 μg/ml) 4. Sugammadex (16.25 μg/ml) 5. Saline (control)
Coagulation assays	Plasmatic coagulation assays (international normalized ratio, activated partial thromboplastin time, thrombin time, and fibrinogen concentration), ROTEM® analyses (*i.e.*, EXTEM®, INTEM®, and diluted EXTEM® assays), and coagulation factor activities (*i.e.*, factors V, VIII, IX, X, XI, XII, and XIII)	DRVVT with low (DRVVT 1) and high (DRVVT 2) phospholipid concentration and activated partial thromboplastin time

Diluted EXTEM® assay (TEM® International GmbH, Germany) = EXTEM® reagent diluted 1:2,000 with HEPES buffer; DRVVT = diluted Russel viper venom time; EXTEM® = thromboelastometric assay activated using tissue factor, phospholipids, and calcium chloride; INTEM® (TEM® International GmbH) = thromboelastometric assay activated using ellagic acid, phospholipids, and calcium chloride; ROTEM® (TEM® International GmbH) = rotational thromboelastometry.

effects on clot lysis. The following ROTEM® variables were assessed: clotting time (CT), clot formation time, maximum clot firmness (MCF), and clot lysis index 60 min after initial clotting. Details on the ROTEM® device, measurement variables, and assays used can be found elsewhere.[10]

To assess thrombin generation, another thromboelastometric whole blood assay, using minimal tissue factor activation and using 20 μl of diluted EXTEM® reagent (1:2,000 dilution with HEPES buffer) as an activator, was performed as described by Sørensen *et al.*[11] For these assays, CT and the area under the curve of the first derivative of the thromboelastometric trace plotted against time were obtained. Measurements were recorded for 60 min.

Experimental Protocols

Standard coagulation assays and analyses of coagulation factor activities were performed in citrated plasma samples obtained by centrifuging whole blood samples at a relative centrifugal force of 1,040*g* for 10 min using a Rotina 48R Centrifuge (Firma Hettich, Germany). To serve as a control, 400 μl saline was added to one tube. To assess drug effects, either Sugammadex alone (200 and 200 μl saline; final Sugammadex concentration: 130 μg/ml), Sugammadex (200 μl, final concentration: 130 μg/ml) and Rocuronium (200 μl, final concentration: 32.5 μg/ml) combined, or Rocuronium (200 μl 200 μl saline, final Rocuronium concentration: 32.5 μg/ml) were added to the tubes containing citrated whole blood before centrifugation.

To assess drug effects on ROTEM® variables, 10-fold smaller amounts of either drug and/or saline were added directly to the measurement cups before measurements, in order to achieve the same final concentrations and a similar degree of hemodilution.

Diluted Russel viper venom time assays and the TriniCLOT HS aPTT analyses were performed in platelet-poor plasma. After addition of either 400 μl of saline (control) or 400 μl of Sugammadex and additional saline as needed (total volume again 400 μl; final concentrations: 16.25, 32.5, 65, and 130 μg/ml, respectively) to the tubes containing whole blood samples, the samples were centrifuged at a relative centrifugal force of 3,080*g* for 10 min. Afterward, about three fourth of the supernatant underwent the same centrifugation process for a second time. Three quarters of the resulting supernatant were then used for the coagulation assays.

Statistical Analysis

Data were analyzed using Prism 6 for Mac OS X, Version 6.0b (GraphPad Inc., USA). Based on a previous study elaborating on the effect of Sugammadex on aPTT and PT measurements, a sample size of 8 was considered to be sufficient to detect significant effects of Sugammadex on coagulation assays.[5] Normal distribution of data could not be demonstrated using a Kolmogorov–Smirnov test with Dallal and Wilkinson approximation to Lilliefors' method. Accordingly, nonparametric Friedman tests with Dunn adjustment of the α-error were used for statistical analyses. Data are shown as median (25th/75th percentile) and mean percentage as compared to the respective controls, with *P* values adjusted for the number of statistical comparisons performed. Values of DRVVT1 and DRVVT2 assays at the various Sugammadex concentrations were compared using the Wilcoxon signed-rank test, considering an adjusted *a priori* α-error *P* value of 0.01 as statistically significant in order to account for multiple testing.

Results

Effects on Plasmatic Coagulation Assays

Addition of Sugammadex but not of Rocuronium or of Sugammadex and Rocuronium in equimolar amounts significantly prolonged the PT (quick) and aPTT measurements. Thrombin time and fibrinogen concentration (Clauss method) were not significantly affected by the addition of either drug or their combination. Data on the effects of administration of Sugammadex, Rocuronium, and Sugammadex and Rocuronium combined on the results of the plasmatic coagulation assays are shown in table 2.

Specifically, addition of Sugammadex resulted in a significantly decreased PT (quick) (74.5 [65/86]; −16.4%; $P < 0.0001$) as compared with controls (91.5 [83/99.25]). In contrast, addition of Rocuronium alone (91 [81.8/99]); +0.4%) and of Rocuronium and Sugammadex (91 [83.8/99]; +0.8%) combined did not significantly affect the PT. In a similar way, addition of Sugammadex alone (30.6 s [29.8/21.5]; +13.1%; $P = 0.0003$) resulted in significant prolongation of the aPTT as compared with controls (27 [26.4/28.3]). Addition of Rocuronium alone (27.2 [26.8/28.5]; +1.1%) and of Rocuronium and Sugammadex combined (27.6 [26.3/28.5]; +1.3%) did not exert significant effects on aPTT measurements.

Effects on Thromboelastometric Variables

Addition of Sugammadex to citrated human whole blood resulted in a significant prolongation in CT in EXTEM® and INTEM® assays, while MCF and clot lysis in EXTEM® assays were not significantly affected by either drug or their combination. Furthermore, neither CT nor the area under the curve of the first derivative of the thromboelastometric trace plotted against time were significantly affected by the addition of Sugammadex, Rocuronium and Sugammadex, or Rocuronium. Data on the effects on ROTEM® variables are shown in table 2.

Specifically, addition of Sugammadex resulted in a prolonged CT in EXTEM® assays (61 [56/71]; +33.1%; $P = 0.0028$) as compared with controls (49 [47/57]). Addition of Rocuronium alone (48 [45/55]; +4.3%) and combined administration of Sugammadex and Rocuronium (49 [43/55]; +1.3%) did not significantly affect CT. In INTEM® assays, CT was significantly prolonged by the addition of Sugammadex (196 [183/214]; +22.4%; $P < 0.0001$) as compared with controls (165 [150/177]) but not by addition of Rocuronium alone (173 [155/179]; +4.4%) or by

Table 2. Results of Plasmatic Coagulation Assays, Thromboelastometric Assays, and Coagulation Factor Activities

Assay/Variable (No. of Patients)	Median (25th/75th Percentile)	Mean Percentage of Respective Control	*P* Value
PT (n = 18)			
Control	91.5 (83/99.3)	100	
Sugammadex	74.5 (65/86)	83.4	< 0.0001*
Sugammadex + Rocuronium	91 (83.8/99)	98.6	0.21
Rocuronium	91 (81.8/99)	98.9	0.21
TT (s) (n = 8)			
Control	18.5 (18.1/19)	100	
Sugammadex	18.7 (18.3/19.3)	101.1	0.24
Sugammadex + Rocuronium	18.7 (18.1/19.5)	101.4	0.2
Rocuronium	18.5 (18.2/18.8)	99.6	> 0.99
aPTT (s) (n = 8)			
Control	27 (26.4/28.3)	100	
Sugammadex	30.6 (29.8/31.5)	113.1	0.0002*
Sugammadex + Rocuronium	27.6 (26.3/28.5)	101.3	0.62
Rocuronium	27.2 (26.8/28.5)	101.1	0.53
Fibrinogen concentration (mg/dl) (n = 8)			
Control	218.5 (204.3/238)	100	
Sugammadex	222 (212.3/246.5)	101.4	0.2
Sugammadex + Rocuronium	222.5 (210.8/244.3)	104.8	0.44
Rocuronium	224.5 (209.8/245.8)	101.9	0.53
EXTEM® CT (n = 18)			
Control	49 (47/57)	100	
Sugammadex	61 (56/71)	133.1	0.0021*
Sugammadex + Rocuronium	49 (43/55)	101.3	0.774
Rocuronium	48 (45/55)	104.3	0.944
EXTEM® MCF (n = 18)			
Control	60 (56/64)	100	
Sugammadex	59 (58/67)	101	0.56
Sugammadex + Rocuronium	60 (58/64)	101	0.27
Rocuronium	60 (56/63)	100	> 0.99
EXTEM® CLI60 (n = 18)			
Control	89 (87/92)	100	
Sugammadex	88 (86/91)	99.4	0.21
Sugammadex + Rocuronium	89 (87/92)	100.3	> 0.99
Rocuronium	89 (87/92)	100.5	> 0.99
INTEM® CT (n = 18)			
Control	165 (150/177)	100	
Sugammadex	196 (183/214)	122.4	< 0.0001*
Sugammadex + Rocuronium	169 (163/189)	107.5	0.051
Rocuronium	173 (155/179)	104.4	0.35
Diluted EXTEM® CT (n = 18)			
Control	524 (447/580)	100	
Sugammadex	553 (459/636)	105.2	0.064
Sugammadex + Rocuronium	548 (442/598)	100.1	> 0.99
Rocuronium	535 (456/612)	95.7	> 0.99
Diluted EXTEM® AUC (n = 18)			
Control	5,500 (5,234/5,990)	100	
Sugammadex	5,413 (5,218/5,969)	99.5	0.86
Sugammadex + Rocuronium	5,379 (5,222/6,197)	102	> 0.99
Rocuronium	5,673 (5,349/6,110)	100.5	0.21
Factor II (n = 18)			
Control	92 (86.25/97.75)	100	
Sugammadex	92 (84.25/94.75)	97.6	0.74
Sugammadex + Rocuronium	92 (86.5/97.25)	100	> 0.99
Rocuronium	92 (87.75/96)	99.2	> 0.99

(*Continued*)

Table 2. (Continued)

Assay/Variable (No. of Patients)	Median (25th/75th Percentile)	Mean Percentage of Respective Control	*P* Value
Factor V (n = 18)			
Control	97.5 (83.5/108.3)	100	
Sugammadex	89.5 (82.25/102.8)	95.7	0.06
Sugammadex + Rocuronium	99.5 (89.25/105)	101.1	> 0.99
Rocuronium	96.5 (87.75/104.5)	101.8	> 0.99
Factor VII (n = 18)			
Control	85 (76/110.3)	100	
Sugammadex	85 (75.75/100)	97	0.072
Sugammadex + Rocuronium	86 (76.5/107.8)	100.2	> 0.99
Rocuronium	90 (76/103)	101.9	> 0.99
Factor VIII (n = 18)			
Control	97.5 (77.53/111.1)	100	
Sugammadex	92.9 (58.9/117.9)	93	0.009*
Sugammadex + Rocuronium	101.4 (68.53/107.9)	98	> 0.99
Rocuronium	93.2 (70.95/114.9)	97	0.21
Factor IX (n = 18)			
Control	89 (77.25/96.75)	100	
Sugammadex	81 (72.5/91)	92.2	< 0.0001*
Sugammadex + Rocuronium	86.5 (74.25/95.25)	98.4	0.82
Rocuronium	90 (77/97)	98.3	0.32
Factor X (n = 18)			
Control	94.5 (83.25/99.5)	100	
Sugammadex	93 (83/100.3)	99.6	> 0.99
Sugammadex + Rocuronium	96 (84.25/103.5)	101.9	0.21
Rocuronium	95 (84.5/102.3)	102.6	0.18
Factor XI (n = 18)			
Control	85 (80.75/92.5)	100	
Sugammadex	78 (73/86.5)	93.1	< 0.0001*
Sugammadex + Rocuronium	83.5 (80/91.5)	99.1	> 0.99
Rocuronium	83.5 (78.5/90.5)	99.1	> 0.99
Factor XII (n = 18)			
Control	96 (89/110.3)	100	
Sugammadex	95 (84.75/103)	95.7	0.011*
Sugammadex + Rocuronium	97 (88.25/110.5)	99.5	> 0.99
Rocuronium	99 (88.75/110.3)	100.9	0.99
Factor XIII (n = 18)			
Control	95.5 (76.75/114.3)	100	
Sugammadex	97 (73.75/113)	98.9	0.41
Sugammadex + Rocuronium	96.5 (77.25/114.8)	100.2	> 0.99
Rocuronium	100 (78.5/104.8)	99.7	> 0.99

Data are presented as median (25th/75th percentile), percentage of the respective control, and adjusted *P* values according to Friedman tests with Dunn adjustment of the α-error.

*Indicates $P < 0.05$.

aPTT = activated partial thromboplastin time; AUC = area under the curve of the first derivative of the thromboelastometric trace plotted against time; CLI60 = clot lysis index 60; CT = clotting time; diluted EXTEM® (TEM® International GmbH, Germany) = EXTEM® diluted 1:2,000 with HEPES buffer; EXTEM® = thromboelastometric assay activated using tissue factor, phospholipids, and calcium chloride; INTEM® (TEM® International GmbH) = thromboelastometric assay activated using ellagic acid, phospholipids, and calcium chloride; MCF = maximum clot firmness; PT = prothrombin time (quick); TT = thrombin time.

administration of Sugammadex and Rocuronium combined (169 [163/189]; +7.5%).

Effects on Activities of Individual Coagulation Factors

Addition of Sugammadex, but not of Rocuronium or of Sugammadex and Rocuronium combined, resulted in a significant decrease in the activities of factors VIII, IX, XI, and XII, respectively. Activities of all other coagulation factors analyzed were not significantly affected by addition of either drug. Data are presented in table 2.

Specifically, the activity of factor VIII was significantly decreased after addition of Sugammadex (92.9 [58.9/117.9]; −7%; $P = 0.012$). Addition of Rocuronium (93.2 [70.95/114.9]; −3%) or Rocuronium and Sugammadex combined (101.4 [68.53/107.9]; −2%) did not significantly affect factor VII activity.

The activity of coagulation factor IX was significantly decreased by addition of Sugammadex (81 [72.5/91]; –7.8%; $P < 0.0001$), as compared with controls (89 [77.25/96.75]) but not by the addition of Rocuronium (90 [77/97]; –1.7%) or of Rocuronium and Sugammadex combined (86.5 [74.25/95.25]; –1.6%).

Similarly, addition of Sugammadex significantly decreased the activity of coagulation factor XI (78 [73/86.5]; –6.9%; $P < 0.0001$). Again addition of either Rocuronium (83.5 [78.5/90.5]; –0.9%) or Rocuronium and Sugammadex combined (83.5 [80/91.5]; –0.9%) did not exert significant effects.

Coagulation factor XII activity was significantly decreased by the addition of Sugammadex (95 [84.75/103]; –4.3%; $P = 0.0148$), as compared with controls (96 [89/110.3]), but not by the addition of Rocuronium (99 [88.75/110.3]; +0.9%) or by Rocuronium and Sugammadex combined (97 [88.25/110.5]; –0.5%).

Effects on DRVVT and on Phospholipid-sensitive aPTT

Sugammadex significantly prolonged the highly phospholipid-sensitive aPTT and DRVVT assay with a low phospholipid concentration (DRVVT1) in a dose-dependent manner (fig. 1). This effect was markedly and significantly mitigated in a second DRVVT assay with a higher phospholipid concentration (DRVVT2). Data on aPTT and DRVVT measurements are presented in table 3 and figure 1.

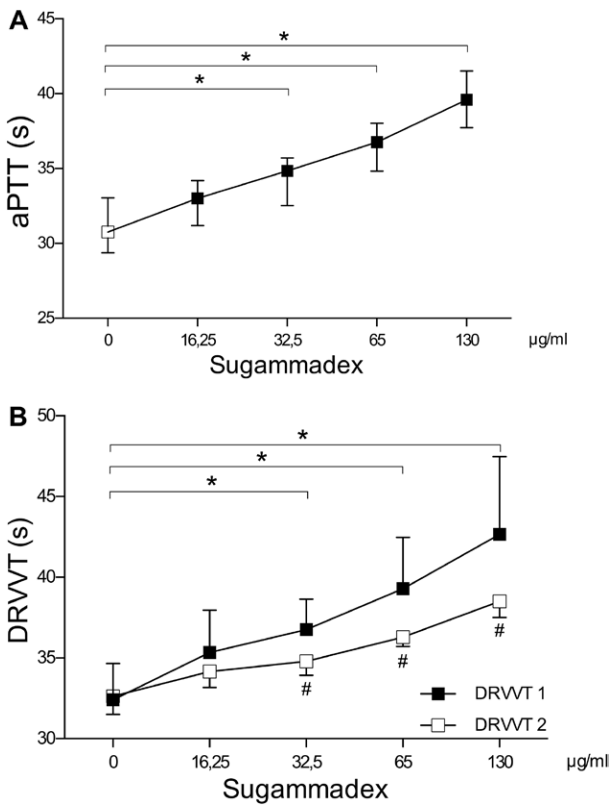

Fig. 1. Sugammadex significantly (*statistically significant) prolongs highly phospholipid-sensitive activated partial thromboplastin time (aPTT) (*A*) and diluted Russel viper venom time (DRVVT) with low phospholipid concentration (DRVVT1; *B*; *black boxes*). High concentrations of phospholipids in another DRVVT assay (DRVVT2; *B*; *white boxes*) significantly decrease this effect (#statistically significant considering an adjusted α-error of 0.01).

Discussion

The present experiments were aimed to characterize the presumed anticoagulant effects of Sugammadex using a broad panel of plasmatic and whole blood coagulation assays. Our data demonstrate that Sugammadex affects numerous of these coagulation assays, suggesting an anticoagulant effect. However, results from DRVVT assays, using low and high phospholipid concentrations, respectively, reveal that these effects of Sugammadex are likely to be exerted by its binding of phospholipids contained in such assays, and thus an *in vitro* artifact is observed in commercial phospholipid-dependent assays.

Effects of Sugammadex on "coagulation" have been reported.[5] In fact, these observations were responsible, in part, for the Food and Drug Administration's decision to not approve Sugammadex in 2008. Meanwhile, a retrospective study in surgical cancer patients, considered at high risk for postoperative bleeding, demonstrated that Sugammadex, at doses of 2 and 4 mg/kg, was not associated with increased bleeding.[12] Furthermore, a recent randomized double-blind trial in patients undergoing orthopedic surgery demonstrated that administration of Sugammadex (4 mg/kg), despite causing prolongations of aPTT and PT assays, was not associated with an increased incidence or severity of postoperative bleeding. Although these latter studies are indicative of the safety of Sugammadex, at least at lower doses, investigations aimed to discover the underlying mechanism of Sugammadex's effects on coagulation are sparse, and available data are essentially limited to aPTT, PT,[5,6] anti-Xa assays,[7] and to collagen-activated whole blood impedance aggregometric measurements.[8] Our present data reconcile the apparent discrepancy between clinical data on bleeding and coagulation measurements.

In our study, addition of Sugammadex but not of Rocuronium or of Sugammadex and Rocuronium combined significantly affected several coagulation assays and suggested anticoagulant effects. Specifically, the aPTT was found prolonged (+13.1%), the PT (quick) increased (+9.1%), and whole blood thromboelastometric CTs were prolonged after both extrinsic and intrinsic activation (EXTEM®: +33.1%; INTEM®: +22.4%, respectively). In contrast, thrombin time assays, fibrinogen concentration, as well as thromboelastometric MCF, and measures of fibrinolysis were not affected. Furthermore, another whole blood thromboelastometric assay reflecting thrombin generation[11] was unaffected by Sugammadex.

These data suggest that the anticoagulant effect of Sugammadex is mediated by this molecules cavity since only addition of Sugammadex but not of Sugammadex and Rocuronium together affected the various coagulation assays. Furthermore, our data on CTs are in accordance with the only prior study using *in vivo* and *in vitro* administration

Table 3. Results from DRVVT Assays and Phospholipid-sensitive aPTT Measurements

	Control	Sugammadex 16.25 μg/ml	Sugammadex 32.5 μg/ml	Sugammadex 65 μg/ml	Sugammadex 130 μg/ml
aPTT (TriniCLOT) (s)	30.8 (29.4/33)	33 (31.2/34.2)	34.9 (32.5/35.7); *P = 0.0006	36.8 (34.8/38); *P < 0.0001	39.6 (37.7/41.5); *P < 0.0001
DRVVT1 (s)	32.4 (31/34.7)	35.4 (33.8/38)	36.8 (35.1/38.7); *P = 0.0009	39.3 (37.5/42.5); *P < 0.0001	42.7 (41.6/47.5); *P < 0.0001
DRVVT 2 (s)	32.6 (31.5/33.3)	34.2 (33.2/35.7); †P = 0.0007	34.8 (33.9/35.8); †P < 0.0001	36.3 (35.7/37); †P < 0.0001	38.5 (37.5/39.4); †P < 0.0001

Data are presented as median (25th/75th percentile). Comparisons for activated partial thromboplastin time (aPTT) and diluted Russel viper venom time (DRVVT) "screening" assay with low phospholipid concentration (DRVVT1) were made using Friedman test with Dunn adjustment of the α-error, and adjusted P values are reported. Results from DRVVT1 and DRVVT "confirmation" assay with high phospholipid concentration (DRVVT2) assays at the respective Sugammadex concentration were compared using Wilcoxon signed-rank tests.

*Significant difference to the respective saline control. †Indicating significant differences between DRVVT1 and DRVVT2 assays, respectively, considering an adjusted α-error of 0.01 as statistically significant.

of similar doses of Sugammadex[5] and with the notion that lower doses exert less effects.[13]

The percentage alteration of the aPTT and the PT (quick) after *in vitro* administration of Sugammadex (130 μg/ml) in our study is only slightly below the alteration reported by others using IV application of Sugammadex (16 mg/kg).[5] Any quantitative differences may be explainable by the use of different coagulation assays (*e.g.*, Dade Actin® *vs.* Dade Actin® FS)[14] or by the differences in Sugammadex concentrations.

Sugammadex prolonged CTs in intrinsically and extrinsically activated thromboelastometric whole blood assays to an even somewhat greater extent than the CTs in plasmatic assays with analogous activation of coagulation. Since one of the main differences between plasmatic and whole blood assays obviously is the presence of platelets in whole blood and since platelets play a major role in thrombin generation, inhibition of platelet function by Sugammadex could be a possible explanation.[15,16] However, Sugammadex (4 mg/kg) has no significant effects on collagen-induced platelet aggregation as assessed by impedance aggregometry.[8] Our data using whole blood thromboelastometric assays rule out major effects of Sugammadex on platelet function, fibrin polymerization, and platelet–fibrin interaction as MCF was unaltered.

Since CTs of both extrinsically and intrinsically activated assays were prolonged by Sugammadex, we expected either an inhibitory effect of Sugammadex on one or more factors of the common coagulation pathway (*i.e.*, factors II, V, and X) or on at least one factor in both the extrinsic and intrinsic pathways.[17] However, activities of factors II (prothrombin), V, and X were unaffected by Sugammadex. A direct thrombin inhibitor effect (anti-IIa-effect) could be ruled out as causative for our observations by unaffected thrombin time and a whole blood thrombin generation assay.[11] In addition, a relevant anti-Xa activity of Sugammadex has been ruled out previously.[7] Furthermore, assessment of coagulation factor activities revealed that only factors of the so-called intrinsic pathway (*i.e.*, factors VIII, IX, XI, and XII) and represented only in aPTT measurements but not in PT assays[17] showed slight but significantly reduced activities after addition of Sugammadex. In contrast, activities of plasmatic coagulation factors located in the so-called extrinsic and common pathways (*i.e.*, factors II, V, VII, and X) were not markedly decreased by Sugammadex. However, it has been demonstrated that deficiency of a single coagulation factor affects aPTT measurements only if the activity of the factor decreases below 40% and affects PT measurements only if the activity decreases below 20%, respectively.[18] Therefore, although combined deficiencies of two or more coagulation factors may act synergistically,[18] the observed decrease in activities of only four coagulation factors exclusively located in the intrinsic pathway, which is less than 8% (4.3 to 7.8%), seemed insufficient to explain the marked prolongations in aPTT and INTEM® CT and cannot be a causative for the effects of Sugammadex on PT and EXTEM® CT. Effects on contact activation such as evoked by kallikrein inhibitors seem unlikely since inhibitors of contact activation do not alter extrinsically activated coagulation assays.[19,20]

Considering diagnostic pathways and prolongations in both aPTT and PT assays[17] and ruling out inhibitor-like effects on specific coagulation factors, as well as thrombin inhibition, we decided to perform a second series of experiments searching for a phospholipid-binding effect of Sugammadex similar to that observed in patients with antiphospholipid antibodies. According to the recent International Society on Thrombosis and Hemostasis guidelines, at least two phospholipid-sensitive assays, based on different principles, must be prolonged for the diagnosis of antiphospholipid antibodies.[9] Furthermore, another assay with an increased phospholipid concentration is recommended for confirmation. As demonstrated by our data, both a highly phospholipid-sensitive aPTT assay and a DRVVT assay with low phospholipid concentration (DRVVT1) were prolonged by the addition of Sugammadex in a dose-dependent manner. Furthermore, a higher phospholipid concentration in another DRVVT assay (DRVVT2) significantly mitigated this effect and decreased CTs compared with those in the DRVVT1 assay, unmasking a phospholipid-binding effect of Sugammadex.

This finding perfectly aligns with the observations made using coagulation assays in our study. According to the manufacturer, the usual aPTT and PT assays are sensitive

to phospholipid-binding antibodies.[5,14,21] According to the manufacturer, both thromboelastometric assays used in our study contain phospholipids as well. Therefore, it is reasonable that these latter assays are affected by the phospholipid-binding effect of Sugammadex. Systematic thromboelastometric measurements in patients with lupus(-like) anticoagulants or antiphospholipid syndromes are not available. However, in a single patient suffering from antiphospholipid syndromes and undergoing cardiac surgery, CTs were prolonged before cardiopulmonary bypass both in EXTEM® and INTEM® assays,[22] and, similar to that in our study, CTs in EXTEM® assays was more prolonged than the CTs in INTEM® assays.

The observed decrease in coagulation factor activities also is fully in line with an antiphospholipid effect of Sugammadex. All coagulation factor activities except the activity of factor XIII are assessed using either the aPTT or the PT assays and calibrated normal plasma deficient of the specific coagulation factor being assessed. Therefore, all coagulation measurements dependent on phospholipids and influenced by antiphospholipid antibodies will be affected by Sugammadex as well.

A thorough review of the literature reveals that binding of various phospholipids to cyclodextrins has been investigated. Using a flexible docking algorithm based on molecular mechanics, binding of phosphatidylinositol, phosphatidylserine, and phosphatidylethanolamine to α -, β -, and γ-cyclodextrins was simulated, and stable complexes driven by van der Waals forces were considered possible.[23] Although these simulations did not investigate the modified γ-cyclodextrin Sugammadex, these findings seem consistent with our findings regarding an antiphospholipid effect of Sugammadex. Of special importance, all the phospholipids included in these molecular simulations are a constituent in the thromboplastins and partial thromboplastins of coagulation assays.[24,25]

Our study has some limitations. First, our data are derived from an addition of Sugammadex *in vitro*. However, as all coagulation assays are performed *ex vivo* and Sugammadex does not undergo metabolization after IV injection, our results seem to be transferable to an *in vivo* situation. Furthermore, the first part of our study investigated only one concentration of Sugammadex. However, since we searched for hints for elucidating the mechanism by which Sugammadex affects "coagulation," it seemed appropriate to base our calculations on the highest dose used clinically to detect any relevant effects. Finally, although high phospholipid concentrations significantly and markedly decreased Sugammadex-evoked prolongation in DRVVT assays, CTs did not fully return to control. This is likely due to a fixed concentration of phospholipids even in the so-called "confirmatory" DRVVT assay. Since coagulation time of whole blood not containing Sugammadex did not differ between DRVVT assays with high or low phospholipid concentrations, there appears no alternative explanation but a phospholipid-binding effect exerted by Sugammadex.

In conclusion, while Sugammadex significantly affects various coagulation assays, our data reveal that these effects are likely to be exerted by the binding of phospholipids contained in such assays. Accordingly, Sugammadex's presumed anticoagulant effects are likely to just represent an *in vitro* artifact observed in commercial phospholipid-dependent assays.

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Competing Interests

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