Evaluating the effects of sugammadex on coagulation in humans: reversed translational research to unravel off-target pharmacology

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CHAPTER 7

Summary and general discussion

This thesis describes the pharmacological studies into the off-target effect of sugammadex (Bridion®, laboratory code ORG 25969) on coagulation. Sugammadex is a modified γ-cyclodextrin which encapsulates steroidal neuromuscular blocking agents rocuronium and vecuronium and thereby dose-dependently, rapidly and completely reverses their pharmacological effect in the post-operative setting.[1–7] An intravenous dose of 2 and 4 mg/kg sugammadex is recommended for routine reversal of moderate and deep blockade in adults, respectively, and 16 mg/kg sugammadex for reversal 3 minutes after an intubating dose of 1.2 mg/kg rocuronium.[7]

In vitro spiking experiments carried out during the development trajectory of sugammadex showed that 100 µg/mL sugammadex (corresponding with a dose of 8 mg/kg) significantly prolonged activated partial thromboplastin time (APTT) and international normalized ratio for prothrombin time PT(INR),[8] but values remained within normal ranges.[9] The effect of sugammadex on coagulation was not further evaluated in any clinical trial rendering its clinical relevance unknown.[10] This raised safety concerns by the European Medicines Agency (EMA)[11] and the United States Food and Drug Administration (FDA) during their review of the application for marketing authorization for sugammadex.[12] In an effort to address this concern, a post-hoc analysis of all adverse events related to hemorrhage in phase 2/3 trials was performed. Such events occurred in 5.7% and 3.1% of the sugammadex- and placebo-treated subjects, respectively. When the analysis was limited to surgery related bleedings and extended to the total sugammadex group, the incidence decreased to 2.8% for sugammadex subjects and 2.3% for placebo subjects (no statistically significant difference).[11,13] However, the same data resulted in different regulatory decisions in July 2008. While marketing authorization was granted in the European Union,[14] the FDA rejected the application because of deficiencies regarding the characterization of sugammadex effects on coagulation and allergic reactions (the latter mainly concerned the lack of data on safety of repeat exposures).[12] The EMA decided, as risk mitigation, to include the effect of sugammadex on APTT and PT(INR) in the summary of product characteristics (SPC). In addition, dedicated pharmacology studies to investigate the off-target effect of sugammadex on coagulation had to be undertaken as post-authorization commitment.[11] Such studies were also required for resubmission to the FDA.[12] Most of these studies were performed by the Centre for Human Drug Research (CHDR) and collaborators, as described in this thesis. These comprised a variety of *in vitro, ex vivo* and *in vivo* (clinical) pharmacology studies.

For evaluation of the potential clinical relevance of sugammadex induced coagulation effects, understanding the underlying mode of action (MoA) is of importance. In **CHAPTER 2**, a stepwise *in vitro* approach was taken to unravel this MoA. The first step was to scrutinize which component of the drug substance sugammadex is driving the APTT and PT prolongations. During the synthesis of sugammadex (ORG 25969), the related γ-cyclodextrin ORG 48302 is formed that is present up to 7% in the drug substance. ORG 25969 was found to be the major determinant of the sugammadex effects on coagulation parameters and was, therefore, selected for the subsequent experiments. APTT and PT increased with approximately 10 and 2.5 seconds, respectively, at a concentration of 200 µg/mL ORG 25969 which corresponds to the mean peak plasma concentration reached at the dose recommended for immediate reversal in emergency situations of 16 mg/kg. Next, the effect of ORG 25969 on a variety of (adapted) clotting assays addressing coagulation aspects such as thrombin activity, thrombin generation, factor Xa activity and factor Xa generation was explored. These showed that sugammadex is likely to decrease factor Xa activity in the common pathway and activation of factor X specifically in the intrinsic pathway.

The effect of sugammadex on coagulation increases the possibility of interaction with anticoagulant/antiplatelet compounds administered in the perioperative setting and may expose surgical patients to an increased bleeding risk. These potential interactions were first explored in a series of *in vitro* experiments as described in **CHAPTER 3**. Sugammadex (ORG 25969) was added to plasma of patients on a vitamin K antagonist with elevated INRs and to plasma of healthy volunteers spiked with either a low or high level of enoxaparin, fondaparinux, rivaroxaban, or dabigatran. In all conditions, sugammadex induced concentration-dependent increases in APTT and PT(INR), mainly in a proportional manner, with the strongest increases recorded for dabigatran and rivaroxaban. Furthermore, sugammadex demonstrated a similar pattern of APTT and PT(INR) prolongations in perioperatively collected patient plasmas and in control plasma. It was also highlighted that both rocuronium and vecuronium counteract the effect of sugammadex on APTT and PT suggesting that the prolongations are completely neutralized when equimolar concentrations of rocuronium or vecuronium and sugammadex

are present. These findings, combined with the transient nature of sugammadex effects on coagulation and the perioperative management of the investigated compounds, are unlikely to translate into an increased bleeding risk in the perioperative setting, although this possibility cannot be excluded for scenarios not clinically studied.

The potential interactions between sugammadex and thromboprophylactic agents used in the perioperative setting were further evaluated in clinical pharmacology studies. **CHAPTER 4** reports on a feasibility study of using *ex vivo* collagen-induced whole blood platelet aggregometry for evaluation of potential aspirin-drug interactions affecting platelet aggregation in preparation of a sugammadex-aspirin interaction study. Healthy male volunteers received a daily oral dose of 75 mg aspirin for 6 consecutive days. Whole blood platelet aggregation in response to various collagen concentrations was assessed during the day before start of the aspirin treatment and on the last day of treatment. This methodology was found to be robust in terms of assay reproducibility and intra-subject variability. Platelet aggregation was inhibited after aspirin administration and the effect size varied with the collagen concentration. Collagen concentrations of 1 to 2 µg/mL rendered sufficient window to evaluate a potential aspirin-drug interaction on platelet aggregation. These findings were taken into account for the design of the sugammadex-aspirin interaction study in healthy male volunteers as described in **CHAPTER 5**. Subjects randomly received 4 mg/kg sugammadex or placebo intravenously in absence (treatment period 1 and 2) or presence (treatment period 3 and 4) of aspirin. The administration in treatment period 3 and 4 occurred after at least 7 and 11 consecutive days of once daily oral treatment of 75 mg aspirin, respectively, with a maximum of 16 consecutive days of aspirin intake. The pharmacodynamic assessments included whole blood platelet aggregation induced by 1.5 µg/mL collagen, APTT, cutaneous bleeding time, and PT(INR). Aspirin inhibited platelet aggregation and prolonged cutaneous bleeding time, while sugammadex prolonged APTT and PT(INR). No clinically meaningful interaction between sugammadex and aspirin was observed.

In **CHAPTER 6**, the potential interaction between 4 and 16 mg/kg sugammadex and enoxaparin or unfractionated heparin (UFH) on anticoagulant activity in healthy male volunteers was evaluated. Subjects received a subcutaneous abdominal injection of 40 mg enoxaparin (study part 1), 5,000 units of UFH (study part 2) or anticoagulant placebo followed by an intravenous dose of 0, 4 or 16 mg/kg sugammadex 3 hours later. Study part 1 consisted of 4 treatment periods in random order with anticoagulant placebo in combination with 4 mg/kg sugammadex and enoxaparin in combination with 0, 4 or 16 mg/kg sugammadex. Study part 2 consisted of 4 treatment periods in random order with anticoagulant placebo in combination with 16 mg/kg sugammadex and UFH in combination with 0, 4 or 16 mg/kg sugammadex. Anti-Xa activity and APTT were selected as primary endpoints for enoxaparin and UFH, respectively. Other assessments included APTT (for enoxaparin), anti-Xa activity (for UFH), and PT(INR). No clinically relevant effect of sugammadex on enoxaparin or UFH anticoagulant activity was revealed. These findings were further substantiated by exploratory pharmacokinetic/pharmacodynamic (PK/PD) modeling, which showed no effect of sugammadex on anti-Xa activity in presence of enoxaparin, UFH or anticoagulant placebo. Furthermore, similar positive relationships between sugammadex concentration and APTT or PT(INR) were observed, regardless of anticoagulant pretreatment.

In order to evaluate the clinical relevance of the anticoagulant effect of sugammadex, the potential impact of sugammadex on several concomitant thromboprophylactic therapies has been addressed by the sugammadex-drug interaction *in vitro* experiments (**CHAPTER 3**) and studies in healthy volunteers (**CHAPTER 5** and **6**). However, drug-induced anticoagulation is not solely determining bleeding risk in the perioperative setting. Other factors such as nature of the surgery and underlying medical conditions (*e.g.* coagulation factor defects) can contribute as well.[15] Therefore, the research into the clinical relevance of sugammadex effects on coagulation continued in the target patient population. A subpopulation of patients at increased bleeding risk due to intraoperative thromboprophylaxis combined with a major surgical procedure was investigated. This included the effect of reversal of neuromuscular blockade with 4 mg/kg sugammadex *versus* usual care (neostigmine or spontaneous recovery) on bleeding risk in patients undergoing hip or knee joint replacement or hip fracture surgery and receiving commonly prescribed thromboprophylaxis (mainly low molecular weight heparin (LMWH)).[16] Sugammadex induced limited, transient APTT and PT(INR) prolongations, but without increasing the incidence of bleeding or severity of bleeding compared to usual care.

Data of this patient study and the sugammadex-enoxaparin/UFH interaction study (**CHAPTER 6**) were used to build PK-APTT and PK-PT(INR) models

for prediction of the anticoagulant effects of sugammadex in the patient population in scenarios not clinically evaluated, such as treatment with 16 mg/kg sugammadex.[17] This dose is rarely used, only in case of rescue reversal where patients are in a potentially life-threatening situation and restoring the airway is the immediate concern[7,18] which clearly outweighs the potential increased bleeding risk associated with sugammadex. Additionally, surgery has generally not been initiated at such stage and is likely to be postponed if the surgery is not urgent. Nonetheless, modeling the APTT and PT(INR) effects upon treatment with 16 mg/kg sugammadex complements the insights on the effects of sugammadex on coagulation. The relationship between sugammadex plasma concentration and anticoagulant activity was in both APTT and PT(INR) models best described by a maximum effect (E_{max}) function, however, the majority of the data fell below the estimated concentration of sugammadex producing the half-maximal response (EC_{50}), indicating that there were limited data to accurately estimate the potentially maximally achievable effect. Nevertheless, these models predict APTT and PT(INR) increases in surgical patients on thromboprophylaxis receiving 16 mg/kg sugammadex well below the threshold considered to be the minimum clinically relevant meaningful effect of anticoagulant treatment.[17] Data of the sugammadex-aspirin study (**CHAPTER 5**) were used for external validation of these models.

Evaluation of the off-target effects of sugammadex on coagulation as described in this thesis were indispensable to overcome the bleeding safety concerns raised by both the EMA and the FDA. FDA approval was granted on 15 December 2015[19] and reflection on this odyssey of 7.5 years may provide an opportunity to learn from the development trajectory of sugammadex. At CHDR, we advocate the so-called question-based drug development approach.[20–22] During a clinical drug development program, a number of generic questions need to be answered on the pharmacology of a drug. These questions range from the compound's absorption, distribution, metabolism and excretion (does the compound get to the site of action?) to the sources of variability in drug response in the target population. These questions are used to design clinical trials and their objectives rather than the traditional successive 4 clinical phases approach. Answering all questions reduces the uncertainty about a drug and thereby minimizes its developmental risk. Question-based drug development addresses both the on-target and off-target pharmacology of a drug; the latter requires for instance knowledge on the etiology of an off-target effect. This knowledge on the *in vitro* effects of sugammadex on APTT and PT(INR) was missing in the original application of sugammadex, which prompted the regulators to require additional studies dedicated to establish the clinical relevance of the anticoagulant effect of sugammadex. An interesting question is what would have happened when the development trajectory had included detailed coagulation assessments in the early clinical studies. It is tempting to speculate that earlier availability of such data would have contributed to earlier clinical acceptance of sugammadex by the regulatory authorities.

In conclusion, this thesis shows that when sugammadex and anticoagulants are administered according to their labels in the perioperative setting, the bleeding risk can be considered negligible. However, off-label use such as in patients with (high) clinically relevant anticoagulant levels in an emergency surgery situation should be cautiously handled. Asking and answering all relevant scientific questions related to off-target effects during the early clinical development phase may have unlocked the true potential of sugammadex earlier.

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