We report a case of a 6-year-old girl with severe protein S deficiency due to a homozygous mutation and recurrent episodes of skin necrosis. She developed purpura fulminans at birth and a catheter-related venous thrombosis complicated by massive pulmonary embolism at the sixth day of life. Long-term oral anticoagulant therapy with a vitamin K-antagonist was started with a therapeutic range of the international normalized ratio of prothrombin time between 2.0 and 3.0. Unfortunately, this common range was not sufficient because recurrent episodes of warfarin-induced skin necrosis developed if the international normalized ratio was <4.0. Vitamin K antagonists decrease plasma level of vitamin K–dependent coagulation proteins, including the natural anticoagulant protein C. In our patient, the hypercoagulable state due to warfarin-induced reduction of protein C, other than severe protein S deficiency, outweighed the anticoagulant efficacy of the inhibition of procoagulant factors II, VII, IX, and X. The switch of anticoagulant therapy from warfarin to rivaroxaban, a direct inhibitor of activated factor X that does not inhibit other vitamin K–dependent proteins, resulted in the disappearance of skin necrosis at 1 year of follow-up. Rivaroxaban may be considered as a valid anticoagulant alternative in patients with severe inherited protein S deficiency and warfarin-induced skin necrosis. 

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**Key Words** protein S, rivaroxaban, anticoagulant, coagulation

Dr Martinelli followed the patient and drafted the initial manuscript; Drs Bucciarelli, Artoni, Fossali, Passamonti, and Gianniello contributed to clinical decisions and critically reviewed the manuscript; Dr Tripodi performed and supervised functional laboratory tests and critically reviewed the manuscript; Dr Peyvandi supervised genetic tests and critically reviewed the manuscript; and all authors approved the final manuscript as submitted.

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Neonatal purpura fulminans is a rare, life-threatening condition caused by severe congenital deficiencies of the natural anticoagulant protein C or S. The clinical presentation is that of skin necrosis and disseminated intravascular coagulation, which can progress rapidly to multiorgan failure caused by thrombotic occlusion of small- and medium-size blood vessels. Its early recognition and prompt treatment with fresh-frozen plasma, anticoagulants such as heparins or vitamin K antagonists, and, in the case of protein C deficiency, concentrate replacement therapy are essential to reduce mortality and prevent major sequelae. After resolution of the acute phase, long-term treatment with vitamin K antagonists is warranted. However, vitamin K antagonists may induce skin necrosis in patients with congenital protein C or S deficiency by further decreasing their plasma concentration. Alternative anticoagulant drugs are therefore claimed for these patients.

CASE REPORT

In January 2006, a preterm female infant weighing 1060 g was born by vaginal delivery at the 31 weeks’ gestation to a 28-year-old primigravid woman with placental abruption. Apgar scores were 6 at 1 minute, 7 at 5 minutes, and 8 at 10 minutes. In the second day of life, she developed skin necrosis of the right-hand fingers and concomitant disseminated intravascular coagulation. Fresh-frozen plasma and platelet units were administered through an indwelling catheter placed in the right innominate vein. On the sixth day of life, she developed a catheter-related vein thrombosis complicated by massive bilateral pulmonary embolism that required intubation and mechanical ventilation. Laboratory tests showed levels of protein S activity (anticoagulant) and free antigen <5%. Both parents, unrelated and asymptomatic for venous thrombosis, had low protein S activity levels (24% the mother and 43% the father). The infant was started on anticoagulant therapy with intravenous heparin and warfarin; the former was stopped after 1 week when the international normalized ratio of prothrombin time was >2.0. Lung computed tomography scanning at hospital discharge showed complete resolution of venous thromboembolism. This first episode of skin necrosis resulted in the loss of part of 3 distal phalanxes of the right hand. At age 14 months, despite an international normalized ratio of 2.5, she developed skin necrosis at the left tibia that was treated with fresh-frozen plasma and low molecular weight heparin. Since then, recurrent episodes of skin necrosis occurred when the international normalized ratio decreased to <4.0. She had no other illnesses or symptoms. In 2011, she had 5 recurrences of skin necrosis, and on April 2012 (at age 6 years), alternative oral anticoagulation with the direct inhibitor of activated factor X rivaroxaban was given after informed consent signed by the parents and approval by the institutional ethics committee of the hospital were obtained. For her body weight of 21 kg, a dose of 5 mg once daily was started the day after warfarin withdrawal, but within few days, she developed multiple areas of skin necrosis on the legs (Fig 1) and disseminated intravascular coagulation. The daily dose of rivaroxaban was therefore gradually increased over a period of 8 weeks to 10 mg, 20 mg, 30 mg, and 40 mg, with only 40 mg being efficacious in preventing skin necrosis at 9 months of follow-up.

METHODS

Thrombophilia screening was performed in the patient and her parents as described previously. Genomic DNA of the patient and her parents was extracted from leukocytes and the 15 exons, including the exon-intron boundary regions, of the protein S gene (PROS1) were sequenced by Sanger method on an ABI 3130 genetic analyzer (PE Applied Biosystem, Foster City, CA). Pharmacokinetics of plasma concentration of rivaroxaban was performed by high-pressure liquid chromatography (Bayer HealthCare, Wuppertal, Germany). Inhibition of activated factor X was measured by an anti-factor Xa assay calibrated with rivaroxaban-calibration samples (Stago, Asnieres, France). Thrombin generation was measured as endogenous thrombin potential by triggering coagulation in platelet-poor plasma with small amounts of tissue factor (1 pM) and phospholipids (1.0 μM) with the addition of thrombomodulin (4 nM) to optimize the in vitro activation of the protein C/protein S system.

RESULTS

Table 1 shows the results of coagulation tests and measurement of the naturally occurring anticoagulant proteins in the patient and her parents. The rest of thrombophilia screening (factor V Leiden, prothrombin G20210A mutations, antiphospholipid antibodies, fibrinogen, factors VIII and IX, and homocysteine) was normal. DNA analysis of the patient revealed homozygosity for a missense mutation in the
PROS1 gene, c.200A>C p.Glu67Ala (previously reported as Glu26Ala)\(^6\) (Fig 2A), that was heterozygous in her parents.

The pharmacokinetic profile showed a rapid absorption of the drug and a mean half-life of 3.5 hours (Fig 2B). Anti-factor Xa activity was correlated with plasma rivaroxaban concentration (\(r = 0.96, P < .001\); Fig 2C), and both showed an inverse correlation with endogenous thrombin potential (\(r = -0.95\) and \(r = -0.88\), respectively, \(P < .001\) for both; Fig 2D), indicating the lowest amount of thrombin generation when the highest anti-factor Xa activity of rivaroxaban concentration was achieved.

**DISCUSSION**

Protein S is a vitamin K–dependent plasma protein that plays an important role in the regulation of blood coagulation. It acts as a nonenzymatic cofactor of activated protein C, which selectively inhibits the procoagulant factors V and VIII in their activated form, thereby downregulating the coagulation cascade.\(^7\) Inherited protein S deficiency is an autosomal dominant disorder associated with an increased risk of venous thromboembolism.\(^8\) The estimated prevalence of heterozygous protein S deficiency, which is associated with plasma levels approximately half of normal, varies from 1 in 1000 to 3 in 10 000 individuals in the general population.\(^9\) Extremely low plasma levels determined by homozygous or compound heterozygous mutations in the protein S gene are rare and associated with the onset of purpura fulminans in the neonatal period.\(^1\) Symptomatic newborns need long-term anticoagulation, and to date the recommended treatment is a vitamin K antagonist. However, despite maintaining an international normalized ratio of the prothrombin time range within the therapeutic range for the treatment venous thromboembolism, or even much higher in our child, patients with severe deficiency of protein S or protein C may develop warfarin-induced skin necrosis. An alternative drug not acting on vitamin K–dependent proteins is therefore warranted for these patients.

The direct oral anticoagulants targeting activated factor II or factor X have at least the same efficacy and safety as vitamin K antagonists in adults,\(^10\) but no data in children are available. Dabigatran etexilate, a direct antithrombin (factor II) inhibitor, has been successfully used for preventing warfarin-induced skin necrosis in a 21-year-old patient with severe protein C deficiency.\(^11\) Because thrombin activates protein C when it binds thrombomodulin,\(^12\) we preferred to preserve this natural anticoagulant mechanism and administered the direct inhibitor of activated factor X rivaroxaban, which also increases thrombomodulin plasma levels, to our patient.\(^13\)

Why does this child require such a high daily dose of rivaroxaban to be free from skin necrosis? The pharmacokinetic profile performed with a daily dose of 30 mg showed a rapid metabolism of the drug, particularly during the day, with a peak of activity reached 1 to 2 hours after oral intake and a mean half-life of 3.5 hours. These figures are approximately half those seen in adults.\(^14\) Because skin necrosis always occurred within a couple of hours from drug intake, we decided to keep the patient at trough plasma concentrations of rivaroxaban for as short a time as possible by giving this drug at a dosage of 10 mg every 6 hours. Although 4 daily administrations are demanding for the child and parents, they are compliant, and at 1 year of follow-up it remained efficacious and safe because she had no recurrent skin necrosis or side effects including bleeding episodes. At present there is insufficient experience on the use of rivaroxaban in children and on the target anti-Xa levels that should be achieved, but it is known that for some drugs, such as warfarin, children require higher doses than adults.\(^15\) We believe that the explanation lies in part in the massive hypercoagulable state caused by homozygous protein S deficiency, which requires strong anticoagulation. The magnitude of the hypercoagulable state of our patient is unknown because she has always been on anticoagulant therapies. However, at trough and peak concentration of rivaroxaban, the endogenous thrombin potential, a marker of thrombin formation, was higher when she was taking 20 mg (1423 nM/min and 989 nM/min) than 40 mg per day (1126 nM/min and 779 nM/min). Similar results were obtained for plasma levels of D-dimer, a marker of thrombin activation.

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**TABLE 1 Global Coagulation Tests and Plasma Levels of the Naturally Occurring Anticoagulant Proteins in the Patient and Her Parents**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Values</th>
<th>Patient</th>
<th>Patient’s Mother</th>
<th>Patient’s Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time (ratio)</td>
<td>0.89–1.18</td>
<td>1.19</td>
<td>0.96</td>
<td>1.12</td>
</tr>
<tr>
<td>Activated partial thromboplastin time (ratio)</td>
<td>0.85–1.18</td>
<td>1.13</td>
<td>0.98</td>
<td>1.15</td>
</tr>
<tr>
<td>Antithrombin activity (%)</td>
<td>&gt;82</td>
<td>109</td>
<td>113</td>
<td>125</td>
</tr>
<tr>
<td>Protein C activity, chromogenic method (%)(^a)</td>
<td>&gt;67</td>
<td>74</td>
<td>89</td>
<td>134</td>
</tr>
<tr>
<td>Protein S activity, anticoagulant method (%)(^b)</td>
<td>&gt;60</td>
<td>4</td>
<td>24</td>
<td>43</td>
</tr>
<tr>
<td>Free protein S antigen (%)(^c)</td>
<td>&gt;60</td>
<td>3</td>
<td>43</td>
<td>60</td>
</tr>
</tbody>
</table>

\(^a\) Chromogenic Protein C, Instrumentation Laboratories, Bedford, Massachusetts.

\(^b\) Hemosil ProS, Instrumentation Laboratory, Orangeburg, New York.

\(^c\) Dako A584 and P419, Glostrup, Denmark.

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confirming the need of high doses of rivaroxaban to counterbalance our patient’s hypercoagulable state.

This is the first description of the use of rivaroxaban, a direct inhibitor of activated factor X, in a child with skin necrosis induced by severe protein S deficiency or warfarin treatment. We believe that rivaroxaban may be considered as a valid anticoagulant alternative to vitamin K antagonists in patients with severe inherited protein S deficiency because it does not reduce the anticoagulant function of activated protein C. At present, efficacy and safety of rivaroxaban are monitored monthly clinically and with laboratory tests in our patient. There is no specific antidote for rivaroxaban. In case of life-threatening bleeding, treatment with prothrombin complex concentrates or recombinant activated factor VII are the most plausible options.16

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