Chapter 13
Zymogen Protein C to Prevent Clotting without Bleeding during Invasive Medical Procedures

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Abstract Thrombophilic disorders that predispose patients to develop blood clots can be life-threatening and result in a large economic burden on healthcare expenditures. Venous Thromboembolism (VTE) (deep vein thrombosis and pulmonary embolism) are the third leading cause of death in the United States. Protein C deficiency is a common thrombophilic condition that affects an estimated 1 in 400 Americans. Zymogen Protein C (ZPC) is the precursor to Activated Protein C (APC), a pivotal endogenous anticoagulant in human blood. Patients with protein C deficiency who have roughly half the normal level of protein C are estimated to be at 10-fold increased risk of VTE. We describe the use of protein C concentrate (Ceprotin®, Baxter, Deerfield, IL) in a patient with protein C deficiency and with a previous pulmonary embolism who developed a life-threatening gastrointestinal bleed after polypectomy. The patient is a 75-year-old male at very high risk for deep vein thrombosis and possible lung emboli. He has heterozygous Protein C deficiency (50%) and heterozygosity for the prothrombin gene G20210A mutation. During a routine colonoscopy, a large 3 cm cecal polyp was identified and resected. Eight days post-procedure while performing abdominal exercise he developed a life-threatening GI bleed originating from the polypectomy site as his warfarin was becoming therapeutic on a Low Molecular Weight Heparin (LMWH) periprocedural bridge. The patient's warfarin was reversed with vitamin K, and LMWH and warfarin were discontinued. To prevent thrombosis, he was started on ZPC until anticoagulation could be safely restarted.

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During endoscopy, the bleeding site was treated with an injection of 1:10,000 dilution of epinephrine, followed by cauterization and placement of endoclips (4 metal staples). Three days after endoscopic repair LMWH was restarted with warfarin. Sixteen months post-bleed, the patient remains on life-long warfarin without further episodes of bleeding or thrombosis. Zymogen Protein C concentrate (Ceprotin®, Baxter Deerfield, IL) should be strongly considered for peri-procedural management of any patient with protein C deficiency and previous thromboembolism.

13.1 Introduction

As with many scientific findings, Protein C was discovered in a serendipitous way. In 1960 an inhibitory to blood coagulation was found and named autoprothrombin II [16]. Originally it was thought to be derived from prothrombin, however in 1972 it was shown that the precursor protein was not prothrombin [17]. It was determined in 1974 that rabbits generated anticoagulant activity in response to intravenous injection of thrombin and the inhibitory activity appeared to be similar to autoprothrombin II A [14]. In 1976, a new vitamin K-dependent protein was purified from bovine plasma and named Protein C because it was the third peak in an ion-exchange column elution [25]. Protein C was shown to be the proenzyme of a serine protease which could be activated by thrombin. Activated Protein C (APC) was then confirmed to be autoprothrombin II A [24] and human Protein C was first purified in 1979 [15].

Zymogen Protein C (ZPC) can be activated by thrombin alone but a more efficient activation process involves a thrombin/thrombomodulin complex bound to endothelial membrane and to the surface of platelets [9, 12]. This process takes place where and when APC is needed, thus preventing clotting with little or no bleeding.

The coagulation cascade includes at least three pathways that regulate coagulants: the antithrombin III inhibition of coagulation proteases, which is accelerated by vascular heparin-like molecules [23], the lipoprotein associated coagulation inhibitor (LACI) that blocks the activity of the factor VIIa-tissue factor complex [22], and, finally, the pivitol pathway which includes activated protein C (APC) that deactivates factors Va and VIIIa [11]. These three inhibitory pathways function together to inhibit both the proteases and their cofactors in the coagulation system. Clinical data suggest that patients with antithrombin III or Protein C deficiency are subject to thrombotic complications [23, 8].

Thrombophilic disorders that predispose patients to develop blood clots can be life-threatening and result in a large economic burden on healthcare expenditures. Venous thromboembolism (VTE) (deep vein thrombosis/DVT, and pulmonary embolism) are the third leading cause of death in the United States. Protein C deficiency (hereditary and acquired) is a condition that can result in patient mortality.

The endogenous anticoagulants and their cofactors play an important role in normal blood hemostasis and tissue health via oxygenation and metabolic waste product removal. Protein C deficiency is a hidden disease that affects 1 in 400 Americans. Approximately 1 in 16,000 are symptomatic, but all are prone to developing DVT

and lung emboli. Zymogen Protein C has a host of beneficial in-vivo characteristics. When activated it not only acts as a natural anti-coagulant but has also been found to have antithrombotic, anti-inflammatory and anti-apoptotic properties. Most importantly APC inhibits red cell coagulation and platelet-dependent thrombosis formation [13].

References giving detailed information on Protein C, it's biochemistry and production are Bertina, 1988; and Bruley and Drohan, 1990 [1, 6]. Earlier work on anticoagulants [2] pre-dated Protein C however the many attributes of ZPC [4] started a focused research effort on the low- cost production of this pivotal trace blood protein.

An important previous clinical research effort, the peri-operative use of ZPC for the left hip replacement of the same patient [5, 7, 26] was a very successful procedure. That work and the present case should lead the way to many safer surgeries in the future.

13.2 Emergency Procedure

The patient, a 75–year-old male with a medical history notable for protein C deficiency (protein C activity level of approximately 50%), had experienced DVT and bilateral pulmonary embolism at age 65. Subsequently, he was noted to be heterozygous for the prothrombin 20210 gene mutation by DNA analysis, had been prescribed life-long warfarin therapy targeted to an international normalized ratio (INR) range 2 to 3, and has not suffered any additional episodes of VTE.

The protein C deficiency was diagnosed in the summer of 1981 during testing suggested by Dr. Rene Bourgain (ISOTT member) [3] via laboratory assays conducted by Dr. Richard Marlar [18, 19], after the patients middle son sustained an idiopathic pulmonary embolism. At the same time, other family members were diagnosed with protein C deficiency: father, brother and two of three sons at approximate protein C activity levels of 48%, 38%, 48%, and 50%, respectively.

On Thursday, September 4, 2008 the patient underwent a routine colonoscopy. During the procedure a large 3 cm diameter cecal polyp was identified and resected. The wound was double cauterized and on the next day warfarin therapy was started using a Low Molecular Weight Heparin (LMWH) bridge to prevent clotting during the recovery to therapeutic INR levels of 2 to 3. Eight days after the polyectomy the patient reached INR of 2.0 and was approved to start exercise (Thursday, September 11, 2008). About one hour after abdominal exercise a strong urge to evacuate created an excessive rectal bleed from the wound site. The patient stopped LMWH shots and warfarin treatment simultaneously to attempt slowing the bleed. He was admitted to the Emergency Room that evening at the Johns Hopkins Hospital, Baltimore, MD and was managed while waiting for the bleed to stop. Sunday, September 14, 2008, vitamin K was administered and later that evening a Go-Lytely prep was taken by the patient to prepare for an emergency colonoscopy, scheduled for early Monday morning, September 15, 2008. Since there was a 10/20 fold increased risk of VTE the patient was bridged during the procedure with ZPC to prevent clotting without

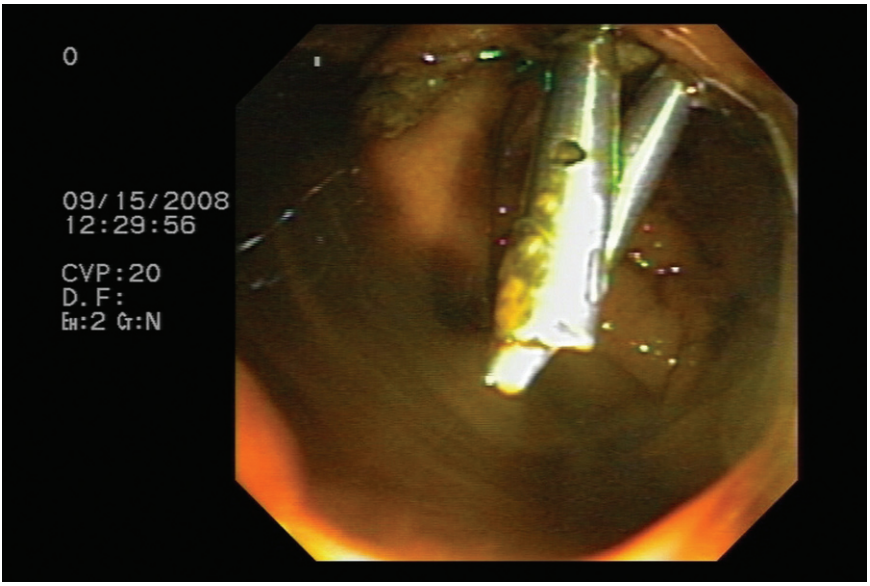

Fig. 13.1 Post polypectomy bleeding controlled with placement of endoclips.

increased bleeding. The initial doses were calculated at 60 units per kilogram twice a day. When Protein C activity peaks reached 205% of normal, the dose was reduced to prevent increasing the supraphysiologic levels of Protein C and using excessive ZPC.

During the endoscopy, the bleeding site was treated with an injection of l:10,000 dilution of epinephrine, followed by cauterization and placement of four metal staples (see Figure 1). Three days after endoscopic repair, while on ZPC protection, warfarin (7 mg/day) was restarted with a LMWH bridge (70 mg two times/day) to prevent thrombosis. Sixteen months post-bleed, the patient remains on life-long warfarin without further episodes of bleeding or thrombosis.

13.3 Discussion/Results

When natural and man-made anticoagulants are used in medical procedures, it is necessary to consider at least three things, first, the safety of the patient, second, the efficacy of the drug used and third, the additional cost involved. For the case of Protein C deficient patients it is very wise to consider the use of ZPC because it is effective against thrombosis and does not cause significant bleeding (if any) because of its activation process. As with the patient cited in this paper, the Protein C activity was assayed at 205% of normal levels without complications. Since this procedure was completed successfully it suggests the ZPC bridging be considered for PC deficient patients whenever invasive medical procedures are required. This

might also be true for patients with normal PC levels for highly invasive orthopedic surgery, brain surgery, etc.

An important unknown is what is the optimal ZPC level sufficient for different medical problems? Therefore, more data must be generated for a spectrum of potential uses. The use of ZPC products beg for refinement of peri-procedural ZPC levels, development of economic production technologies and patient compliant administration technologies.

Because of the unknown pharmacokinetics of the blood factors [27] while returning to accepted INR level, bridging with LMWH is necessary for at least protein C deficient patients. Therefore, there is a need for further studies regarding LMWH dosing levels to enhance patient safety. Presently, there is little or no data regarding dose level interaction during the return period to accepted INR levels while using a combination therapy of warfarin and LMWH. It is possible that using full-dose warfarin along with full-dose LMWH until therapeutic values of INR are reached, could contribute to unnecessary bleeds and possible patient death.

13.4 Product Cost/Production

For wider use of ZPC and APC it is necessary to explore technologies to produce less expensive products. At the present time, Eli Lilly is producing activated Protein C (Xigris) employing human cell culture and Baxter International is using Immunoaffinity Chromatography to recover ZPC (Ceprotin) from human blood plasma. Both processes are extremely expensive which limits the use of Protein C products to only very special medical emergencies. Past work [28, 29] is being continued to research the application of immobilized metal affinity chromatography, IMAC [20, 21] to replace Immuno-affinity Chromatography, using the best combination of absorption and elution buffers for optimal bio-downstream processing to achieve low cost production of Protein C products. The use of transgenic animals might also be a viable alternative [10] for high volume, lower cost bio-processing. With low cost products and the development of novel administration techniques it might be possible to use prophylactic ZPC to treat Protein C deficiency.

13.5 Conclusion

Based on the success of this case, Zymogen Protein C concentrate (Ceprotin, Baxter, Deerfield, IL) should be strongly considered for invasive peri-procedural management of any patient with protein C deficiency and previous thromboembolism.

Clinical medicine should be aware of other medical procedures that would be safer using ZPC. In all cases, dosing studies should be done to ensure optimal safety, efficacy and cost.

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