JOURNAL OF SURGICAL RESEARCH 187 (2014) 334–342

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

A totally recombinant human fibrin sealant

Mark A. Carlson, MD,[a,c,*] Jennifer Calcaterra, PhD,[d] Jason M. Johanning, MD,[b,c] Iraklis I. Pipinos, MD,[b,c] Crystal M. Cordes, PhD,[e] and William H. Velander, PhD[d]

[a] Department of Surgery, University of Nebraska Medical Center, Omaha, Nebraska
[b] Department of Vascular Surgery, University of Nebraska Medical Center, Omaha, Nebraska
[c] Department of Surgery, VA Nebraska–Western Iowa Health Care System, Omaha, Nebraska
[d] Department of Chemical and Biomolecular Engineering, University of Nebraska–Lincoln, Lincoln, Nebraska
[e] Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, Nebraska

ARTICLE INFO

Article history:
Received 10 June 2013
Received in revised form
25 September 2013
Accepted 26 September 2013
Available online 2 October 2013

Keywords:
Fibrin sealant
Hemostasis
Hemorrhage
Swine
Fibrinogen
Factor XIII
Thrombin
Recombinant
Human

ABSTRACT

Background: Applications of plasma-derived human fibrin sealants (pdhFS) have been limited because of cost, limited supply of pathogen-screened plasma, the need for bioengineering improvements, and regulatory issues associated with federal approval. We describe a totally recombinant human fibrin sealant (rhFS), which may engender an abundant, safe, and cost-effective supply of efficacious fibrin sealant.

Materials and methods: A first-generation rhFS made from recombinant human fibrinogen (rhFI; produced in the milk of transgenic cows), activated recombinant human factor XIII (rhFXIIIa; produced in yeast), and recombinant human thrombin (rhFIIa; purchased, made in animal cell culture) was formulated using thromboelastography (TEG). The hemostatic efficacy of rhFS *versus* commercial pdhFS was compared in a nonlethal porcine hepatic wedge excision model.

Results: The maximal clot strength of rhFS measured *in vitro* by TEG was not statistically different than that of pdhFS. TEG analysis also showed that the rhFS gained strength more quickly as reflected by a steeper α angle; however, the rhFS achieved this clot strength with a 5-fold lower factor I content than the pdhFS. When these fibrin sealants were studied in a porcine hepatic wedge excision model, the hemostatic scores of the rhFS were equivalent or better than that of the pdhFS.

Conclusions: The bioengineered rhFS had equivalent or better hemostatic efficacy than the pdhFS in a nonlethal hemorrhage model, despite the factor I concentration in the rhFS being about one-fifth that in the pdhFS. Because the rhFS is amenable to large-scale production, the rhFS has the potential to be more economical and abundant than the pdhFS, while having a decreased risk of blood-borne pathogen transmission.

Published by Elsevier Inc.

1. Introduction

The use of dried plasma as a topical hemostatic aid was documented in 1909 [1]. The combination of relatively pure fibrinogen (factor I or FI) with thrombin to make fibrin glue or foam was described in 1944 [2], but it was not until improved purification technology became available that fibrin sealants (FS) became commercially available in the 1970s [1]. Since that time, the efficacy of FS products as a topical hemostat or tissue adhesive has been demonstrated in numerous elective clinical

Presented in part at the 6th Annual Academic Surgical Congress; February 3, 2011; Huntington Beach, California.

* *Corresponding author*. Surgery 112, VA Medical Center, 4101 Woolworth Ave, Omaha, NE 68105. Tel.: +1 402 995 5371; fax: +1 402 995 5370.
E-mail address: macarlso@unmc.edu (M.A. Carlson).

0022-4804/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.jss.2013.09.039

scenarios, including peripheral vascular procedures [3], total knee arthroplasty [4], reoperative cardiac procedures [5], pulmonary resection [6], bleeding duodenal ulcer [7], and partial nephrectomy [8]. FS alone was not useful during hepatectomy [9], but FS combined with a collagen matrix applied during liver resection reduced blood loss and/or postoperative drainage compared with standard operative care [10]. Examples of currently available FS formulations that use plasma-derived fibrinogen include Evicel (Ethicon, Inc; Somerville, NJ) and Tisseel (Baxter Healthcare; Deerfield, IL).

The United States Department of Defense has maintained an interest in the development of hemostatic devices using FS for control of traumatic hemorrhage [11]. There has been particular interest in hemostatic FS devices for use under coagulopathic conditions [12]. Topical hemostatic treatments that incorporate FS have been successfully used in porcine trauma models, including femoral vessel injury [13], aortic injury [14], and hepatic injury [15]. One notable FS-containing device for traumatic hemorrhage was the Dry Fibrin Sealant Dressing, produced by the American Red Cross [16]. The Dry Fibrin Sealant Dressing was efficacious in porcine models of lethal hemorrhage [12,17] and was anecdotally successful in military trauma but was discontinued due to fragility and cost issues [18].

The availability of a relatively abundant FS might increase innovation into FI-based hemostatic devices for the treatment of severe hemorrhage. The essential components of FS are: FI, the biomonomer from which fibrin polymer is made [19]; activated thrombin (factor IIa or FIIa), which catalyzes the formation of soluble fibrin from FI and also activates factor XIIIa [20]; and activated factor XIII (FXIIIa), which cross-links the fibrin polymer to itself (rendering it insoluble) and to the wound surface [21]. One abundant source for these clotting factors can be large-scale recombinant protein production.

The complexity of FI and FIIa necessitates that recombinant versions of these proteins be made in animal cells [22]. We recently reported the production of recombinant human FI (rhFI) made at high concentrations in the milk of dairy cows [23]. Recombinant human FIIa (rhFIIa) already is commercially available (Recothrom; ZymoGenetics, Inc, Seattle, WA) and has been approved for topical hemostatic therapy in the United States and in Europe [24]. In contrast to FI and FIIa, FXIIIa is less complex; its core catalytic unit (FXIIIA2), which is kinetically faster than the more complex tetrameric plasma-borne FXIII [21], has been produced at large scale in yeast. FXIII nomenclature and specific activity are summarized in Table 1. Recombinant FXIIIA2 (rFXIIIA2) currently is in clinical studies of FXIII replacement therapy [25]. In the present study, we used a porcine hepatic wedge resection model to compare the hemostatic efficacy of a fully recombinant human FS (rhFS), containing rhFI, rhFIIa, and recombinant human FXIIIa (rhFXIIIa), against a commercially available, plasma-derived human FS (pdhFS).

Table 1 – Sources of factor XIII activity.

Factor XIII species	Abbreviation	Activity (U/mg)
Plasma-derived tetrameric factor XIII	FXIII	40[*]; 6–8[†]
Plasma-derived, dimeric, catalytic subunit factor XIII	FXIIIA2	N/A
Recombinant dimeric catalytic subunit factor XIII	rFXIIIA2	140[‡]
Plasma-derived, activated, dimeric factor XIII	FXIIIA2a	N/A
Recombinant human, activated factor XIII	rhFXIIIa	7000[§]

N/A = not applicable.
[*] Activity based on normal plasma pool, which by definition is 1 U/mL.
[†] Reported activity of plasma FXIII [37].
[‡] Reported activity of FXIIIA2 made in *Saccharomyces cerevisiae* [25].
[§] Reported activity of rFXIIIa made in *Pichia pastoris* [23].

2. Materials and methods

2.1. Animal studies

The use of swine was approved by the Subcommittee of Animal Studies and by the Research and Development Committee at the Omaha VA Medical Center. The number of swine ($n = 6$) used for each group in the two-group comparison of rhFS *versus* pdhFS (with hemostatic score as the outcome measurement) was determined with a statistical power analysis [26], using Δ/σ (Cohen d, in which Δ is the desired difference in means set by the observer and σ is the estimated standard deviation) = 2.0, false-positive rate (α) = 0.05, false-negative rate (β) = 0.2, and power (π, or $1 - \beta$) = 0.8.

2.2. Clotting factor sources

rhFI was produced in the milk of transgenic cows by inserting the primary sequence of the human transgenes for the α-, β-, and γ-chains of fibrinogen into the cow genome by nuclear transfer [23]. Southern blot analysis confirmed the presence of the three transgenes. The rhFI expressed in the milk of the transgenic cows was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot, γ and γ′-chain content, fibrinopeptide phosphorylation, glycosylation, thrombin-catalyzed activation, thrombin-catalyzed protofibril formation, factor XIIIa–catalyzed molecular cross-linking, viscoelasticity, scanning electron microscopy, and tissue sealant function [23]. The main differences between rhFI made in transgenic cow milk and plasma-derived fibrinogen (pdFI) was the γ′-chain content [23].

The human FXIIIA1 gene was expressed in *Pichia pastoris* [23,27]. The expressed rhFXIIIa was characterized by SDS-PAGE, Western blot, Pefakit FXIII incorporation assay, FXIIIa-catalyzed molecular cross-linking of fibrin, and viscoelasticity [27]. Human rhFIIa (Recothrom) was purchased from ZymoGenetics, Inc. Human pdFI depleted of plasminogen, von Willebrand factor, and fibronectin was purchased from Enzyme Research Laboratories (South Bend, IN). Commercial human pdhFS (Tisseel, unless otherwise specified) was purchased from Baxter BioSurgery (Deerfield, IL).

2.3. Determination of clotting factor concentration and activity

The concentrations of the purified stocks of rhFI, pdFI, and rFXIIIa were determined by OD_{280} and the bicinchoninic acid