Chapter 40
Pancreaticoduodenectomy Using Perioperative Zymogen Protein C to Help Prevent Blood Clotting: A Trilogy on Increased Patient Safety

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Abstract The blood clotting mechanism is a very important and complex physiologic process. Blood flow must be continuous through the blood vessels to provide essential oxygen and nutrients to the cells of the body. Dr. Melvin H. Knisely (Honorary First President of ISOTT, 1973) named and pioneered research in blood sludging and clotting which led to his nomination for the Nobel Prize by Dr. August Krogh in 1948. Abnormal clotting is a pathological state that can inhibit and prevent normal blood flow, leading to reduced oxygen transport to tissue from the microcirculation. It can result in the death of cells and tissues, including entire organs as well as the patient. Blood clotting and sludging are common occurrences during and after invasive surgery; thus, it is imperative to find safe procedures to reduce or prevent these deadly phenomena. All anticoagulants used today, for clot prevention and dissolution, can cause excessive bleeding that can lead to enormous medical expense to provide control, otherwise causing patient death. Protein C is a natural protein and is the pivotal anticoagulant in the blood. Due to the mechanism of converting the zymogen protein C (ZPC) to active protein C (APC), only when and where it is needed, and their respective half-lives in the body, the natural anticoagulant, antithrombotic characteristics of APC can be utilized without causing bleeds.

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40.1 Preamble

This is the final chapter of the trilogy that demonstrates the use of perioperative zymogen protein C to achieve safer patient results in invasive surgery. The same patient has experienced three surgical procedures: (1) total hip replacement, (2) life-threatening internal bleed, and (3) pancreaticoduodenectomy (PD) for pancreatic cancer. These procedures occurred over a period of several years without any complications due to clotting or bleeding. The patient is at very high risk for clotting problems because of age (77 years), previous venous thromboembolism (VTE), protein C deficiency, factor II gene mutation, severe surgery, and pancreatic adenocarcinoma. The percentage chance of VTE occurring increases significantly with the number of risk factors involved. In this case, without appropriate intervention, it was predicted that the patient had a 100 % risk of having a VTE.

The result is especially meaningful since there was no clotting or out-of-the-ordinary bleeding, even though the PD procedure is one of the most invasive of abdominal surgeries. The management of congenital and acquired thrombophilia with multimodality antithrombotic therapy is recommended for most surgeries. It is strongly suggested that ZPC be considered, along with standard practice anticoagulation, for any patient with protein C deficiency. It remains to be seen if all invasive surgeries would be safer with the use of ZPC.

40.2 Introduction

This chapter is a continuation of the application of the hypothesis for increased patient safety using ZPC for invasive medical procedures. Although we have proposed this hypothesis since the early 1980s, the first formal presentation of this strategy was delivered at the 2007 ISOTT meeting held in Uppsala, Sweden. The resulting paper first appeared in the Springer Publishing Company Experimental Medicine and Biology series, Oxygen Transport to Tissue [1]. This hypothesis was based on the attributes of the blood anticoagulant ZPC. Two important characteristics result in its action locally rather than globally in the body. First, the mechanism for ZPC to activated protein C (APC) occurs only where and when the APC is needed. Second, the half-life of ZPC in the body is approximately 8–10 h, and the half-life of APC is on the order of minutes. These two attributes allow local anticoagulant activity without exacerbating bleeding. Therefore, we defined ZPC as the "silver bullet" of anticoagulation [2].

This study represents the third chapter in a trio of clinical testing of the hypothesis. The same patient experienced all three of the major surgeries using perioperative ZPC. The first surgery was a total hip replacement (12 Nov 2007). The second was for the repair of a life-threatening internal bleed at the site where a flat polyp was removed from a location near the cecum (15 Sept 2008). The bleed was partially due to the administration of heparin and warfarin and began as the patient,

who was on lifetime warfarin, was progressing toward an INR of 2.0 [3, 4]. This chapter involving the PD surgery procedure for pancreatic cancer utilizing perioperative ZPC (1 Oct 2010) is the final chapter of the trilogy. These cases illustrate the importance of controlling abnormal functions (pathology) leading to tissue deprivation of oxygen, which is of particular interest to the objectives of the International Society on Oxygen Transport to Tissue (ISOTT).

ISOTT research focuses on all processes of normal and pathological oxygen transport within the blood and tissue [5, 6]. Foremost of interest is blood hemostasis to ensure optimal tissue oxygenation. This includes the coagulation cascade, particularly the significance of natural anticoagulants and substitutes for them in cases of pathology. The phenomenon of "blood sludging" (agglutination) was first observed by Dr. Melvin H. Knisely via his quartz rod crystal optical technology [7]. Our initial studies included mathematical modelling of oxygen transport in the microcirculation along with polarographic microelectrode oxygen sensing [8–10]. This work was complemented with studies on anti-adhesive drugs to enhance tissue oxygenation [11].

Historically, protein C (PC) was not so named until Stenflo isolated the protein from bovine plasma and labeled it protein C because it was the third peak in the chromatograph elution [12]. Originally, activated protein C (APC) was coined autoprothrombin IIA (APIIA) because it was believed to be derived from prothrombin [13]. Twelve years later, it was shown that APIIA's precursor was not only distinct from prothrombin, but it inhibited the factor Xa-mediated activation of prothrombin as well as facilitating fibrinolysis [14]. Shortly after Stenflo isolated the bovine PC, Seegers verified it to be identical to APIIA [15]. Three years later, Kisiel was able to purify human PC [16].

Zymogen protein C (ZPC) is the pivotal anticoagulant and antithrombotic in the human blood coagulation cascade [17]. PC is a glycoprotein with a molecular weight of 62,000 Da. Human ZPC is synthesized in the liver as a single-chain precursor and circulates in the blood primarily as a two-chain inactive zymogen until it is activated by proteolytic cleavage. The protein is a serine protease that requires vitamin K for normal biosynthesis [13]. It is a member of the vitamin K-dependent (VKD) family also consisting of coagulation proteins such as factors VII, IX, proteins S and Z, and prothrombin.

ZPC is a trace protein in human blood with a concentration of 4 μg/mL [12, 18, 19]. Serious problems can occur when the PC level in the blood is lowered. For example, patients deficient in PC are at risk of deep vein thrombosis (DVT) [20] and other clotting complications, resulting in tissue oxygen deprivation, some of which can be life threatening. When these blood clots break away from the surface of the vein and enter the bloodstream, they will induce strokes, heart attacks, and pulmonary embolisms. Venous thrombosis is one of the most frequent complications in medical patients [21]. It is the most common cause of death in patients undergoing major orthopaedic operations. In the United States, it has been estimated that 300,000 hospitalizations and 50,000 deaths occur [21], and this amounts to millions and possibly billions of dollars in medical expenses annually.

Although very complex, three main pathways are involved in regulating coagulation [22]. One pathway that utilizes heparin-like molecules and antithrombin III results in the inhibition of coagulation proteases. A second pathway, referenced as lipoprotein-associated coagulation inhibitor, or extrinsic pathway inhibitor, blocks the activity of factor VIIa-tissue factor complex [23]. The third and most important pathway involves APC which neutralizes factors Va and VIIIa [17]. These major pathways function together to inhibit both the proteases and regulatory proteins (cofactors) of the clotting system. It has been shown through clinical studies that antithrombin III, protein C, and factor S deficiencies all exhibit thrombotic complications [20, 24].

Although inefficient, PC can be activated by thrombin alone. This activation process can be enhanced by a factor of at least 1000 via a complex between thrombin and a membrane protein called thrombomodulin on the surface of endothelial cells [17, 25]. The activated PC is a potent serine protease that regulates blood coagulation by forming a complex with protein S (PS) on both endothelial and platelet surfaces. This deactivates factors Va and VIIIa, thus preventing generation of the enzymes factors Xa and thrombin [26, 27].

Cofactor PS circulates in the blood as a free agent and in complex with C4bBP which is a regulatory protein of the complement system. The PS-C4bBP complex is not functional as a cofactor for activated PC in factor Va inactivation and therefore downregulates the effectiveness of PC in the inactivation of Va and VIIIa. Va is required to produce thrombin. Once thrombin is produced, it activates factor I (fibrinogen) to form fibrin, which synthesizes a soft clot. VIIIa is then required to solidify the clot. So by inactivating Va and VIIIa, you downregulate the clotting process.

At present time, heparin and warfarin are used to treat ZPC deficiency and other hypercoagulable conditions. The disadvantage of these drugs is that both have dangerous side effects. Excessive internal bleeding is a major problem that can result from their use, possibly causing a stroke or major organ failure. Additionally, warfarin-induced skin necrosis and heparin-induced skin necrosis can lead to amputation of extremities and death. Also, pregnant women cannot use warfarin.

Previous animal testing [28–31] and clinical trials indicate that PC is an effective anticoagulant/antithrombotic for many medical indications without harmful side effects. This unique feature is a function of the mechanistic behavior of PC in the body. Differing from all other anticoagulants, PC circulates the blood in an inactive form and is activated only at the site where it is needed and at the time when it is needed. Also, ZPC can be used at greater than normal blood concentrations without bleeding complications.

PC has many clinical applications. Not only can it be used to treat genetically deficient patients, but it can also be used to treat septic shock [32], hip and knee replacement patients, warfarin-induced skin necrosis patients, heparin-induced thrombocytopenia, patients doing fibrinolytic therapy, and patients undergoing angioplasty or suffering from unstable angina, etc. [33]. Additionally, research has shown that the use of safe anticoagulants could lower the rate of strokes in the USA

from 80,000 to 40,000 per year, reduce patient complications, and save the medical industry an estimated $600 million per year [34].

ZPC concentrate has been shown to be successful for the prevention and treatment of thrombosis in individuals with inherited and acquired PC deficiency and to avoid the problems associated with fresh and frozen plasma administration [35–38]. When one considers that PC is the only known anticoagulant/antithrombotic without bleeding side effects and 1 in 300 people are hereditary PC deficient [21], it is easy to see the enormous benefit of having inexpensive PC available to medical patients. Additionally, there are innumerable patients with acquired PC deficiency.

40.3 Experimental Medical Procedure

The patient experienced painless obstructive jaundice and was found to have a mass in the periampullary area. The tumor biopsy revealed adenocarcinoma. As a result, it was proposed that the mass be surgically removed. The procedure recommended was pancreaticoduodenectomy, most often referred to as the Whipple surgery, named after Dr. Allen O. Whipple, who was the first American to perform it and reported it in 1935 [39]. This operation was actually first performed by Dr. Walther Kausch of Germany in 1909 and reported in 1912 [40]. This is a very extensive operation that involves more than just removing parts of the pancreas. Because the pancreas is anatomically connected to other organs and ducts, multiple organs are involved in the operation. The surgery typically removes the head of the pancreas, the duodenum, the common bile duct, the gall bladder, and often, part of the stomach.

The surgical procedure was complicated by the fact that the patient had multiple risk factors for a VTE, including the patient's age (77 years), pancreatic cancer, major surgery, previous VTE, protein C deficiency, and factor II mutation. This combination of risk factors results in a markedly increased risk of VTE formation during and after surgery. Evidence shows that patients with five or more risk factors approach a 100 % chance of developing VTE.

To prevent blood clotting for the management of congenital and acquired thrombophilia, a multimodality, antithrombotic therapy was used [41]. The patient was on lifetime warfarin, which was stopped 5 days before surgery and replaced with Lovenox until 24 h before surgery. Six hours prior to surgery, PC concentrate at 50 μ/kg IV was administered every 6 h until after surgery, then every 8 h for 24 h, and then every 12 h until post-op day 8. Two days after surgery, warfarin was administered orally, and heparin was administered 5,000 units SQ every 8 h until post-op day 5, then Lovenox 10 units/kg/h IV to aPTT 50–65 s. The Lovenox was discontinued when the INR reached a value of 2.0. Thus, the VTE prophylaxis consisted of pre- and postoperative administration of ZPC, warfarin, and heparin. However, only ZPC was administered in the 2+ days encompassing the surgery.

40.4 Outcome

According to medical statistical data, this patient had a probability of experiencing a VTE that approached 100 %, in particular because of the radical invasiveness of this surgery and the multiple risk factors present. However, the patient had no thrombotic events and no bleeding complications throughout the entire procedure and recovery. This demonstrates the value of multimodality VTE prophylaxis with ZPC as the bridge. The patient moved on to warfarin therapy and continues to function normally without incidence.

40.5 Production Research Note

As presented in this trilogy of chapters, it would be beneficial to future surgical patients as well as the many hereditary and acquired protein C-deficient patients to develop innovative upstream and downstream bioprocessing strategies for the low-cost, high-volume production of ZPC. This would make ZPC available to a wider patient population.

The two existing protein C products (Ceprotin, Baxter International; Xigris, Eli Lilly) are so expensive that they are rarely used even in cases where patient survival is in question. If cost were not an issue, the zymogen could be used for prophylactic treatment of PC deficiency, as well as other disease states or in standard medical and surgical procedures. The significant examples of perioperative procedures utilizing ZPC to prevent clotting without exacerbating bleeding as presented in this trilogy support the need to produce inexpensive ZPC.

There is ongoing research toward achieving this goal. The three sources of PC that are available from upstream bioprocessing include rDNA cell culture technology [42], blood plasma [43–46], and transgenic animals [47, 48]. Our research continues to optimize the downstream processing for these raw materials via immobilized metal affinity chromatography (IMAC) [49–58]. The correct combination of ion exchange, IMAC, and absorption and elution buffers are being investigated for the optimal bio-downstream processing.

40.6 Conclusion

The three chapters of this trilogy establish the need for multimodality VTE prophylaxis utilizing ZPC as a bridge during invasive medical procedures. This is an important indicator of the need to produce a low-cost ZPC for many invasive medical procedures and utilization by heterozygote protein C-deficient patients. The ultimate goal would be either to produce this low-cost ZPC product or to design a substitute that mimics ZPC's "silver bullet" ability to act as an anticoagulant without increasing bleeding [2].

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References

1. Bruley DF (2009) Zymogen protein C concentrate for safer heterozygote surgery, "I am a guinea pig!". Adv Exp Med Biol 645:115–121
2. Bruley DF, Streiff MB (2013) Nature's "silver bullet" for anticoagulation: mechanism of zymogen protein C to activated protein C. Adv Exp Med Biol 765:15–21
3. Bruley DF, Mears SC, Streiff MB (2010) Safer surgery using zymogen protein C concentrate. Adv Exp Med Biol 662:439–445
4. Bruley DF, Jagannath SB, Streiff MB (2011) Zymogen protein C to prevent clotting without bleeding during invasive medical procedures. Adv Exp Med Biol 701:91–97
5. Bruley DF (2012) The history of ISOTT. Adv Exp Med Biol 737:1–9
6. Bruley DF (2008) ISOTT: roots, founding and beyond. Adv Exp Med Biol 614:1–8
7. Goro FW (1948) Blood sludge. Life Mag 24(22):49–59
8. Thews G (1960) Oxygen diffusion in the brain. A contribution to the question of the oxygen supply of the organs. Pflugers Arch 271:197–226
9. Reneau DD, Bruley DF, Knisely MH (1967) A mathematical simulation of oxygen release, diffusion and consumption in the capillaries and tissue of the human brain. In: Chemical engineering in medicine and biology. Plenum Press, New York, pp 135–241
10. Artigue RS, Bruley DF (1984) The transport of oxygen, glucose, carbon dioxide and lactic acid in the human brain: mathematical models. Adv Exp Med Biol 159:1–4
11. Bicher HI, Bruley DF, Knisely MH (1973) Anti-adhesive drugs and tissue oxygenation. Adv Exp Med Biol 37:657–667
12. Stenflo J (1976) A new vitamin K-dependent protein. J Biol Chem 251(2):355–363
13. Mammen EF, Thomas WR, Seegers WH (1960) Activation of purified prothrombin to autoprothrombin II (platelet cofactors II or autoprothrombin IIA). Thromb Diath Haemorrh 5:218–249
14. Marcianik E (1972) Inhibitor of human blood coagulation elicited by thrombin. J Lab Clin Med 79(6):924–934
15. Seegers WH, Novoa E, Henry RL, Hassouna HI (1976) Relationship of "new" vitamin K-dependent protein C and "old" autoprothrombin II-a. Thromb Res 8(5):543–552
16. Kisiel W (1979) Human plasma protein C: isolation, characterization and mechanism of activation by a thrombin. J Clin Invest 64(3):761–769
17. Esmon CT (1989) The roles of protein C and thrombomodulin in the regulation of blood coagulation. J Biol Chem 264(9):4743–4746
18. Fernlund P, Stenflo J (1982) Amino acid sequence of the light chain of bovine protein C. J Biol Chem 257(20):12170–12179
19. Kisiel W, Carnfield WM, Ericsson LH, Davie EW (1977) Anticoagulant properties of bovine plasma protein C following activation by thrombin. Biochemistry 16(26):5824–5831
20. Clouse LH, Comp PC (1986) The regulation of hemostasis: the protein C system. N Engl J Med 314(20):1298–1304
21. Bertina RM (1988) Protein C and related proteins: biochemical and clinical aspects. (Contemporary issues in haemostasis and thrombosis). Churchill Livingstone, New York
22. Esmon CT (1990) Regulation of coagulation: the nature of the problem. In: Bruley DF, Drohan WN (eds) Protein C and related coagulants. Gulf, Houston, p 3
23. Rapaport SI (1989) Inhibition of factor VIIa/tissue factor-induced blood coagulation: with particular emphasis upon a factor Xa-dependent inhibitory mechanism. Blood 73(2):359–365
24. Rosenberg R, Marcum JA (1985) Heparin-like molecules and thrombotic disease. ASAIO J 8:215

25. Zushi M, Gomi K, Yamamoto S, Maruyama I, Hayashi T, Suzuki K (1989) The last three consecutive epidermal growth factor-like structures of human thrombomodulin comprise the minimum functional domain for protein C-activating cofactor activity and anticoagulant activity. J Biol Chem 264(18):10351–10353
26. Walker FJ, Sexton PW, Esmon CT (1979) The inhibition of blood coagulation by activated protein C through the selective inactivation of activated factor V. Biochim Biophys Acta 571(2):333–342
27. Fulcher CA, Gardiner JE, Griffin JH, Zimmerman TS (1984) Proteolytic inactivation of human factor VIII procoagulant protein by activated human protein C and its analogy with factor V. Blood 63(2):486–489
28. Colucci M, Stassen JM, Collen D (1984) Influence of protein C activation on blood coagulation and fibrinolysis in squirrel monkey. J Clin Invest 74(1):200–204
29. Comp P, Esmon CT (1981) Generation of fibrinolytic activity by infusion of activated protein C into dogs. J Clin Invest 68(5):1221–1228
30. Gruber A, Griffin JH, Harker LA, Hanson SR (1989) Inhibition of platelet-dependent thrombosis formation by human activated protein C in a primate model. Blood 73(3):639–642
31. Okijima K, Koga S, Kaji M et al (1990) Effect of protein C and activated protein C on coagulation and fibrinolysis on normal human subjects. Thromb Haemost 63(1):48–53
32. Sharma GR, Gerlitz B, Berg DT et al (2008) Activated protein C modulates chemokine response and tissue injury in experimental sepsis. Adv Exp Med Biol 614:83–91
33. Bruley DF, Drohan WN (1990) Protein C and related anticoagulants, Advances in applied biotechnology series. Gulf Publishing Company (Portfolio Publishing Company, The Woodlands
34. Winslow R (1995) Increased use of blood-thinning drugs could prevent 40,000 strokes a year. Wall St J
35. Vukovich T, Auerger K, Weil J, Engelmann H, Knöbl P, Hadorn HB (1988) Replacement therapy for a homozygous protein C deficiency-state using a concentrate of human protein C and S. Br J Haematol 70(4):435–440
36. Dreyfus M, Magny JF, Bridey F et al (1991) Treatment of homologous protein C deficiency and neonatal purpura fulminans with a purified protein C concentrate. N Engl J Med 325(22):1565–1568
37. Manco-Johnson M, Nuss R (1992) Protein C concentrate prevents peripartum thrombosis. Am J Hematol 40(1):69–70
38. Marlar RA, Montgomery RR, Broakman AW (1989) Diagnosis and treatment of homozygous protein C deficiency. Report of the working party on homozygous protein C deficiency of the subcommittee on protein C and protein S, international committee on thrombosis and haemostasis. J Pediatr 114(4 Pt 1):528–534
39. Harvard Health Letter (2009) The Whipple procedure: better outcomes for pancreatic cancer surgery. www.health.harvard.edu Apr 2009
40. Whiple AO, Parsons WB, Mullins CR (1935) Treatment of carcinoma of the ampulla of vater. Ann Surg 102(4):763–779
41. Streiff MB (2011) "In the Eye of a Thrombotic Storm": management of congenital and acquired thrombophilia with multimodality anti-thrombotic therapy. ISOTT Meeting 2011, Georgetown University, Washington, DC
42. Ganesh RS,Grinnell BW et al (2008) Activated protein C modulates chemokine response and tissue injury in experimental sepsis. Adv Exp Med Biol 614:83–92
43. Wu H, Bruley DF, Kang KA (1998) Protein C separation from human blood plasma Cohn fraction IV-1 using immobilized metal affinity chromatography. Adv Exp Med Biol 454:697–704
44. Wu H, Bruley DF (1999) Homologous blood protein separation using immobilized metal affinity chromatography: protein C separation from prothrombin with application to the separation of factor IX and prothrombin. Biotechnol Prog 15(5):928–931
45. Wu H, Bruley DF (2002) Chelator, metal ion and buffer studies for protein C separation. Comp Biochem Physiol A Mol Integr Physiol 132(1):213–220
46. Tadepalli SS, Bruley DF, Kang KA et al (1997) Immobilized metal affinity chromatography process identification and scale-up for protein C production. Adv Exp Med Biol 428:31–43

47. Velander WH, Johnson JL, Page RL et al (1992) High-level expression of a heterologous protein in the milk of transgenic swine using the cDNA encoding human protein C. Proc Natl Acad Sci USA 89(24):12003–12007
48. Drohan WH, Wilkins TD, Lattime E et al (1994) A scalable method for the purification of recombinant human protein C from the milk of transgenic swine. In: Advance bioprocess engineering. Kluwer Academic Publishers, Dordrecht, pp 501–507
49. Porath J (1992) Immobilized metal ion affinity chromatography. Protein Expr Purif 3(4):263–281
50. Porath J, Carlsson J, Olsson I, Belfrage G (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. Nature 258(5536):598–599
51. Andersson L (1991) Recognition of phosphate groups by immobilized aluminum (III) ions. J Chromatogr A 539(2):327–334
52. Andersson L, Porath J (1986) Isolation of phosphoproteins by immobilized metal (Fe^{3+}) affinity chromatography. Anal Biochem 154(1):250–254
53. Ramadan N, Porath J (1985) Fe3+− hydroxamate as immobilized metal affinity-adsorbent for protein chromatography. J Chromatogr 321(1):93–104
54. Sulkowski E (1988) Immobilized metal ion affinity of proteins on IDA-Fe3+. Makromol Chem Macromol Symp 17(1):335–348
55. Wong JW, Albright RL, Wang NH (1991) Immobilized metal ion affinity chromatography (IMAC) chemistry and bioseparations applications. Sep Purif Rev 20(1):49–106
56. Winzerling JJ, Patrick B, Porath J (1992) How to use immobilized metal ion affinity chromatograph. Methods 4(1):4–13
57. Thiessen EE, Bruley DF (2003) Theoretical studies of IMAC interfacial phenomena for the purification of protein C. Adv Exp Med Biol 540:183
58. Lee J, Thiessen EE, Bruley DF (2005) Anticoagulant/anti-inflammatory/antithrombotic protein C production-metal ion/protein interfacial interaction in immobilized metal affinity chromatography. Adv Exp Med Biol 566:381–387