Chapter 2
A Compelling Case for the Use of Perioperative Zymogen Protein C for Increased Patient Safety

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Abstract It is imperative to maintain normal blood flow to provide adequate oxygen supply to specific organs and cells, as well as for the removal of metabolic byproducts. Therefore, any situation that results in blood clotting can injure or kill living tissues. In this paper, we describe a case where a protein C deficient subject who would, by all medical indicators, be at 100 % risk of experiencing thrombophlebitis, deep vein thrombosis, and or lung emboli, is able to escape all pathologies by using perioperative zymogen protein C (ZPC). This protein C deficient patient has a long history of blood clotting, particularly from surgical procedures. The patient is 81 years old and first experienced clotting due to hernia surgery in 1964, when he was hospitalized for 16 days post-surgery with life threatening complications. It was later determined in 1980, after many episodes, that the patient had hereditary protein C deficiency at the 38 % level. In his hernia surgery, perioperative ZPC was used along with accepted anticoagulation procedures with no blood clots or other related side effects occurring. This procedure can greatly benefit protein C deficient patients, and could potentially find use for non-PC deficient patients in surgeries and a variety of other medical treatments. This particular case helps to validate the importance of ZPC in effecting safer surgery in high-risk patients. It also supports the mechanism of ZPC acting as an anticoagulant without causing bleeding. Most importantly, each clinical case study represents a unique combination of surgeon, hematologist, medical staff, and patient

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functioning as a coordinated team. In this case, smaller amounts of very expensive ZPC achieved safe and efficacious results, which is hugely important for future clinical applications when considering the production cost of ZPC. More studies must be done to establish minimum dosing while achieving safe and efficacious outcomes.

Keywords Zymogen protein C (ZPC) • Surgery • PC deficiency • Patient safety • Optimal dosage

1 Introduction

The National Blood Clot Alliance (NBCA) has recently stated that there are as many as 900,000 cases of deep vein thrombosis (DVT) and pulmonary embolism (PE) diagnosed in the United States each year, resulting in nearly 300,000 deaths annually [1]. A large portion of the blood clots are due to blood coagulation protein surplus/deficiency (*via* hereditary or acquired conditions) or interference with the mechanisms for protein activation/deactivation [2, 3]. This is seen in numerous disease states where Dr. Melvin H. Knisely demonstrated blood sludging in malaria and over 100 other disease conditions [4]. Some examples of inherited thrombophilia (hypercoagulability) include factor II gene mutation, antithrombin III, plasminogen activator inhibitor-1 (PAI-1), protein S deficiency, protein C (PC) deficiency, and Factor V Leiden [5]. Examples of acquired thrombophilia include antiphospholipid syndrome, and hyperhomocysteinemia. Other factors may trigger or cause blood clots, including surgery, trauma, fractures, bed rest, sitting or lying still for several hours at a time, cancer, chemotherapy, intravenous catheters, estrogen use, pregnancy, giving birth, and air/car travel.

PC is the pivotal blood factor anti-coagulant [6–9]. It also serves as an apoptotic, anti-inflammatory and anti-thrombotic agent [10]. Its function, when activated, is to deactivate factor Va and factor VIIIa, thus downscaling the clotting process. Because Zymogen PC (ZPC) is activated only when and where it is needed, and the activated half-life is on order of minutes, ZPC has been coined the "silver bullet" of anticoagulation and has been used successfully perioperatively in major surgeries [11–16]. In the case of hereditary PC deficiency, babies that are born homozygous PC deficient die within weeks without PC concentrate supplements [17]. Those that are heterozygous for the gene typically have their first episode between the ages of 12 and 25 [18] or after 60.

2 The Patient and His Thrombotic History

Patient K is an 81-year old Caucasian male and a retired naval aviator. He was admitted for recurrent left inguinal hernia repair. Table 2.1 represents the history of thrombotic episodes that the patient has suffered from past surgeries or trauma to

Table 2.1 Patient K's thrombotic history

1964	Hernia repair—thrombus found at site of I.V. in left arm
6/24/1969	Hernia repair—saphenous vein thrombus—extended hospitalization
1980	Protein C on family. Patient K's value at 38 %, daughter's value similar, son's 80 %, other daughter at an excess
9/15 –9/28/1983	Left deep vein thrombosis (DVT). Apparent embolus. Heparin for 13 days
1/2/1987	ER visit with clot. Protein C level of 38 %
8/20/1989	ER visit with clot. History of DVTs. Travelling in car with pain in left leg
8/16/1991	Hospital visit with clot—history of DVT 9/83 and several other episodes of superficial thrombus usually after long car ride, as with today
3/9/1993	Fell off ladder; developed clot a month later. Patient K hospitalized and on heparin for 11 days
1995	SVT ablation procedure; evidence of an embolus during the procedure
11/2007	Superficial thrombosis of left calf
12/2/2010	Emergency laparoscopic surgery for appendix. Two (2) units plasma to avoid clotting
9/2012	Cataract surgeries, remained on Coumadin through procedure
2/6/2013	Surgery for left inguinal hernia. Zymogen PC perioperative—NO CLOTS (*subject of this paper*)

different parts of the body, resulting in blood clotting. The patient suffers from protein C deficiency at the 38 % level (heterozygote) and is on chronic anticoagulation therapy (warfarin). From this clotting history and other medical indicators for clotting, the patient was at 100 % risk of developing a superficial thrombophlebitis (STP), a deep vein thrombus (DVT), and/or a pulmonary embolus (PE) during or after surgery. Because of the potential clotting complications in this case, it was decided to use perioperative zymogen protein C (ZPC) along with standard anticoagulation therapy [11–16].

3 Medical Procedure

Major surgery is an environmental thrombotic risk factor that can lead to deep vein thrombosis and pulmonary embolism. These risks are enhanced in patients with underlying thrombophilic defects such as protein C deficiency, particularly for patients with previous thrombotic events. For the purposes of this paper, protein C deficiency refers to the heterozygote type. In preparation for the surgery, the patient's warfarin was discontinued 7 days prior to the surgery and the next day he was started on enoxaparin sodium (Lovenox; Sanofi-AventisUS LLC, Bridgewater, NJ; 1 mg/kg twice daily) until 24 h before the surgery. The patient was admitted to the hospital the morning of surgery on February 6, 2013 and started on intravenous ZPC concentrate (human) (Ceprotin; Baxter International Inc., Deerfield, IL). The night before surgery and before administration of protein C, the patient's protein C activity level was 32 % and his INR was 1.0 with a prothrombin time of 10.6.

The ZPC was ordered at 450 units bolus every 6 h at a rate of 108 cc/h. His morning PC activity level immediately before surgery and before the bolus was 32. This was taken at 8:13 am but was not known until 48 h later, as the analysis had a long processing time. His PC activity level at 2:51 pm was 85 and at 4:54 am the next day was 124. He was maintained on ZPC until his last dose at 5:43 am on 2/8/2013 (POD 2), and then transitioned back to his normal anticoagulation therapy using Lovenox and warfarin according to the following procedure. Coumadin was started on 2/7/13 (POD 1) at 7:45 pm with a first dose of 5 mg. Lovenox was started on 2/7/13 (POD 1) at 8:00 pm, dosing 80 mg SQ every 12 h. He was given instructions to take 5 mg Coumadin every Tuesday, Thursday, Saturday, and Sunday, and 2.5 mg on Monday, Wednesday, and Friday and to continue with the Lovenox until his INR was greater than 2.0.

During the procedure, he received endotracheal general anesthesia. A 15-bladed knife was used to make an 8-cm curvilinear incision over the inguinal canal. The patient had numerous, engorged, collateral veins from chronic venous stasis disease on the left side. After an extensive procedure with a good landing zone, the mesh was placed and it was sutured into place. After sterilization, the patient recovered from general anesthesia and was transported to the post-anesthesia care unit (PACU) in stable condition. Sponge, instrument, and needle counts were reported as correct times two. He was stable throughout the procedure and was released from the hospital for home care on the afternoon of 2/8/13.

4 Results and Discussion

As reported, Patient K was a naval aviator. Therefore, he was often in very cramped, stressful conditions for relatively long periods of time. It is well known that people in thrombophilic circumstances are prone to developing thrombotic episodes. As can be seen from Patient K's history (Table 2.1), his first detected thrombosis was found after hernia repair in 1969. Expanding on his history, he has experienced at least 12 thrombotic events over the course of his lifetime as a result of major and minor interventions and traumas. Because of the high risk of thrombosis in this hernia surgery, it was decided to employ the use of perioperative ZPC to cover the period before, during, and after surgery. Notably, during the time of surgery, ZPC was the only anticoagulant present and it protected him from clotting without increased bleeding.

Throughout the surgery, blood samples were taken and analyzed for ZPC levels in the blood. Unfortunately, the individual sample assays required 48 h or more, so that the results were not immediately available during the procedure and in fact, the last sample taken was never recorded to indicate the ultimate ZPC level in the blood. The highest recorded level was 124 %, which appeared to be sufficient and may have been more than enough. Therefore, we are unable to determine the effective level of ZPC for such a procedure, and more ZPC was probably used than necessary. It has been demonstrated that supraphysiologic levels (238 %) have been used in past studies without increased bleeding or other negative results [12]. Further research must be done to optimize safe dosing and reduce the cost for ZPC.

It has been established that ZPC concentrate has achieved promising results with a very good safety record [19, 20] for treating such conditions as severe sepsis and neonatal purpura fulminans [21, 22]. It has also been used to prevent vascular thrombosis for patients with familial PC deficiency undergoing renal transplantation without noted side effects [23]. Given Patient K's personal and family history of idiopathic PE and PC deficiency, as well as the substantial thrombotic and bleeding risks posed by this surgery, it was decided the off label use of PC concentrate represented the most efficacious approach. The resulting favorable endpoint helps establish the procedure as a treatment strategy for patients who are highly thrombophilic with PC deficiency. There may be other medical indications that could be treated more safely with the protocol used in this case study.

Based on evidence from several major surgeries involving perioperative PC administration [11–14, 16], it is clear that this procedure increases patient safety. In all cases there was no hypersensitivity reaction with Ceprotin administration, and despite the high levels of PC during the perioperative period, there was no increased surgical site bleeding. In our present case, however, a hematoma did occur postoperatively. But this condition presented itself after the patient was started on warfarin and was therapeutic on heparin [Lovenox].

This report presents the use of human protein C concentrate, in an off-label fashion, to safely perform a hernia surgery in a highly thrombophilic patient deficient in protein C. It should be clear that this strategy is not only promising but should be considered in treating thrombophilic individuals with PC deficiency who require major surgery. Patient K has survived and is presently leading a normal life without any consequences from his hernia repair surgery.

5 Economics of ZPC

It is obvious that using perioperative PC (Ceprotin) is very costly. Based on the approximate shelf value of $34.40/unit of Ceprotin, this surgery for ZPC alone cost approximately $134,160. Considering that the patient's ZPC level during that time period may have risen above 124 % (last measured value) of normal, it is important to find the optimal safe dose to minimize the expense of ZPC.

Alternatively, it is important to continue research and development to establish improved bioprocessing strategies that will reduce production costs of ZPC from blood. One approach that shows promise is the replacement of immunoaffinity chromatography (IAC) with immobilized metal affinity chromatography (IMAC) [24–27]. It is estimated that this approach could reduce product costs by a factor of 500–1000. Other possible approaches might include the use of mini-antibodies (mini-Mab) leading to the development of inexpensive ligands to reduce the PC production cost [28]. Additionally, the use of transgenic animals has been demonstrated to produce less expensive blood proteins [29]. Although less attractive from an economic point of view, further investigation of recombinant DNA technology *via* bioreactors is another possibility. Because of the need to reduce cost of important life-saving blood proteins, it is encouraged that research and development be funded and promoted in this arena.

6 Conclusion

This paper presents an important example of safer surgery through the use of ZPC. The procedure has great benefits for protein C deficient patients and could find use in a variety of medical treatments. This particular case helps to validate the importance of ZPC in effecting safer surgery in high risk patients. It also supports the mechanism of ZPC acting as an anticoagulant without causing bleeding. Most importantly, this paper contributes clinical results indicating that smaller amounts of expensive ZPC can be used to achieve the same satisfactory results. More clinical studies must be completed to target the optimum levels of ZPC with respect to cost and effectiveness. These case studies are rare because of the cost of each experiment and the difficulty in getting a willing patient along with a surgeon, hematologist, and medical team to carry out the procedure. Whatever it takes, the world needs a safe anticoagulant that will not result in costly bleeds and mortality.

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