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Comparison between in vivo and in vitro pharmacokinetics of succinylcholine in humans

Masato Kato[1], Takaaki Shiratori[2], Makoto Yamamuro[3], Shinobu Haga[1], Kunihiko Hoshi[4], Shu Matsukawa[4], Izadi Mobarakeh Jalal[1], and Yasuhiko Hashimoto[1,4]

[1] Department of Anesthesiology, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan
[2] Department of Anesthesia, Sendai Social Security Hospital, Japan
[3] Department of Anesthesia, Miyagi Cancer Center Hospital, Japan
[4] Department of Intensive Care Medicine, Tohoku University School of Medicine, Japan

Abstract
Purpose. To compare the in vivo and in vitro pharmacokinetics of succinylcholine (SCh) in humans.
Methods. A bolus of SCh 1 mg·kg^{-1} ($n = 7$) or 2 mg·kg^{-1} ($n = 11$) was given to 18 patients anesthetized with thiopental. Arterial blood samples for determination of in vivo SCh concentrations were collected every 30 s for 5 min. Another 20-ml blood sample was obtained before induction of anesthesia for determination of in vitro SCh. Concentrations of SCh were measured by high-performance liquid chromatography. In vivo and in vitro concentrations of SCh *vs* time data were analyzed by the one-compartment model.
Results. The respective in vivo and in vitro pharmacokinetic parameters (SCh 1 mg·kg^{-1} *vs* SCh 2 mg·kg^{-1}) were as follows: Plasma clearance was 4.17 ± 2.37 and 1.85 ± 0.28 l·min^{-1}, $P < 0.05$, *vs* 2.91 ± 2.01 and 1.27 ± 0.43 l·min^{-1}, $P < 0.05$. Elimination half-life was 25.4 ± 10.6 and 47.4 ± 5.4 s, $P < 0.002$ *vs* 26.3 ± 10.0 and 75.2 ± 21.8 s, $P < 0.00005$.
Conclusion. These results suggest that the rapid disappearance of SCh from the circulation is due to diffusion out of the blood vessels rather than to enzymatic hydrolysis.

Key words: Cholinesterase, Succinylcholine, Pharmacokinetics

Introduction

Succinylcholine (SCh), a depolarizing neuromuscular relaxant, has been thought to disappear from the circulation due to rapid enzymatic hydrolysis by plasma cholinesterase (ChE). Indeed, in patients with normal plasma ChE activity, 1 mg·kg^{-1} of SCh given intravenously was hydrolyzed by plasma ChE within 1 min in vitro [1]. However, in a canine model, 80% of SCh disappeared from the blood in 5 min, even after administration of hexaflorenium with anti-ChE activity [2]. Thus, plasma ChE activity per se cannot account for the rapid disappearance of SCh. In addition, there is little information regarding the pharmacokinetics of SCh in humans. Using a sensitive and specific high-performance liquid chromatographic (HPLC) assay to determine the levels of SCh in human blood and other biological fluids [3], we compared the in vivo and in vitro pharmacokinetics of SCh in anesthetized patients.

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Materials and methods

Study protocol

After approval had been obtained from the institutional Human Investigation Committee, we studied 18 consenting patients (9 men and 9 women), ASA Physical Status 1 or 2, undergoing general anesthesia for elective surgical procedures. Patients were between 29 and 59 years of age and had normal cardiac, hepatic, renal, and pulmonary function. None of the patients was receiving medication, was grossly obese, or had a history of neuromuscular disease. Premedication consisted of intramuscular meperidine 50 mg and atropine 0.5 mg, given 1 h prior to the scheduled time of induction of anesthesia. On arrival of the patient in the operating room, an intravenous infusion of lactated Ringer's solution was commenced, and a 22-G cannula was inserted into the left radial artery for continuous measurement of blood pressure and collection of blood samples.

Before the induction of anesthesia, 20 ml of heparinized arterial blood was collected, and the plasma was separated by centrifugation. The plasma was stored in a freezer until assayed for plasma ChE activity and used for determination of in vitro pharmacokinetics of SCh. Anesthesia was induced with thiopental 250–

500 mg i.v., and supplemental doses of thiopental were given as needed to maintain anesthesia. Ventilation was controlled manually with 100% oxygen to maintain normocapnia throughout the study period. When consciousness was lost, a bolus of i.v. SCh 1 mg·kg^{-1} (n = 7) or 2 mg·kg^{-1} (n = 11) was given using a randomized allocation. Arterial blood samples for determination of SCh concentration were collected every 30 s for 5 min after the administration of SCh. One milliliter of blood was immediately mixed with 1 ml of 0.2 N perchloric acid to inactivate plasma ChE.

Determination of in vivo pharmacokinetics of SCh

The concentration of arterial blood SCh was measured by HPLC, as previously described [3,4]. Briefly, the mixture of arterial blood and 0.2 N perchloric acid, with 50 μg butyrylcholine added as an internal standard, was centrifuged at 4000 rpm for 20 min at 4°C, and the pH of 1 ml of supernatant fluid was adjusted to between 6 and 7 by titrating with 5 M $KHCO_3$. The fluids were centrifuged again at 4°C at 4000 rpm for 20 min, then 0.5 ml of the supernatant fluid was centrifiltrated as above with a micro-filter (MF-1; Bioanalytical System, Tokyo, Japan). Fifty microliters of the filtrate was injected onto the HPLC column.

For determination of the in vivo pharmacokinetics of SCh, chromatographic analysis was performed with a Bioanalytical Systems 400A HPLC device equipped with an electrochemical detector consisting of an electrolytic cell and LC-4B amplifier (Bioanalytical Systems, Tokyo, Japan). For the electrolytic cell, a dual platinum action electrode and Ag/Ag Cl_2 electrode were used as comparative electrodes. The setting voltage was +500 mV and the sensitivity of the detector was 20 nA. The recorder was a D-2500 chromatodata detector (Hitachi, Tokyo, Japan). Separation was carried out with a separation column of SCh and fixing enzyme columns of ChE and choline oxidase. The measurements were done in duplicate at a column temperature of 35°C. The mobile phase was 0.05 M Na-phosphate buffer solution (pH 8.4) with 1 mM EDTA, and 0.7 mM Na-octyl sulfate was infused at a rate of 0.8 ml·min^{-1}. The lower detection limit of SCh was 0.2 μg·ml^{-1}. The intraassay and interassay coefficients of variation of the measurements were less than 10%.

Analyses of in vitro pharmacokinetics and plasma ChE activities

Plasma from each patient before anesthesia (range, 9.2–10.4 ml) was used for determination of the in vitro pharmacokinetics of SCh. The plasma was warmed in a water bath at 37°C. SCh was added and mixed. To determine the dose of SCh in the in vitro experiment, we assumed a circulating blood volume for each patient of 80 ml·kg^{-1} body weight. The estimated circulating plasma volume was then calculated using the preoperative hematocrit, and the dose that would be equivalent to the plasma concentration of SCh (1 or 2 mg·kg^{-1}) administered by i.v. bolus to each patient was determined. Plasma ChE activities were measured with ACA-SX (DuPont, Wilmington, DE, USA), using oxygenation-reduction index agent methods. Consequently, we compared the half-life of SCh in the in vitro pharmacokinetics with the plasma ChE activities.

Pharmacokinetic analysis

All measurements were performed in duplicate. Concentration *vs* time data were fitted to a one-compartment model by linear least-squares regression programs for microcomputer using the Damping Gauss-Newton method, as previously described [3]. The pharmacokinetic parameters of the apparent volume of distribution at steady state (Vd_{ss}), total body clearance (Cl), area under the plasma concentration-time curve (AUC), and elimination half-lives ($t_{1/2}$) were calculated for each patient. In addition, the kinetics of SCh in the in vivo study were compared with the half-life of SCh in plasma in vitro.

Statistical analysis

Data are expressed as means ± SD, unless otherwise stated. Data were compared using analysis of variance and Student's paired t-test. Differences were considered significant when $P < 0.05$.

Results

There were no significant differences in demographic data and plasma ChE activities between the two groups (Table 1).

The arterial blood SCh concentrations after intravenous administration and the plasma SCh concentrations in the in vitro determinations after i.v. SCh

Table 1. Patient characteristics (mean ± SD)

Group	SCh 1.0 mg·kg^{-1} (n = 7)	SCh 2.0 mg·kg^{-1} (n = 11)
Age (yr)	46 ± 10	46 ± 10
Sex (M/F)	4/3	5/6
Height (cm)	156 ± 4	156 ± 5
Weight (kg)	52 ± 4	56 ± 8
Plasma ChE activities (IU·ml^{-1})	9.3 ± 1.7	10.3 ± 1.6

SCh, Succinylcholine; ChE, cholinesterase.

1 mg·kg^{-1} administration decreased progressively over time (Fig. 1). The average $t_{1/2}$ values for the in vivo and in vitro pharmacokinetics were 25.4 and 47.4 s, respectively. The mean Cl was lower in the in vitro than in the in vivo determinations ($P < 0.05$), and the $t_{1/2}$ value was longer in the in vitro than in the in vivo analysis ($P < 0.002$; Table 2).

The arterial blood SCh concentrations after i.v. administration and the plasma SCh concentrations in the in vitro determinations after i.v. SCh 2 mg·kg^{-1} administration decreased progressively over time (Fig. 2). The average $t_{1/2}$ values for the in vivo and in vitro pharmacokinetics were 26.3 and 75.2 s, respectively. The mean Cl was lower in the in vitro than in the in vivo determinations ($P < 0.00005$), and the $t_{1/2}$ value was longer in the in vitro than in the in vivo analysis ($P < 0.05$; Table 2).

At a dose of 1 mg·kg^{-1} SCh, there were no significant correlations between the plasma ChE activities and the elimination half-life of SCh in plasma determined by both analyses. However, when SCh was administered at a dose of 2 mg·kg^{-1}, the plasma ChE activities correlated significantly with the half-life of SCh in the in vitro analysis ($Y = -0.15X + 165.8$; $r = -0.64$, $P < 0.05$). There was no significant correlation between the plasma half-lives by in vivo and in vitro determinations at dosages of SCh of either 1 mg·kg^{-1} or 2 mg·kg^{-1}.

Discussion

The results of this study showed that SCh disappeared from the blood faster in vivo than in vitro in human subjects. The disappearance rate of SCh from the

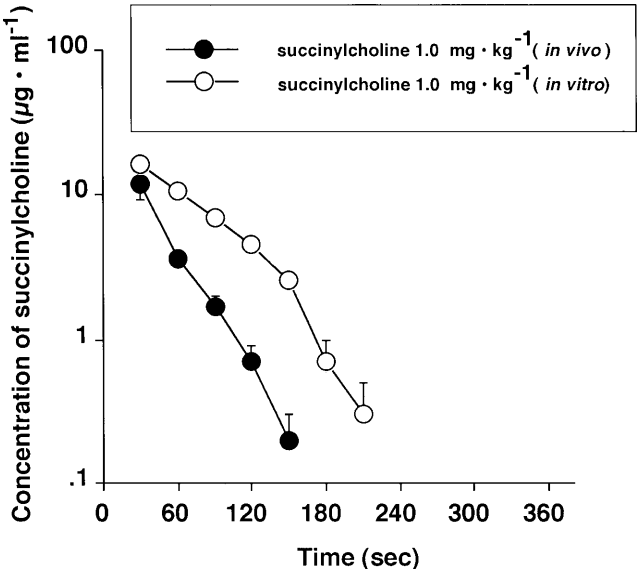

Fig. 1. Kinetics of plasma succinylcholine concentrations in vivo and in vitro at a dosage of 1 mg·kg^{-1}. Values are means ± SE ($n = 7$). Mean in vivo and in vitro elimination half-lives were 25.4 and 47.4 s, respectively

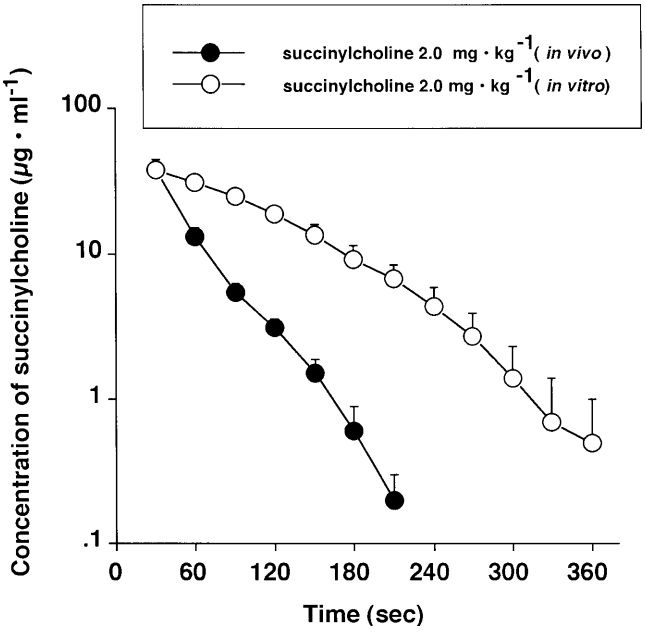

Fig. 2. Kinetics of plasma succinylcholine concentrations in vivo and in vitro at a dosage of 2 mg·kg^{-1}. Values are means ± SE ($n = 11$). Mean in vivo and in vitro elimination half-lives were 26.3 and 75.2 s, respectively

Table 2. Pharmacokinetic analysis of succinylcholine (SCh) at doses of 1 and 2 mg·kg^{-1} (mean ± SD)

	SCh 1 mg·kg^{-1} ($n = 7$)		SCh 2 mg·kg^{-1} ($n = 11$)	
Value	In vivo	In vitro	In vivo	In vitro
$t_{1/2}$ (s)	25.4 ± 10.6	47.4 ± 5.4†	26.3 ± 10.0	75.2 ± 21.8‡
Cl (l·min^{-1})	4.17 ± 2.37	1.85 ± 0.28*	2.91 ± 2.01	1.27 ± 0.43*
Vd (ml·kg^{-1})	3.12 ± 2.83	2.14 ± 0.54	2.21 ± 2.11	2.12 ± 0.50
AUC (min·μg·ml^{-1})	18.5 ± 12.1	29.1 ± 4.8	58.6 ± 37.7	99.1 ± 39.3*

* $P < 0.05$, $^{\dagger}P < 0.002$, $^{\ddagger}P < 0.00005$ *vs* in vivo.

circulation depends on hydrolysis by plasma ChE, diffusion out of the blood vessels to the tissues, plasma protein binding, and renal excretion. Because the last two factors are negligible within the first minute after injection [2], the rapid disappearance of SCh from plasma appears to be due to hydrolysis by plasma ChE [1].

An in vivo rhesus monkey study showed that most of the SCh was hydrolyzed by ChE in plasma before reaching the motor endplates during constant intravenous infusion of SCh to maintain a 50% reduction of twitch tension [5]. However, when one arm was occluded with a tourniquet immediately before i.v. SCh $1\,mg\cdot kg^{-1}$ and the occlusion was relieved after a period of 1 to 3 min, neuromuscular block occurred 5–10 s after the tourniquet was released, even after 3 min of occlusion [6]. These results suggest that the in vivo hydrolysis rate should have been less than the in vitro rate, and that diffusion of SCh out of the circulation may have occurred rapidly.

The metabolic rate of SCh in human plasma is 12–$30\,\mu g\cdot ml^{-1}\cdot min^{-1}$ [7]. In a canine in vivo model, intravenous administration of SCh at $1\,mg\cdot kg^{-1}$ resulted in a peak plasma concentration of $76\,\mu g\cdot ml^{-1}$ [8]; this value is consistent with our study results in humans. In our previous clinical study, $t_{1/2}$ was 16.6 s after i.v. SCh of $1\,mg\cdot kg^{-1}$ [3]. The plasma half-life of SCh was even shorter (11.7 s) when the dose was $2\,mg\cdot kg^{-1}$, and there were no correlations between the plasma ChE activities and the $t_{1/2}$ of SCh. The elimination half-life of SCh in the in vivo pharmacokinetics was one-half to one-third of that in the in vitro analysis at both doses. Although the half-life was much shorter in vivo than in vitro, the fact that the in vivo and in vitro plasma concentrations of SCh 30 s after administration were quite similar in pharmacokinetics strongly suggests that the decline of the blood concentrations of SCh may be due to the rapid distribution of SCh to extravascular tissues, rather than to enzymatic hydrolysis.

There were no correlations between plasma ChE activities and the value of $t_{1/2}$ at the SCh dose of $1\,mg\cdot kg^{-1}$ but there were at the SCh dose of $2\,mg\cdot kg^{-1}$. The reason for these results remains to be elucidated. In experiments in vitro, the potency of ChE to metabolize SCh has been reported to be maximal when the concentration of SCh is $360\,\mu g\cdot ml^{-1}$, and further increases in SCh levels resulted in marked substrate inhibition [9]. In the present study, it is assumed that a correlation was noted between the plasma ChE activities and elimination half-life only in the $2\,mg\cdot kg^{-1}$ group, because the peak level of SCh in the group receiving $2\,mg\cdot kg^{-1}$ was closer to the concentrations at which the maximal potency of ChE should be ob-served, as compared with those of the group receiving $1\,mg\cdot kg^{-1}$. In addition, when administering SCh to patients with abnormal ChE values, one must take account of the possibility of diffusion into tissues, as well as the conventional pharmacokinetics of the drug.

In summary, we compared the in vivo and in vitro pharmacokinetics of SCh in anesthetized patients. The results suggest that the rapid disappearance of SCh from the circulation is due to diffusion out of the blood vessels rather than to enzymatic hydrolysis.

References

1. Kalow W (1959) The distribution, destruction and elimination of muscle relaxants. Anesthesiology 20:505–518
2. Dal Santo G (1968) Kinetics of distribution of radioactive labeled muscle relaxants. 3. Investigations with ^{14}C-succinyldicholine and ^{14}C-succinylmonocholine during controlled conditions. Anesthesiology 29:435–443
3. Hoshi K, Hashimoto Y, Matsukawa S (1993) Pharmacokinetics of succinylcholine in man. Tohoku J Exp Med 170:245–250
4. Ikarashi Y, Sasahara T, Maruyama Y (1984) A simple method for determination of choline (Ch) and acetylcholine (ACh) in rat brain regions using high-performance liquid chromatography with electrochemical detection (HPLC-ED). Nippon Yakurigaku Zasshi 84:529–536
5. Peck AW (1972) Relative importance of the enzymic hydrolysis of sexamethonium in plasma and tissues: studies in rhesus monkeys. Br J Pharmacol 45:64–70
6. Holst-Larsen H (1976) The hydrolysis of suxamethonium in human blood. Br J Anaesth 48:887–892
7. Foldes FF, Norton S (1954) The urinary excretion of succinyldicholine and succinylmonocholine in man. Br J Pharmacol 9:385–388
8. Baldwin KA, Forney R Jr (1988) Correlation of plasma concentration and effects of succinylcholine in dogs. J Forensic Sci 33:470–479
9. Fujino A, Wakisaka K, Inagaki M, Saito T (1971) Degradation mechanism of succinylcholine in man (in Japanese with English abstract). Masui (Jpn J Anesthesiol) 20:939–940