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# Analysis of the safety and pharmacodynamics of human fibrinogen concentrate in animals

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ABSTRACT

Fibrinogen, a soluble 340 kDa plasma glycoprotein, is critical in achieving and maintaining hemostasis. Reduced fibrinogen levels are associated with an increased risk of bleeding and recent research has investigated the efficacy of fibrinogen concentrate for controlling perioperative bleeding. European guidelines on the management of perioperative bleeding recommend the use of fibrinogen concentrate if significant bleeding is accompanied by plasma fibrinogen levels less than 1.5–2.0 g/l. Plasma-derived human fibrinogen concentrate has been available for therapeutic use since 1956. The overall aim of the comprehensive series of non-clinical investigations presented was to evaluate i) the pharmacodynamic and pharmacokinetic characteristics and ii) the safety and tolerability profile of human fibrinogen concentrate Haemocomplettan P® (RiaSTAP®). Pharmacodynamic characteristics were assessed in rabbits, pharmacokinetic parameters were determined in rabbits and rats and a safety pharmacology study was performed in beagle dogs. Additional toxicology tests included: single-dose toxicity tests in mice and rats; local tolerance tests in rabbits; and neoantigenicity tests in rabbits and guinea pigs following the introduction of pasteurization in the manufacturing process. Human fibrinogen concentrate was shown to be pharmacodynamically active in rabbits and dogs and well tolerated, with no adverse events and no influence on circulation, respiration or hematological parameters in rabbits, mice, rats and dogs. In these non-clinical investigations, human fibrinogen concentrate showed a good safety profile. This data adds to the safety information available to date, strengthening the current body of knowledge regarding this hemostatic agent.



## Introduction

Fibrinogen is a soluble plasma glycoprotein with a molecular weight of 340 kDa. It is synthesized and secreted by the liver, and is critical in achieving and maintaining hemostasis. The structure of fibrinogen is complex; each molecule consists of two identical subunits composed of three pairs of non-identical polypeptide chains ($\alpha_2$, $\beta_2$, $\gamma_2$). During coagulation fibrinogen is cleaved by thrombin, which results in the release of fibrinopeptide A and fibrinopeptide B; its subsequent polymerization to form fibrin strands provides the structural network required for clotting. Fibrin fibers are covalently cross-linked by activated coagulation factor XIII (Muszbek et al., 1999). In addition, fibrinogen promotes platelet aggregation by binding platelet glycoprotein IIb/IIIa receptors; these platelets become enmeshed within the fibrin strands, strengthening the clot. Its contribution to both primary and secondary hemostasis led it to be described as a "potential universal hemostatic agent" (Fenger-Eriksen et al., 2009a).

Congenital fibrinogen deficiency, a rare bleeding disorder, affects either the quantity (afibrinogenemia and hypofibrinogenemia) or quality (dysfibrinogenemia) of circulating fibrinogen. Fibrinogen levels are associated with clinical bleeding severity (Peyvandi et al., 2012). Replacement therapy with human fibrinogen concentrate has been shown to be effective and generally well tolerated in treating bleeding episodes in congenital fibrinogen deficiency (Kreuz et al., 2005a). Guidelines recommend that the dose and frequency of administration should be adjusted to maintain a plasma fibrinogen level >0.5–1.0 g/l (Bolton-Maggs et al., 2004).

In a healthy individual, normal plasma levels of fibrinogen range from 2.0 to 4.5 g/l (Fenger-Eriksen et al., 2009a). Reduced fibrinogen levels appear to be correlated with an increased risk of bleeding in different clinical settings (Blome et al., 2005; Charbit et al., 2007; Karlsson et al., 2008). Recent studies have shown that human fibrinogen concentrate is efficacious in controlling perioperative bleeding, restoring clotting and reducing both post-operative blood loss and requirement for allogeneic blood products (Görlinger et al., 2011; Karlsson et al., 2009; Rahe-Meyer et al., 2013a,b; Solomon et al., 2010).

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Fibrinogen supplementation may even compensate for low platelet levels (Lang et al., 2009; Velik-Salchner et al., 2007). Consistent with these findings, European guidelines on the management of severe bleeding in the perioperative (Kozek-Langenecker et al., 2013) and trauma (Spahn et al., 2013) settings recommend the use of fibrinogen concentrate if significant bleeding is accompanied by plasma fibrinogen concentrations less than 1.5–2.0 g/l.

A plasma-derived human fibrinogen concentrate (CSL Behring GmbH, Marburg, Germany) was first approved for use in Brazil in 1963. In 1985, pasteurization was introduced in the manufacturing process, and the pasteurized product has been available since 1986 (Fenger-Eriksen et al., 2009a). Throughout the product's licensing history it has been known under the following trade names: Haemocomplettan® HS (heat sterilized) or P (pasteurized; Europe), and RiaSTAP® (USA). Currently, under the trade name Haemocomplettan® P, the product is approved for the treatment and prophylaxis of hemorrhagic diatheses in congenital and acquired fibrinogen deficiency (Haemocomplettan® PI, 2008) in several countries all over the world (Solomon and Wendt, 2014). The same product, licensed under the trade name RiaSTAP®, is also available in a number of countries world-wide (Solomon and Wendt, 2014) for the treatment of acute bleeding episodes in patients with congenital fibrinogen deficiency, including afibrinogenemia and hypofibrinogenemia (Riastap® PI, 2011). Further details of the current licensing information for human fibrinogen concentrate are described in a recent review by Levy et al. (2014).

The product is subjected to pasteurization (heat treatment at 60 °C for 20 h in aqueous solution), to reduce the risk of viral transmission, (Gröner, 2007) and precipitation and purification. Recent data from prospective controlled studies support the safety of human fibrinogen concentrate across a variety of clinical settings, including cardiovascular surgery, trauma and burn patients (Görlinger et al., 2012; Rahe-Meyer et al., 2013a,b; Schaden et al., 2012; Weber et al., 2012). Additional evidence from retrospective studies (Schöchl et al., 2010, 2011) and case series (Bell et al., 2010) have also been reported.

The overall aim of the comprehensive series of investigations in healthy non-bleeding animals presented here was to evaluate: i) the pharmacodynamic and pharmacokinetic characteristics; and ii) the safety and tolerability profile of human fibrinogen concentrate. With the growing interest and increased clinical use of human fibrinogen concentrate, the data provided by the studies presented here will be valuable and further extends the current body of knowledge on this hemostatic agent.

## Materials and methods

The studies were approved by the local governmental ethics committee. Testing was performed according to relevant European guidelines applicable to the non-clinical safety assessment of human plasma-derived products. Animal care and treatment were in compliance with all pertinent regulations of the European Union and Germany. The characteristics of the animals used in each study and the doses of human fibrinogen concentrate administered (Haemocomplettan® P, CSL Behring GmbH, Marburg, Germany) are described in Table 1.

The animal species used for these studies (e.g., dogs, mice, rabbits, and rats) were chosen because human fibrinogen is pharmacologically active in these species (Karges et al., 1994; Siller-Matula et al., 2008). Coagulation factors are highly conserved and there is evidence that human coagulation factors work in the animal coagulation system (e.g., in hemophilia A or B). The human fibrinogen used in these studies is cleaved by its target animal serine protease, thrombin. It has already been shown that impaired thromboelastography in rats can be normalized by the administration of human fibrinogen (Kaspereit et al., 2004). In accordance with regulatory requirements, both genders were investigated in the safety pharmacology study in beagle dogs and in the single-dose toxicity studies in mice and rats. The doses of human fibrinogen concentrate used in the pharmacodynamic and pharmacokinetic studies correspond to a standard human dose of 4–5 g for an 80 kg person. For the safety pharmacology study and all toxicity studies a number of multiple doses of the standard human dose were used to investigate worse case scenarios for accurately evaluating the toxicity of human fibrinogen concentrate.

**Table 1**
Characteristics of the animals used and doses of human fibrinogen concentrate administered in each of the studies.

| Study | Species/strain/sex | Human fibrinogen concentrate (mg/kg) | Body weight |
|---|---|---|---|
| *Pharmacodynamic* | | | |
| 1 | Rabbit/CHB or NZW/f | Cumulative dosing 50/100/150/200/250 — IV | 1.8–3.5 kg |
| *Pharmacokinetic* | | | |
| 2 | Rabbit/CHB/f | 50 — IV | 2.5–3.0 kg |
| 3 | Rat/CD/f | 60 — IV | >180 g |
| *Safety pharmacology* | | | |
| 4 | Dog/Beagle/f and m | 20/100/200 — IV | 8.5–13.5 kg |
| *Single-dose toxicity* | | | |
| 5 | Mouse/NMRI/f and m | 250/500/1000 — IV | 19.1–23.2 g |
| 6 | Rat/Wistar/f and m | 100/200/300 — IV | 110.3–137.6 g |
| *Local tolerance* | | | |
| 7, 8, 9 | Rabbit/NZW/f | 40 — IV/40 — IA/0.8 — PV | 220–268 g |
| *Neoantigenicity* | | | |
| 10, 11, 12 | Rabbit/house/m | 4/8/8 — IV | ~2.5 kg |
| | Guinea pig/albino or Hartley/f and m | | 300–400 g |
| 13 | Rabbit/NZW/f and m | 100 — IV | 2–3 kg |

f, female; IA, intra-arterial; IV, intravenous; m, male; PV, paravenous.

### *Pharmacodynamic and pharmacokinetic studies*

One study, using thromboelastometry (ROTEM), analyzed the pharmacodynamic efficacy of human fibrinogen concentrate (Study 1). Rabbits (n = 8; all females) were restrained; a 14 G catheter was introduced into the carotid artery for blood sampling and a 20 G catheter was introduced into the jugular vein for blood withdrawal. Rabbits were injected successively with 50 mg/kg (2.5 ml/kg body weight over 1 min) human fibrinogen concentrate at five minute intervals, resulting in a cumulative dose of 250 mg/kg. One milliliter blood samples (10% v/v sodium citrate) were withdrawn at baseline (time = 0) and 1 min after each infusion; the effects of human fibrinogen concentrate on ROTEM parameters were monitored. FIBTEM was performed in whole blood using a ROTEM® analyzer (Tem International, Munich, Germany); the test reaction was initiated through the extrinsic pathway and cytochalasin D was added to inhibit platelet activity. The primary variable for assessing the quality of the fibrin-based clot was maximum clot firmness (MCF) using the FIBTEM test.

Two studies analyzed the pharmacokinetic properties of human fibrinogen concentrate. In the first of these studies (Study 2), rabbits (n = 10; all females) received 50 mg/kg human fibrinogen concentrate as a single bolus intravenous injection. Blood samples were obtained at the following time points: before injection and 5 min, 30 min, and 1, 2, 4, 6, 8, 24, 32, 48, 72 and 96 h after administration. In the second of these studies (Study 3), rats (n = 6; all females) received a single bolus intravenous injection of 60 mg/kg human fibrinogen concentrate. Blood samples were obtained at the following time points: 5 min, 30 min, 60 min, and 2, 4, 8, 24 and 32 h after administration. In both studies, blood samples were collected into 20% sodium citrate (3.13% w/v) and plasma was separated immediately. Each plasma sample was used to quantify human fibrinogen antigen concentration using a validated ELISA technique according to the manufacturer's instructions (Study 2: AssayPro LLC, St. Charles, MO; Study 3: Cedarlane Laboratories Ltd., Burlington, NC). The following pharmacokinetic variables of