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ORIGINAL ARTICLE

Degradation and elimination of succinylcholine and succinylmonocholine and definition of their respective detection windows in blood and urine for forensic purposes

Uta Kuepper · Frank Herbstreit · Jürgen Peters · Burkhard Madea · Frank Musshoff

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Abstract The muscle relaxant succinylcholine (SUX) evokes respiratory paralysis, and numerous cases of fatal SUX intoxication have been reported. Detection of SUX and its metabolite succinylmonocholine (SMC) is difficult, both due to their (bis-) quaternary structure and the extreme hydrolytic susceptibility of SUX, and data on degradation kinetics of SUX and SMC is scarce. The present study investigates the in vivo and in vitro degradation as well as elimination of both target analytes using authentic blood and urine samples from anesthetized patients. With a special focus on the urinary data and stabilization issues, this work intends to considerably enhance the forensic knowledge concerning SUX intoxications and to present the reader with practical analytical strategies to cope with such difficult cases. Eighteen subjects undergoing surgery and requiring arterial as well as bladder catheters were included in this study. Muscle relaxation was initialized with a bolus injection of 80–100 mg SUX. Blood and urine samples were either collected using paraoxonized (n=15) or non-modified (n=3) tubes. Sampling was performed within 6 h after SUX application following a pre-assigned schedule. Samples were processed according to a validated isotope dilution HPLC–MS/MS method using ion-pair solid-phase extraction. In blood, SUX was usually detectable for up to 10 min post-injection, while detection of SMC was possible over the whole observation period of 6 h. Effectiveness of organophosphate stabilization was proven for both analytes and is therefore recommended. In freshly secreted urine, detection windows of a minimum of 2 h as opposed to 6 h have been determined for SUX versus SMC, respectively. Considering SMC plasma kinetics, detection of the metabolite in blood and freshly secreted urine appears to be possible over a period of at least 8–24 h. Paraoxon did not enhance the stability of either target substance in urine, stabilization of urine samples is nonetheless recommended. In summary, SMC was proven to be the most promising target analyte in SUX analysis, with urine being the proposed matrix of choice for forensic applications. Furthermore, our work defines meaningful detection windows for SUX and SMC in blood and urine as routine matrices and presents sampling recommendations as well as guideline values for forensic toxicological analysis.

Keywords Succinylmonocholine · Succinyldicholine · Detection window · Plasma · Urine · Forensic toxicology

Abbreviations

SUX	Succinylcholine
SMC	Succinylmonocholine
HPLC-(ESI-)MS/MS	High performance liquid chromatography-(electrospray ionization-)tandem mass spectrometry
LOD	Limit of detection
SPE	Solid-phase extraction

Uta Kuepper and Frank Herbstreit share equal authorship.

U. Kuepper (✉)
Institute of Forensic Medicine, University Hospital Essen,
Hufelandstrasse 55,
45147 Essen, Germany
e-mail: Uta.Kuepper@uk-essen.de

F. Herbstreit · J. Peters
Klinik für Anästhesiologie & Intensivmedizin,
Universität Duisburg-Essen and Universitätsklinikum Essen,
Hufelandstrasse 55,
45147 Essen, Germany

U. Kuepper · B. Madea · F. Musshoff
Institute of Forensic Medicine, University of Bonn,
Stiftsplatz 12,
53111 Bonn, Germany

Introduction

Succinylcholine (SUX) is a bis-quaternary ammonium compound which structurally resembles the physiological transmitter acetylcholine. SUX acts as an agonist at postsynaptic acetylcholine receptors in skeletal muscle, where its biological activity is prolonged because of its comparatively slow local degradation by acetylcholinesterase (EC 3.1.1.7). As a result of this pharmacological profile, SUX is used as a short acting depolarizing muscle relaxant in anesthesia, with the ensuing respiratory paralysis being compensated by mechanical ventilation. However, in absence of respiratory assistance, a therapeutic dose of SUX may cause a potentially lethal respiratory paralysis [1–3]. The drug is degraded within minutes [4–10] by unspecific cholinesterases (butyrylesterase; EC 3.1.1.8), yielding succinylmonocholine (SMC) as the first, and succinate and choline as subsequent degradation products [11–13]. Its fast degradation to eventually endogenous compounds as well as the fact that both SUX and SMC are analytically challenging compounds led to SUX having the reputation of an undetectable poison. Furthermore, recent reports on detection of the more stable SMC in SUX negative postmortem control tissues questioned the metabolites' suitability as a SUX marker [14, 15].

Our group previously reported the development and validation of an HPLC-ESI-MS/MS method using ion-pair solid-phase extraction [16, 17]. Having established that no endogenous SMC is present in serum or urine [18], an investigation of in vivo as well as in vitro elimination of SUX and especially SMC seemed worthwhile. A previous work already employed the described analytical tools to establish the degradation kinetics of SMC in blood [19]. Complementing and completing this model, the present study addresses the degradation and elimination of both SUX and SMC in plasma as well as urine, aiming to define significant detection windows for these analytes in the forensically most relevant sample matrices. Additionally, the importance of sample stabilization will be investigated. Collected data will provide reference information for application in toxicological case work.

Materials and methods

Study cohort

Following institutional approval by the local ethics committee and the patients' written informed consent, 18 patients (10 female, 8 male), aged 19–79 years (mean 60.0 years) and weighing 55–101.4 kg (mean 77.1 kg), were enrolled in the Department of Anesthesiology and Intensive Care Medicine, University Hospital Essen, Germany. All patients underwent surgery requiring arterial as well as bladder (Fooley) catheters. Patients were classified as physical status class II (11 patients) or III (7 patients) according to the American Society of Anesthesiologists.

Table 1 presents the individual patient characteristics.

Routine medical treatment (anxiolysis/sedation, anesthesia, analgesia, volume substitution etc.) of patients was essentially as described before [19].

Table 1 Cohorts for stabilized (top) and non-stabilized (bottom) sample series

	Subject [#]	Age [years]	Sex [M/F]	Weight [kg]	Abs. SUX dose [mg]	Rel. SUX dose [mg/kg]
Stabilized sampling	3	67	M	95	100	1.1
	4	69	F	58	100	1.7
	7	69	F	62.5	100	1.6
	8	66	M	101.4	100	1.0
	9	19	M	65	100	1.5
	10	33	F	63	100	1.6
	11	58	F	85	100	1.2
	12	74	M	95	100	1.1
	13	64	F	75	100	1.3
	14	75	F	62	100	1.6
	16	45	M	73	100	1.4
	17	79	F	90	100	1.1
	18	72	M	74	100	1.4
	19	40	F	55	80	1.5
	21	61	F	74	100	1.4
Non-stabilized sampling	5	67	F	80	80	1.0
	23	74	M	95	100	1.1
	24	52	M	85	100	1.2

Muscle relaxation was initiated by an intravenous bolus injection of 80–100 mg SUX (corresponding to 1.0–1.7 mg/kg, mean 1.3 mg/kg). Disturbed metabolism of SUX due to BChE depletion or atypical forms of BChE, resulting in atypically prolonged neuromuscular blockade, was ruled out in each patient by quantitative neuromuscular monitoring. Following recovery from initial neuromuscular blockade, further muscle relaxation was evoked by using rocuronium.

As before [19], the patients' individual diagnosis and medication shall not be given in detail; however, health issues and therapy regimens were known to the authors and have been carefully considered during data interpretation.

Patients requiring infusion of blood products were excluded from the study; other exclusion criteria were not defined.

Study protocol

Before intravenous induction of anesthesia (t_0), a cannula was inserted into the radial artery under local anesthesia. Urethral catheterization was performed immediately after onset of anesthesia.

Arterial blood samples were withdrawn following a predefined schedule: within the first 2 min, blood sampling was carried out as fast as possible (approx. every 20 s), and further blood samples were taken at approx. 2, 2.5, 3, 3.5, 4, 4.5, 5, 7, 9, 11, 15, 20, 25, and 30 min, as well as 1, 2, 4, and 6 h after SUX injection.

Freshly secreted urine was sampled 1, 2, 4, and 6 h post-injection and was withdrawn directly from of the catheters' extension tube. In nine subjects, an additional sample of pooled urine, i.e., urine which had been accumulated during the first hour after SUX injection, was taken from the catheters' reservoir pouch.

Any divergence from this schedule, i.e., cancelled or delayed sampling caused by necessary medical interventions, was thoroughly documented and carefully considered during data analysis.

To assess a possible impact of chemical stabilization, blood and urine samples were collected with or without a stabilizing agent (paraoxon) for later comparison.

Blood was drawn into commercially available tubes (S-Monovette® EDTA-K, 4 ml, Ref.: 03.1068, Sarstedt, Nümbrecht, Germany). For use in the stabilized sample series, these tubes had been pre-treated with 10 μl of a 40-μg/ml aqueous paraoxon solution (corresponding to 100 ng/ml paraoxon in a 4-ml sample); vials for the non-stabilized sample sets remained unaltered.

Freshly secreted urine was collected in 2 ml plastic vials (Eppendorf, Hamburg, Germany) and was either combined with 50 μl of a methanolic paraoxon solution (4 μg/ml, corresponding to 100 ng/ml paraoxon in a 2-ml sample; 15 patients) or processed without modifications (three individuals).

Accumulated (pooled) urine was left unstabilized in three patients and was collected from six subjects using paraoxon stabilization.

Directly after sampling, specimens were vigorously mixed and cooled on ice. Blood samples were treated as detailed before [19]; urine samples were snap-frozen in liquid nitrogen without any prior treatment. Samples were stored at −20°C until analysis.

Sample extraction

Extraction and analysis of samples as well as chemicals, buffer composition, and calibration procedures were performed according to a previously published and fully validated method. Briefly, SUX and SMC were extracted from plasma using ion-pair extraction on polymeric reverse phase SPE cartridges, and analyzed by HPLC-ESI-MS/MS in positive ion mode. Quantitation was achieved by an isotope dilution approach using a set of non-interconvertible internal standards (SUX-d_{18} and SMC-d_3, [17]). Limits of detection and quantitation were well below 10 ng/ml for both target substances; precision and accuracy was constantly below 15% over the whole concentration range (12.5 ng/ml–100 μg/ml) [16].

Matrix-specific calibrations were performed as extracted 6-point calibrations covering the expected concentration ranges (i.e., 12.5, 37.5, 125, 500, 10,000, 40,000 ng/ml) for each analyte in both plasma and urine.

Data processing and statistics

Determination of detection windows in plasma

To better structure and interpret analysis results, the incidence of positive SUX as well as SMC findings in unaltered sample sets was related to the respective sampling time, and resulting data were compared to corresponding values for stabilized specimens. For this purpose, the results of all stabilized as well as non-stabilized sample sets were each combined and subgrouped according to the time of sampling. Subgroups were designed to comprise only samples taken within a time slot as narrow as possible, for reasons of statistical power, however, group size was kept maximized. For the stabilized samples, 18 versus 16 subgroups of 11–21 values each (mean 14.6 versus 16.4 values) were defined for SUX and SMC, respectively. For the unaltered sample series, raw data was subdivided into 11 versus 9 groups of 3–11 values each (mean 5.4 versus 7.1 values) for SUX and SMC, respectively. Due to the size of the non-stabilized cohort ($n=3$), a minimum group size of three single values could not be avoided at later sampling times ($t\approx6$ h).

Group sizes were optimized to account for the differences in degradation speed of SUX as compared to SMC.

For SUX, rather small group sizes—favoring nearly identical sampling times—were chosen to capture even the most abrupt changes in detection frequency of this compound. For SMC, slightly bigger time windows could be incorporated in a single subgroup, thus enhancing informative power and significance.

Continuous raw data (analyte concentrations) were eventually transformed into categorical data ("positive", "negative"), and the percentage of positives in each subgroup was determined.

Evaluation of stabilized versus non-stabilized urine samples

Forensic relevance of paraoxon stabilization in pooled urine was assessed using a two-tailed, unpaired *t* test.

To investigate the forensic relevance of sample stabilization in freshly secreted urine, the results of all stabilized as well as non-stabilized sample sets were each combined and sub-grouped according to the time of sampling as already detailed above. For both SUX as well as SMC in stabilized samples, five subgroups of 15 values each were defined. A single sampling at 40 min could not be sensibly included into any of the subgroups and was thus examined individually. For the unstabilized sample series, raw data for both SUX and SMC were subdivided into five groups of three values each. Standard deviation in sampling time was determined to assess the degree of homogeneity of corresponding data. Equivalent samples were evaluated using a two-tailed, unpaired *t* test.

For a determination of detection frequencies of both target analytes in freshly secreted urine, all samples (irrespective of stabilization status, for reasons see below) were combined and divided into five subgroups of 18 single values each according to sampling time. Continuous data were again transformed into categorical data, and the percentage of SUX and SMC positives in each of the five subgroups was determined.

Results

Analyte concentrations in stabilized plasma

To assess plasmatic detection windows of both target substances under ideal sampling conditions, raw data from 15 stabilized sample series were evaluated.

Usually, SUX was detectable approx. 10 min post-injection; however, in a single patient, a positive result was obtained in a plasma sample taken 65 min after SUX application. Generally, peak-plasma concentrations of no more than 1 μg/ml were observed. An exemplary concentration-time profile of SUX is presented in Fig. 1.

As shown previously [19], SMC was detectable in plasma during the whole 6 h observation interval.

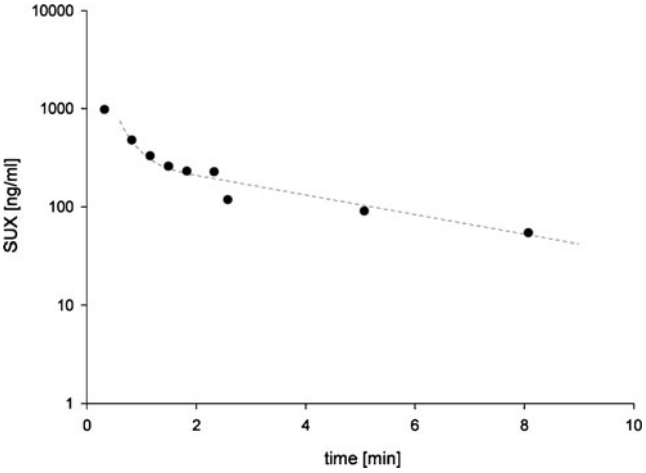

Fig. 1 Representative concentration-time profile of SUX (subject #11)

Concentration profiles in stabilized versus non-stabilized plasma samples

To assess the impact of paraoxon stabilization, three non-stabilized sample sets were compared to results obtained with stabilized sampling. SUX degradation in non-stabilized samples was shown to be substantial, leading to highly erratic concentration-time curves. Accordingly, implausible up- and downward (zigzag) trends in detectable plasma concentrations were observed even between samples that had been taken within a very short time frame (Fig. 2). Compared to chronologically equivalent but stabilized samples, SUX was only detectable in far lower concentrations, or not at all.

For SUX, significantly decreased detectability was observed in all non-stabilized sample sets.

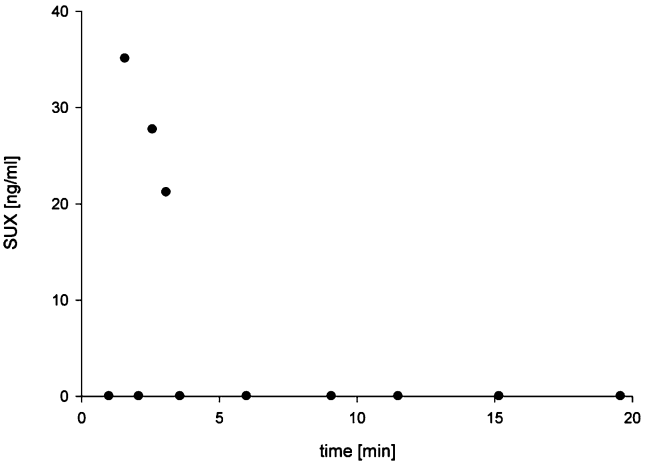

Fig. 2 SUX in unstabilized plasma (subject #23). On a very low concentration level, measured SUX concentrations are highly erratic. Hence, at a given point in time SUX may go undetected, whereas detection may be possible in a subsequent sample

In contrast, effects of paraoxon (or the lack thereof) on SMC degradation were subject to large interindividual differences: in one of the patients (#23) in the non-stabilized cohort, the observed concentration-time profile of SMC was hardly different from those of the stabilized collective (Fig. 3c; cp. [19]); in a second subject (#24), however, no more than 100 ng/ml of SMC were ever detected without stabilization (Fig. 3a). Figure 3 depicts a juxtaposition of SMC concentration-time profiles in the three individuals undergoing non-paraoxonized sampling.

Detection windows in plasma

Forensic relevance of paraoxon stabilization in plasma was assessed as described above. In stabilized plasma samples, the incidence of positive SUX findings quickly decreased to 25% during the first 10 min after SUX injection. After this initial decline, detection frequency decreased more slowly and remained positive over the first hour.

With up to 30% of positive samples, SUX detection in non-stabilized plasma was maximal around the time of C_{max} (T_{max}≈0.5 min, [10]). No SUX positive results were observed whenever sampling was performed later than 4 min post-injection.

Figure 4 illustrates the detection frequencies of SUX in stabilized versus non-paraoxonized plasma samples.

In stabilized plasma, SMC was detectable over the whole observation period. Contrastingly, the incidence of SMC positives in non-stabilized samples decreased to 50% at 4 h post-injection, while at 6 h, only a third of tested samples still yielded a positive result.

Figure 5 depicts the detection frequencies of SMC in stabilized versus non-paraoxonized plasma samples.

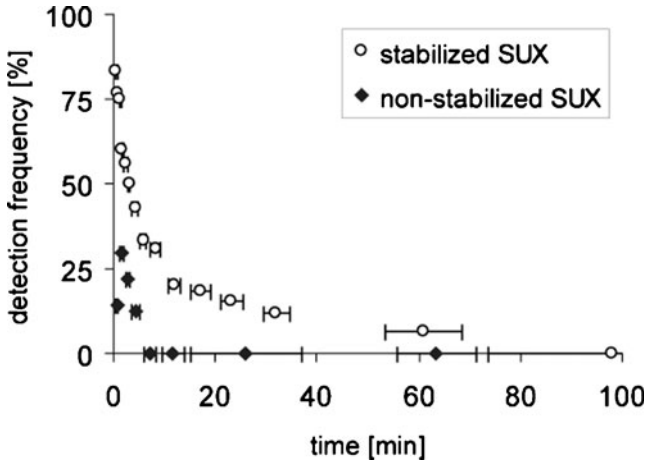

Fig. 4 Detectability of SUX in plasma. Error bars indicate the standard deviation of sampling time for each subgroup of single values

Analyte concentrations in urine

In all stabilized as well as non-stabilized pool urine samples (accumulated during 40–70 min post-injection, mean 62.9 min), SUX was detected in concentrations ranging from 5.7 to 56.9 μg/ml. SMC was detectable in every sample with concentrations of 1.3–189.4 μg/ml. SUX and SMC concentrations in accumulated urine are depicted in Figs. 6a and 7a, respectively.

In freshly secreted urine, i.e., in stabilized as well as non-stabilized samples, SUX and SMC were detected in concentrations of (0–) 0.02–160.7 and 1.3–186.0 μg/ml, respectively. Concentration-time profiles for SUX and SMC in freshly secreted urine are displayed in Figs. 6b and 7b, respectively.

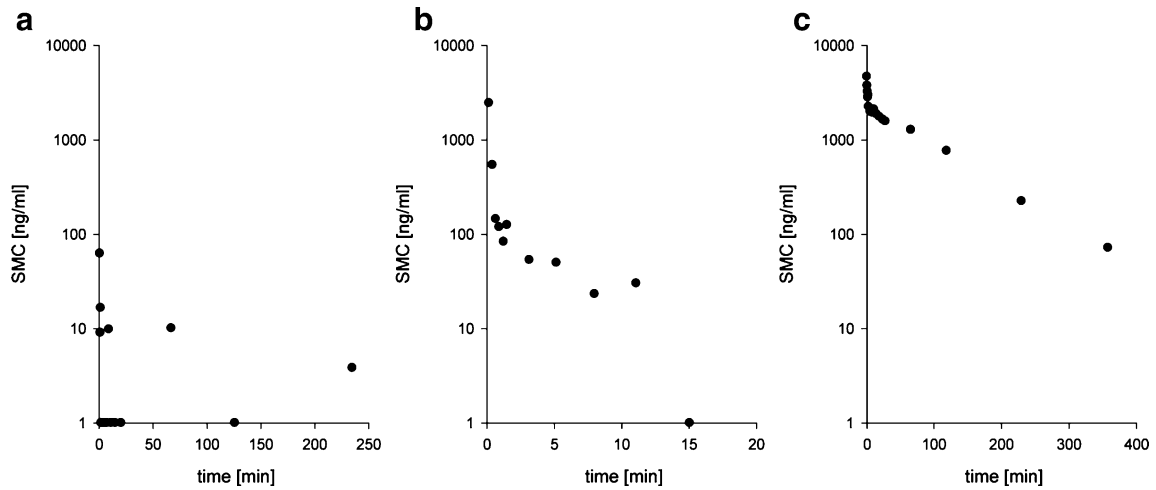

Fig. 3 Differences in the effect of absent paraoxon on SMC degradation. Concentration-time profiles of SMC in the non-paraoxonized cohort are depicted (subject #24, **a**; subject #5, **b**; subject #23, **c**) in semi-logarithmic scale. For clarity, ordinates were adjusted to focus on the most relevant time frames. Lacking "zero" due to the logarithmic scale, negative analysis results are represented by a dot on the ordinate. Interindividual differences in the benefit of stabilization are visible

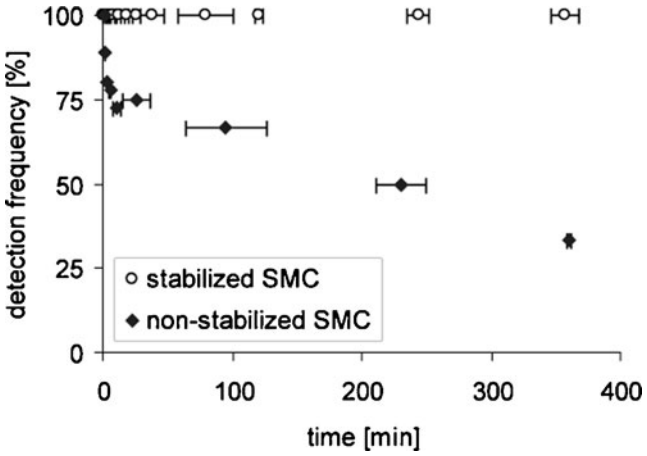

Fig. 5 Detectability of SMC in plasma. Error bars indicate the standard deviation of sampling time for each subgroup of single values

Effect of stabilization in urine samples

Forensic relevance of paraoxon stabilization in pooled urine was assessed as described above. Due to large standard deviations of values within each group, a statistically significant difference of mean concentrations between stabilized and non-stabilized samples could be ascertained for neither SUX nor SMC (p=0.69 for SUX; p=0.42 for SMC).

For freshly secreted urine, the forensic relevance of sample stabilization was investigated as detailed above. Consistently small standard deviations in sampling times (6.3–12 min, i.e., 2.1–8.1%) confirmed the homogeneity of grouped data. Again, large standard deviations in analyte concentration were observed within each subgroup. With probability values usually exceeding 0.41, a statistically significant difference of mean concentrations between stabilized and non-stabilized samples could be ascertained for neither SUX nor SMC. In a single case (stabilized versus non-stabilized SUX in the data subgroup of $t\approx$ 240 min), statistical significance (p=0.02) was shown, but ascribed to a type I statistical error, i.e., a single outlier in the non-stabilized subgroup led to a higher mean concentration in the non-stabilized versus the stabilized subgroup. Due to this bias of means, scientific relevance of the statistical findings can be confidently rejected.

It is to be noted that the absence of statistically detectable differences between stabilized and non-paraoxonized urine samples is not to be confused with their equivalence: the results do legitimize the following, uniform analysis of all urines for the determination of detection windows. However, a detailed discussion on the relevance of urine stabilization in forensic case work will accompany the sampling recommendations given at the end of the following section (see "Discussion").

Detection windows in urine

In all accumulated urine samples, i.e., stabilized as well as non-stabilized pool urines, both SUX and SMC were ubiquitously detected.

In freshly secreted urine samples (again irrespective of stabilization), SMC was detectable over the whole observation period, whereas the incidence of SUX positives decreased over time: 4 h after injection of SUX, the parent compound was still detected in 89% of samples whereas at 6 h post-application only 56% of analyses returned a positive SUX result. Figure 8 depicts the detection frequencies of SUX as well as SMC in freshly secreted urine.

Discussion

For long, SUX intoxications have been difficult to prove, especially in postmortem cases. For the first time, the

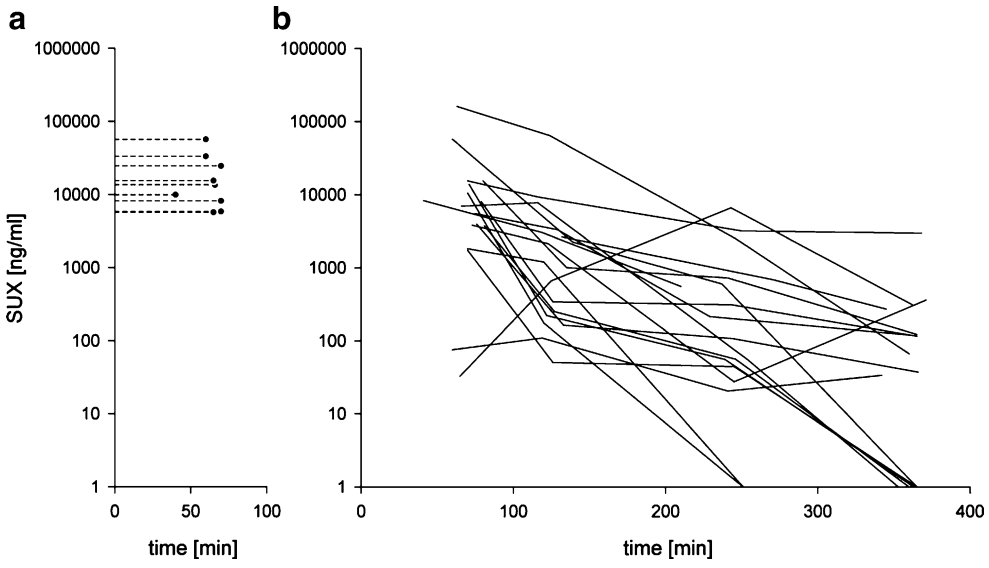

Fig. 6 Overlay of SUX concentration profiles in pooled (**a**) as well as freshly secreted urine (**b**). **a** SUX concentrations in nine pooled urine samples. The accumulation interval is defined by the length of the *dotted lines*, while the *end points* indicate the time of sampling. **b** Overlay of SUX concentration-time profiles of all 18 urinary sample sets. Between individuals, SUX concentrations of chronologically equivalent samples may deviate by a factor of more than 100. Lacking "zero" due to the logarithmic scale, the contact of a curve with the ordinate implies a negative analysis result at the indicated time

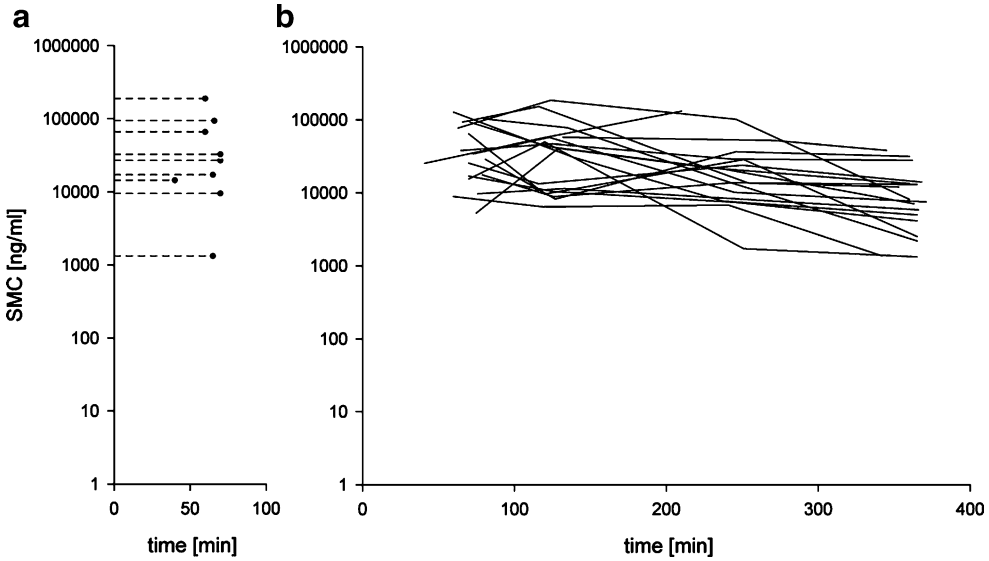

Fig. 7 Overlay of SMC concentration profiles in pooled (**a**) as well as freshly secreted urine (**b**). **a** SMC concentrations in nine pooled urine samples. The accumulation interval is defined by the length of the *dotted lines*, while the *end points* indicate the time of sampling. **b** Overlay of SMC concentration-time profiles of all 18 urinary sample sets. Between individuals, SMC concentrations of chronologically equivalent samples may deviate by a factor of approx. 100

present study uses both authentic blood and urine samples (from individuals without evidence of abnormal SUX metabolism) to define meaningful detection windows, guideline concentration values, and sampling recommendations for forensic toxicological case work.

SUX in blood/plasma

With a usual detection window of approx. 10 min in blood plasma, our results were found to correspond well with previously published values, in particular when considering possible constraints of earlier techniques: in 1993, Hoshi et al. [20] postulated a single-compartment kinetic model with a mean elimination half-life of 16.6 s for SUX, with the analyte being no longer detectable than 150 s. With 25.4 s, the half-life as determined later by Kato et al. [4] was comparatively short. The strikingly brief period of possible SUX detection in these studies may be attributed to the rather insensitive HPLC-methods used. In this context, it seems plausible that the resulting curtailed observation period of a maximum of 5 min led to the deduction of kinetic constants describing distribution rather than elimination processes, and interpretation based on these data therefore should be made with caution.

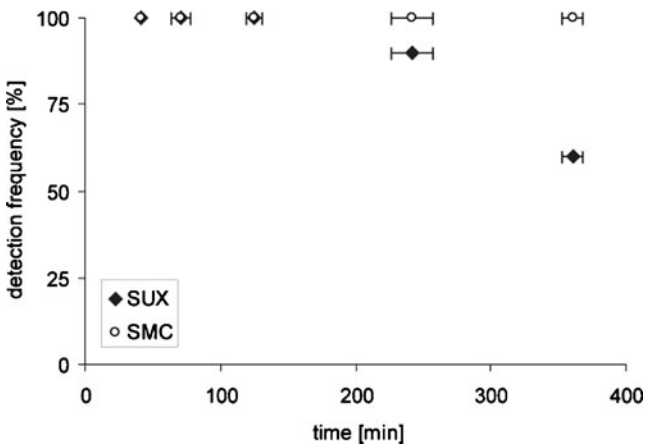

Fig. 8 Incidence of SUX and SMC detection in freshly secreted urine. Error bars indicate the standard deviation of sampling time for each subgroup of single values

In contrast to the above literature values, Lagerwerf et al. [8] postulated a tri-phasic SUX degradation with half-lives of 0.4, 1.2, and 8 min. Especially the long terminal half-life as well as the reported detection of almost 200 ng/ml of SUX at 15 min after application of a single therapeutic dose (2 mg/kg, $n=1$) is remarkable. Due to the reported methodology, however, doubts concerning the study's ability to capture in vivo conditions seem again legitimate. As no selectivity data has been reported, it cannot be excluded that the HPLC method using fluorescence detection may have been subject to interferences. In this context, especially any interference of the structurally related SMC may have led to elevated (apparent) SUX levels and even false positive results, and could therefore explain the largely extended detectability of SUX.

Using different approaches and/or refined methods, ensuing in vivo as well as in vitro studies yielded almost identical elimination half-lives of 41 [9] and 47.6 s [4] for SUX. These values were correlated with a detection window of approx. 7 min following application of a single therapeutic SUX dose (1 mg/kg, limit of detection 25 ng/ml, [9]). Additionally, a pharmacodynamic study on anesthetized patients established an effect compartment half-life of 47 s [7]. The present paper blends in very well with the aforementioned works, although a reduction in the LOD as compared to Roy et al. [9, 10] served to even expand (if only marginally) the detection window of SUX. Nonetheless, detection of SUX in blood/

plasma is highly unlikely if more than 10 min have passed since injection of the drug. In this context, the isolated case of a SUX positive plasma sample taken at 65 min post-injection must seem rather odd; however, apart from elimination also distribution plays an important role in SUX pharmacokinetics.

Within the framework of a two-compartment model [10], an extensive distribution of SUX has been reported, and may be seen as a contributing—if not the most dominant—factor influencing the initial decrease in plasma concentration of this substance [4, 5, 20]. The extent of SUX distribution, however, is hard to define, as analyte degradation in the central compartment (and most probably in the periphery as well) is likely to cause an underestimation of its true distribution volume [10]. As a result thereof, but also depending on the applied kinetic model, extremely divergent steady state distribution volumes of SUX, i.e., 2.2 [4], 16.4 [20], and 39 ml/kg [10] were reported. Again, it has to be kept in mind that older studies may not accurately represent the conditions in vivo, which is why the work of Roy et al. [10], being able to at least prove a dispersion of SUX in the entire intravascular space, seems most plausible. A comparatively slow re-distribution from peripheral compartments [19, 21, 22], especially if assuming an esterase-poor or -inactive periphery [21], may therefore serve as a satisfactory explanation for positive SUX findings in blood samples taken long after injection of the drug.

SMC in blood/plasma

Compared to SUX, pharmacokinetic characteristics of SMC were shown to be more favorable for detection in forensic case work [19]. Using SMC, the time frame to possibly confirm prior SUX application is extended from a few minutes to at least several hours, the metabolite was thus proven to be the only realistic plasmatic SUX marker in a forensic context.

For more detailed information on the pharmacokinetics of SMC, the reader is referred to the above-cited publication.

SUX and SMC in urine

Detection of analytes in urine requires their unchanged elimination with this medium, which has been described for both SUX as well as SMC [12, 23]. However, data on the renal elimination of both target analytes is scarce, with the present study being the first to use authentic patient data to evaluate urine for its potential as a SUX marker in forensic toxicological case work.

Subjects in this study exhibited significantly different urinary SUX and SMC levels, with analyte concentrations of chronologically equivalent samples sometimes deviating by a factor of more than 100.

In this context, Foldes et al. already reported a large variation in the extent of urinary SMC elimination. While consistently less than 3% of a SUX dose was excreted in urine, between 3.8% and 22.9% of injected SMC were eliminated this way [12, 23]. The authors attributed these discrepancies to a non-uniform metabolization of SMC in blood and discussed the possible impact of heterogeneous enzyme endowment, i.e., varying amounts or activity of BChE or other unknown esterases [23].

The variance of urinary analyte concentrations in the present study might be consistent with such differences in enzymatic metabolization; however, other explanations are likewise conceivable. Apart from the extent and speed of degradation in blood, urinary substance concentrations are dependent on a multitude of influencing factors, especially kidney function (e.g., filtration rate, extent of excretion versus resorption, total amount of diuresis, and even enzyme permeability leading to esterase-active urine [24]). The present study draws its forensic importance from exactly this enormous complexity and (potential) variance of involved processes. For the first time, relevant data was collected using a comparatively large number of subjects, eventually allowing plausibility control of analysis results in forensic toxicological case work.

Urine was generally proven to be a very good matrix for SUX analysis, with accumulated pool urine being shown to act as a reservoir for administered SUX as well as for its metabolite SMC. In spite of these characteristics, employing urine as a target matrix in forensic routine work may entail difficulties with data interpretation, particularly in cases of survived SUX intoxications. Here, due to often only slowly developing suspicions and thus delayed sampling, surviving victims may void their bladder contents one or even several times until a urine sample is eventually secured for later analysis. For this scenario, only one previous publication has reported that after a single bladder movement (ascribed to SUX-induced incontinence) SUX detection in urine was no longer possible [24]. However, SMC was not analyzed in the cited article, and the chronological order of SUX administration, micturition, as well as sampling was not documented. In this context, the present work yields more insight by showing that as long as a urine sample is taken within at least 6 h following SUX application, intoxications can be proven via detection of SMC irrespective of any (even several) interim urinations. For the parent compound, such proof can only be obtained if a sample is collected within 2 h post-application.

In fatal intoxications, perimortally secreted SUX as well as SMC will accumulate in the urine, and, whenever muscle relaxation does not result in a complete emptying of the bladder at the time of death, will be detectable in collected urine despite the usually rather short agony [16, 24]. Our

data indicate an already large scatter of urinary concentrations even under well-defined conditions; in forensics, however, results may additionally be subject to a range of uncontrollable influences, i.e., (among others) survival time and the extent of (post-mortem) residual kidney activity. As a consequence, different analyte concentrations as well as concentration ratios of SUX versus SMC seem possible.

Effectiveness of sample stabilization

Addition of paraoxon to urine did not result in a significant increase of either SUX or SMC stability. This is not surprising since urine can be considered a usually esterase-free sample matrix [24]; however, possible destabilizing effects have to be considered.

Contamination of urine with trace amounts of blood and thus enzymes may occur during autopsies yet seems less probable in cases of survived intoxications. Nonetheless, considering patients with an indwelling bladder catheter and thus possible cellular injury, a realistic danger of enzyme contamination has to be acknowledged. Additionally, even in a cohort of healthy subjects a case of an esterase-active urine sample has been reported [24]. In light of these considerations, unexceptional stabilization of urine samples is recommended (see below).

In blood, effectiveness of paraoxon stabilization of SUX was considerable. While the parent compound was detectable for a maximum of 4 min in non-stabilized samples, SUX detection in paraoxonized plasma was usually possible for approx. 10 min post-injection. These results were to be expected [14] but further emphasize that sample stabilization is an absolute prerequisite for SUX detection in blood or blood products (see recommendations).

For the first time, stabilizing effects were observed for SMC as well. In paraoxonized plasma, the metabolite was detectable over the whole study interval of 6 h, whereas the detection frequency of SMC in non-stabilized samples decreased to 50 % within the first 4 h, and only a third of samples was still positive at 6 h after SUX application.

Considering the effectiveness of paraoxon treatment, large interindividual differences were apparent. While the effects of absent stabilization were virtually negligible in one subject, another patient presented extremely decreased SMC concentrations and a concomitantly narrow detection window for this metabolite. Although the explicitly forensic objectives of the present study were already met by proving the potential relevance of SMC stabilization in biological samples, our results are strikingly inconsistent with the reported pharmacology of SMC and thus merit further consideration.

The observed and partially fulminant instability of SMC stands in clear contrast to literature data reporting that in vitro SMC degradation by serum esterases proceeds far more slowly than that of SUX [4, 11–13, 25, 26]. Although these data were based solely on experiments using serum, plasma, or purified BChE, a generally enhanced in vivo stability of the metabolite was often inferred [14, 25, 27]. In this context, however, it has to be acknowledged that not only the soluble butyrylesterases but also true cholinesterases (e.g., the membrane bound acetylcholinesterases of erythrocytes or lymphocytes) may influence SMC degradation. While the BChE undoubtedly does act faster on SUX than on SMC, true cholinesterases hydrolyze even low concentrations of SMC but do not degrade SUX [11]. Such effects are impossible to grasp using cell free sample matrices (e.g., serum or plasma), and their relevance for the pharmacology of SMC have thus never before been detected or investigated. The present work is the first to present stability data based on experiments in full blood samples. Reasons for the observed variance in SMC concentration profiles of different non-stabilized sample series remain yet unclear; however, interindividual differences in enzyme endowment seem plausible. Considering the results on urinary SMC elimination in a similar cohort (n=3), such differential functionality of BChE and/or other unidentified esterases was already postulated by Foldes and Norton [23].

With a distribution volume of SMC approximating total body water [19], not only soluble or membrane bound esterases have to be considered as possible candidates for its degradation in blood. In fact, intracellular metabolization, as described for some xenobiotics in neutrophil granulocytes [28, 29], cannot be excluded.

The present work establishes interindividual differences in the pharmacology of SMC and thus seems to support Foldes' position; however, for a full understanding of this complex issue, the observed differences will have to be traced back to physiological entities. Detailed pharmacological analysis is needed for clarification, but with such information still lacking the origin of interindividual variance remains yet to be pinpointed.

Detection windows

To determine detection windows for SUX as well as SMC, detection frequencies were interpreted as the probability of

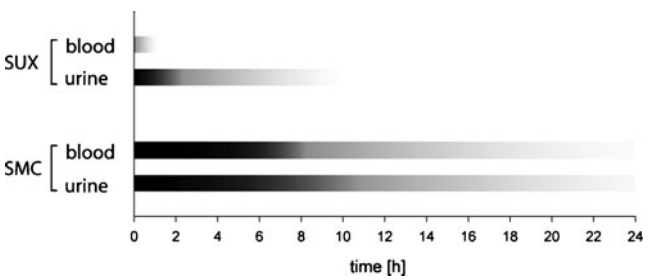

Fig. 9 Detection windows of SUX and SMC in plasma and freshly secreted urine

obtaining a positive analysis result at a given point in time. Upon quick stabilization of drawn blood samples, SUX was usually detectable for 10 min post-injection, and in isolated cases may even be traceable for up to approx. 1 h after its application.

Detection of SMC was possible in all subjects and over the whole observation period of 6 h, and kinetic data [19] suggest that positive results may be obtained for 8–24 h after SUX application.

In freshly secreted urine, minimum detection windows of 2 and 6 h have been determined for SUX versus SMC, respectively. Considering SMC plasma kinetics, detection of the metabolite in freshly secreted urine appears to be possible over a period of at least 8–24 h. As the bladder usually functions as a stable analyte reservoir, detection of target compounds may even be possible upon violation of these margins and can be regarded as mainly limited by possible bladder movements.

Last but not least, detection windows of SUX and SMC must also be assumed to be dose dependent. Application of SUX in either lower or higher dosages than reported here may result in significant deviations from the presented model. Therefore, each case of presumed SUX application has to be considered carefully and individually according to available dosage information.

Figure 9 summarizes the detection windows for SUX and SMC in both tested matrices as established in the present study.

Sampling recommendations

As has been demanded before [9, 27], sampling as well as storage of biological matrices throughout this project was executed using plastic materials only. Although adsorption issues were not investigated in this study, the exclusive use of plastic containers during SUX analysis can be recommended.

Without fast sampling and addition of paraoxon, the highly unstable SUX [6, 9, 10, 14, 30], but obviously also SMC, may be completely consumed both in vivo as well as in vitro. In order to minimize in vivo degradation, sampling has to be performed as early as possible after the presumed SUX application. To further diminish in vitro degradation of target analytes, stabilization of blood samples is mandatory. Esterase-inactivated samples are to be cooled, processed, and snap-frozen (using liquid nitrogen) as fast as possible. Thereafter, the cooling chain (−20°C or below) is not to be broken until analysis.

If single items of this recommendations list cannot be realized in forensic case work, care should be taken to abide by the others even more carefully (e.g., if stabilization is impossible, sample processing should be performed especially fast, and utmost importance should be attached to the uninterrupted cooling/freezing of samples).

Critical delays in sampling (due to death investigation formalities) or sample stabilization (due to sample shipping), however, can hardly be influenced by the analyst. As these delays tend to be longer than the known in vivo and in vitro stabilities of the labile target compounds in blood, this sample matrix is destined to play a minor role in forensic toxicological SUX analytics.

Therefore, care should be taken to appropriately collect and store urine samples whenever a SUX intoxication is suspected. Since urine specimens may contain esterases, routine stabilization of urine samples and fast sample processing is advisable. Snap-freezing in liquid nitrogen can again be considered the gold standard.

Conclusion

The present study considerably extends our knowledge on the in vivo and in vitro stability of SUX as well as SMC in humans by defining meaningful detection windows for both target analytes in blood and urine for use in forensic toxicological case work.

With a detection window of several hours for SMC as compared to a few minutes for SUX, SMC was proven to be the only realistic SUX marker in blood samples. However, due to possible sampling delays in forensic routine, the probability of positive SUX or even SMC findings is likely to be very low in blood samples. Thus, urine has to be considered the best matrix to prove application of SUX in forensic casework, and care should be taken to enable its optimal sampling (including stabilization) and storage. With appropriate preservation, positive detection of SUX and SMC is mainly limited by prior bladder discharge.

References

1. Maltby JR (1975) Criminal poisoning with anaesthetic drugs: murder, manslaughter, or not guilty. Forensic Sci 6:91–108
2. Strock D, Kuczkowski KM, Greenberg M (2004) Accidental administration of succinylcholine for the treatment of hypotension in a labouring parturient. Can J Anaesth 51:853–854
3. Somogyi G, Varga M, Prokai L, Dinya Z, Buris L (1989) Drug identification problems in two suicides with neuromuscular blocking agents. Forensic Sci Int 43:257–266
4. Kato M, Shiratori T, Yamamuro M, Haga S, Hoshi K, Matsukawa S, Jalal IM, Hashimoto Y (1999) Comparison between in vivo and in vitro pharmacokinetics of succinylcholine in humans. J Anesth 13:189–192
5. Dal Santo G (1968) Kinetics of distribution of radioactive labeled muscle relaxants. Anesthesiology 29:435–443
6. Pitts NI, Deftereos D, Mitchell G (2000) Determination of succinylcholine in plasma by high-pressure liquid chromatography with electrochemical detection. Br J Anaesth 85:592–598
7. Torda TA, Graham GG, Warwick NR, Donohue P (1997) Pharmacokinetics and pharmacodynamics of suxamethonium. Anaesth Intensive Care 25:272–278

8. Lagerwerf AJ, Vanlinthout LE, Vree TB (1991) Rapid determination of succinylcholine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr 570:390–395
9. Roy JJ, Boismenu D, Gao H, Mamer OA, Varin F (2001) Measurement of succinylcholine concentration in human plasma by electrospray tandem mass spectrometry. Anal Biochem 290:238–244
10. Roy JJ, Donati F, Boismenu D, Varin F (2002) Concentration–effect relation of succinylcholine chloride during propofol anesthesia. Anesthesiology 97:1082–1092
11. Lehmann H, Silk E (1953) Succinylmonocholine. Br Med J 1:767–768
12. Foldes FF, Vandervort RS, Shanor SP (1955) The fate of succinylcholine in man. Anesthesiology 16:11–21
13. Foldes FF (1953) Succinylmonocholine iodide: its enzymatic hydrolysis and neuromuscular activity. Proc Soc Exp Biol Med 83:187–189
14. Ballard KD, Vickery WE, Nguyen LT, Diamond FX, Rieders F (2006) An analytical strategy for quaternary ammonium neuromuscular blocking agents in a forensic setting using LC-MS/MS on a tandem quadrupole/time-of-flight instrument. J Am Soc Mass Spectrom 17:1456–1468
15. LeBeau M, Quenzer C (2003) Succinylmonocholine identified in negative control tissues. J Anal Toxicol 27:600–601
16. Kuepper U, Musshoff F, Madea B (2008) A fully validated isotope dilution HPLC-MS/MS method for the simultaneous determination of succinylcholine and succinylmonocholine in serum and urine samples. J Mass Spectrom 43:1344–1352
17. Kuepper U, Musshoff F, Madea B (2007) Synthesis and characterization of succinylcholine-d(18) and succinylmonocholine-d(3) designed for simultaneous use as internal standards in mass spectrometric analyses. J Mass Spectrom 42:929–939
18. Kuepper U, Musshoff F, Madea B (2008) Succinylmonocholine analytics as an example for selectivity problems in high-performance liquid chromatography/tandem mass spectrometry, and resulting implications for analytical toxicology. Rapid Commun Mass Spectrom 22:1965–1970
19. Kuepper U, Musshoff F, Hilger RA, Herbstreit F, Madea B (2011) Pharmacokinetic properties of succinylmonocholine in surgical patients. J Anal Toxicol 35:302–311
20. Hoshi K, Hashimoto Y, Matsukawa S (1993) Pharmacokinetics of succinylcholine in man. Tohoku J Exp Med 170:245–250
21. Forney RB Jr, Carroll FT, Nordgren IK, Pettersson BM, Holmstedt B (1982) Extraction, identification and quantitation of succinylcholine in embalmed tissue. J Anal Toxicol 6:115–119
22. Nordgren I, Baldwin K, Forney R Jr (1984) Succinylcholine-tissue distribution and elimination from plasma in the dog. Biochem Pharmacol 33:2519–2521
23. Foldes FF, Norton S (1954) The urinary excretion of succinyldicholine and succinylmonocholine in man. Br J Pharmacol Chemother 9:385–388
24. Stevens HM, Moffat AC (1974) A rapid screening procedure for quaternary ammonium compounds in fluids and tissues with special reference to suxamethonium (succinylcholine). J Forensic Sci Soc 14:141–148
25. Goedde HW, Held KR, Altland K (1968) Hydrolysis of succinyldicholine and succinylmonocholine in human serum. Mol Pharmacol 4:274–287
26. Schmidinger S, Held KR, Goedde HW (1966) Hydrolysis of succinyldicholine by pseudocholinesterase at low concentrations. Humangenetik 2:221–224
27. Tsutsumi H, Nishikawa M, Katagi M, Tsuchihashi H (2003) Adsorption and stability of suxamethonium and its major hydrolysis product succinylmonocholine using liquid chromatography-electrospray ionization mass spectrometry. J Health Sci 49:285–291
28. Mtairag EM, Abdelghaffar H, Labro MT (1994) Investigation of dirithromycin and erythromycylamine uptake by human neutrophils in vitro. J Antimicrob Chemother 33:523–536
29. Uetrecht JP (1992) Metabolism of clozapine by neutrophils. Possible implications for clozapine-induced agranulocytosis. Drug Saf 7(Suppl 1):51–56
30. Baldwin KA, Forney R Jr (1988) The influence of storage temperature and chemical preservation on the stability of succinylcholine in canine tissue. J Forensic Sci 33:462–469