To the editor:

A modified Folts model or the original Folts model to evaluate new antithrombotics?

I read with interest and some misgivings the report by Wu et al. They used a modified Folts model in baboons receiving either a murine antihuman von Willebrand factor (VWF) monoclonal antibody (mAb) 82D6A3 (600µg/kg, n = 2, or 300µg/kg, n = 3) or a placebo (normal saline, n = 2). Animals receiving 82D6A3 had a 100% decrease in the cyclic flow reductions (CFRs). The study suggests that a blockade of VWF-collagen interaction by 82D6A3 reduced platelet thrombus formation in the injured and stenosed baboon femoral arteries. They state that “the VWF-collagen interaction...might be a new target for preventing arterial thrombosis.”

The methods and results presented can be accepted without reservations; however, I would like to make the following comments. First, Wu et al. presented the original Folts model. In 1974, John D. Folts developed a canine model of coronary stenosis. A Medline search was performed, and the first report in the literature was in 1976, not in 1991. Wu et al. state that the experimental procedure followed was that of the original Folts model except that the artery was injured with a spring-loaded forceps. It is true that Folts produced a stenosis in the artery by an externally applied plastic cylinder. However, this was not the only difference between the original Folts model and the “modified Folts model” presented by Wu et al. Folts described CFRs in coronary arteries of dogs. Coronary blood flow showed cyclical reductions to near zero, with a sudden spontaneous return to near control levels. In contrast, Wu et al. studied baboon femoral arteries, and when flow was reduced by at least 50%, blood flow was restored by pushing the spring of the clamp to physically dislodge the thrombus. However, the Folts model has been evaluated in several animal species and vascular territories, and mechanically dislodging the thrombus to restore blood flow has been successfully accomplished by other groups.

Regarding potential bias, blood pressure monitoring and arterial blood gases could not be obtained. Information concerning the vaporizer used was lacking (was it periodically calibrated?) and unfortunately, it was not a blind study. Moreover, Bertha et al. studied halothane in Folts model and reported in 8 of 8 mongrel dogs that CFRs were abolished at a low concentration of inspired halothane (halothane 0.5%, n = 5; halothane 0.25%, n = 3).

Finally, as an anesthesiologist, I must remember that lungs are ventilated, not animals (nor are they intubated; their tracheas are). Similarly, the correct term is “cuffed tracheal tube,” not “cuffed endotracheal tube.”

Maurizio Fattorutto
Correspondence: Maurizio Fattorutto, Department of Anesthesiology, Hospital Erasme, Route de Lennik 808 Brussels 1070, Belgium; e-mail: m.fattorutto@swing.be

Response:

A modified Folts model

We would like to express our appreciation to Dr. Fattorutto for going through our paper so thoroughly, and we are happy that his remarks do not really question the validity or the interpretation of our findings. As far as we see, he seems to be concerned mainly with the fact that we have used a model which is quite different from the original Folts model, perhaps too different to still call it a “modified Folts model.” It is correct that we did not refer to the first paper on this model published in 1976, but rather to a 1991 review paper by Folts, where he gives a summary of 15 years of experience with the model, bringing together the improvements on the model and summarizing the data obtained. We are convinced this provides more information to the interested reader and, at the same time, gives a means to readily access the original references.

On the other hand, our model indeed differs considerably from the original model developed by Folts, and even in more aspects then Dr. Fattorutto puts forward: the animal species used was different, as was the artery, the anesthesia, systemic and coronary blood pressures, and ECG measurements; we damaged the vessel, and Folts, in his original paper, did not; we applied a stenosis not with a cylinder but with a clamp; we dislodged the thrombus mechanically... However, as correctly stated by Dr. Fattorutto, similar and other adaptations have been described by other groups, but it seems that whenever cyclic flow reductions (CFRs) are measured in a stenosed artery, this is considered a modification of the original Folts model. Even the injured femoral artery itself has been used already in pigs, a model that itself was simply referred to as “the Folts model on femoral arteries.”
Our sole intention was to test whether inhibition of binding of von Willebrand factor (VWF) to collagen was antithrombotic, for which we needed a relatively easy (hence the femoral instead of the coronary artery) high-shear thrombosis model, in a species whose VWF did crossreact with the antibody. We wanted to give credit to Folts, and we definitely had no intention to propose our modified model as a new or better model.

Finally, the study would have been better indeed if blinded; however, the measurements of blood flow reductions are quite straightforward and do not leave that much room for interpretation. And as for the other remarks are concerned, we believe that we used the proper control animals to overcome potential artifacts; in addition, the animals served as their own controls, as we first determined baseline CFRs before administration of compounds.

The study nevertheless could have been even better; however, perfection is a privilege not bestowed upon man.

Hans Deckmyn, Karen Vanhoorelbeke, and Harry Kotzé

Correspondence: Hans Deckmyn, Laboratory for Thrombosis Research, Interdisciplinary Research Centre (IRC), KU Leuven Campus Kortrijk, E. Sabbelaan 53, B-8500 Kortrijk, Belgium; e-mail: hans.deckmyn@kuleuven.be

References

To the editor:

Monoclonal myelopoiesis and subnormal erythropoietin concentration are independent risk factors for thromboembolic complications in essential thrombocythemia

Essential thrombocythemia (ET), one of the chronic myeloproliferative disorders, was recently shown to consist of a heterogeneous cohort of patients. Indeed, when clonality analyses were performed using X-chromosome inactivation patterns (XCIP) in samples of ET patients, about 50% showed constitutive skewing, about 25% had monoclonal myelopoiesis, and 25% had polyclonal myelopoiesis. Further, about 50% of newly diagnosed ET patients presented with subnormal plasma erythropoietin (EPO) concentrations. ET patients with monoclonal myelopoiesis were, compared with those with polyclonal disease, shown to have a significantly higher risk for developing thrombotic complications by Harrison et al; this finding was confirmed by Chiusolo et al. Also, ET patients with subnormal EPO concentration at the time of diagnosis were shown to have a significantly higher requirement for myelosuppressive treatment. Further, in the group of ET patients with subnormal EPO, 10 out of 13 developed cerebrovascular or other macrovascular events compared with 7 of 18 patients with normal plasma EPO concentration at diagnosis. The difference between the groups was statistically significant (P < .02). The aim of the present study was to investigate whether the group of ET patients with monoclonal myelopoiesis was identical to the group of ET patients with subnormal EPO concentration at diagnosis, or whether these 2 variables are independent risk factors for the development of thromboembolic complications in ET.

Female patients with ET, all fulfilling the Polycythemia Vera Study Group (PVSG) criteria, were recruited from the Hematology Outpatients Clinic at Sahlgrenska University Hospital, Göteborg, Sweden, and from the University College London Medical School, United Kingdom. XCIP analyses on neutrophils and T cells were performed using the human androgen receptor (HUMARA) assay as described by Gale et al. At the time of sampling, some patients had a platelet count of less than 600 × 10^9/L, but all patients had platelet counts greater than this limit at the time of diagnosis. None of the patients had been treated with myelosuppressive agents. All patients were on low-dose aspirin prophylaxis.

Eight English patients were selected among earlier XCIP-tested patients who had frozen serum stored and venous blood counts taken. Twenty-two Swedish patients were investigated, using the HUMARA assay, at the Department of Clinical Genetics at Lund University Hospital Lund, Sweden; of these, 9 were not interpretable due to skewing, 2 were excluded due to anemia (ie, hemoglobin concentration < 120 g/L, which possibly could affect the EPO concentration), and 11 patients were found to be evaluable. All serum or plasma EPO concentrations were analyzed at the Clinical Chemistry Laboratory at Sahlgrenska University Hospital, using an immunoenzymometric assay (Quantikine IVD Human Erythropoietin DEP 00; R&D Systems, Minneapolis, MN). The reference range for healthy individuals, given by the manufacturer, is 3.1-14.9 IU/L.

No significant differences between means (Student t test) for the groups of monoclonal and polyclonal ET patients were found when hemoglobin concentration, EPO, concentration, or disease duration were compared. Two of the 7 ET patients with monoclonal myelopoiesis had a subnormal EPO, compared with 2 of the 12 ET patients with polyclonal myelopoiesis; this difference did not reach statistical significance (Fisher exact test). However, the mean platelet count was significantly higher in the monoclonal group of patients compared with the mean for the ET patients with polyclonal myelopoiesis (P = .02; Table 1).

The present results consequently do not support the hypothesis that the group of ET patients with monoclonal myelopoiesis is identical to the group of ET patients in whom the EPO concentrations were subnormal at diagnosis. Also, not all ET patients exhibiting monoclonal myelopoiesis present with subnormal EPO levels at diagnosis. The XCIP analyses were done with DNA,

<table>
<thead>
<tr>
<th>Hemoglobin level, g/L (range)</th>
<th>Platelet count, × 10^9/L (range)</th>
<th>EPO concentration, IU/L (range)</th>
<th>Disease duration, y (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal ET, n = 7</td>
<td>137 ± 15 (120-160)</td>
<td>888 ± 317 (621-1458)</td>
<td>6.5 ± 4.8 (0.8-14.1)</td>
</tr>
<tr>
<td>Polyclonal ET, n = 12</td>
<td>137 ± 12 (120-152)</td>
<td>629 ± 122 (424-772)</td>
<td>6.0 ± 4.6 (0.1-16.8)</td>
</tr>
<tr>
<td>P, Student t test</td>
<td>.94</td>
<td>.02</td>
<td>.8</td>
</tr>
</tbody>
</table>

Table 1. Results for hemoglobin, platelet, and erythropoietin concentrations in ET patients with monoclonal and polyclonal myelopoiesis.