Reply to Dr. Nowak's Letter

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In reference to the expected circulating blood levels of recombi-nant hirudin for certain clinical indications [1, table 3], we do not totally agree with Dr. Nowak's statement [2] that these levels do not reflect the actual concentrations in experimental and clinical investigations. It is, however, true that the listed concentrations are based on projections and do not represent data from experimental or clinical results. As indicated in the tables, we have approximated these levels on the basis of experimental data obtained for hirudin in various valid animal models of thrombosis and hemostatic processes [3-5]. The information obtained was compared to that of heparin and then extrapolated to the various clinical situations. The levels represented in these tables were not meant to be absolute concentrations.

In contrast to heparin, recombi-nant hirudin is a short-lived, single-target (thrombin) drug. These characteristics are reflected in the wide range of dosages found to produce antithrombotic actions in different models of thrombosis [6-9]. Several recent studies have reported ranges of 5 to > 100 µg/kg for ED₅₀ antithrombotic actions. These values nearly represent our earlier projections. In a recent review, Gray et al. [7] also showed that varying levels of recombinant hirudin are needed to prevent different experimentally induced thromboses and that the stasis time was one important factor in determining a dose.

For the prophylaxis of deep vein thrombosis, different dosages of hirudin are needed for different clinical indications. As shown by Kaiser et al. [10], in comparison to heparin, recombinant hirudin is a relatively poor inhibitor of thrombin generation. Because of this relatively inefficient thrombin generation inhibition, higher amounts of hirudin are indicated to achieve similar pharmacologic effects as heparin. Dr. Nowak suggests that the literature pertinent to this discussion shows that relatively lower levels of hirudin are required; however, specific references were not quoted by him.

Regarding therapeutic antico-agation, our projected levels of hirudin (5-10 µg/ml) were perceived as too high by Dr. Nowak. We do not believe that 20-50 times lower levels as stated by Dr. Nowak (0.1-0.5 µg/ml) would be sufficient for this purpose. If a 2-2.5 time prolongation of activated partial thromboplastin time is considered

as the level of therapeutic anticoagulation [11, 12], then at least 2-3 $\mu\text{g/ml}$ hirudin is needed. By the same rationale our ex vivo whole-blood clotting studies revealed that at least 5-10 $\mu\text{g/ml}$ of recombinant hirudin would be required for therapeutic anticoagulation. Therefore, we projected a dose of 5-10 $\mu\text{g/ml}$ for this indication. We feel that the dosages referred to by Dr. Nowak are subtherapeutic and may not be optimal for therapeutic use in clinical settings.

We do not feel that our projected levels of hirudin for hemodialysis in the range of 3-8 $\mu\text{g/ml}$ are any different than what Dr. Nowak (5 $\mu\text{g/ml}$) has suggested.

In reference to major surgery and cardiovascular bypass surgery, we have projected a broad range of hirudin levels (10-30 $\mu\text{g/ml}$). We fully agree with Dr. Nowak that at this concentration blood is incapable of clotting. This is the aim of such a dosage. In cardiopulmonary bypass surgical procedures, concentrations of heparin in the neighborhood of 3-5 IU/ml are reached. Such a degree of anticoagulation is only achievable by > 15 $\mu\text{g/ml}$ of hirudin. This was clearly demonstrated by us in a canine model of bypass surgery where blood clotting was obvious at circulating

levels of hirudin below 10 $\mu\text{g/ml}$ [5,13].

Moreover, in contrast to heparin there were no major bleeding complications noted in the dogs treated with these high dosages of hirudin. Dr. Nowak refers to these dosages used as 'absolutely nonsense' and to be 'associated (with) fatal hemorrhage'. In our studies, none of over 100 dogs operated experienced bleeding problems. This positive outcome can also be underscored by the organ autopsies performed on these animals where no microscopic or macroscopic hemorrhages were detected. Therefore, we stand by our statement that for the strong thrombogenicity of an extracorporeal circuit, the dogs required dosages between 10 and 30 $\mu\text{g/ml}$ which clearly did not lead to fatal bleeding episodes.

The reference to experimentally implanted artificial hearts in calves is not relevant to the discussion on major surgical procedures. The cardiopulmonary bypass circuit is much more complex than the artificial heart and is thus not comparable to the model suggested by Dr. Nowak where he recommends that 0.2-0.5 $\mu\text{g/ml}$ recombinant hirudin would provide adequate anticoagulation.

The blood loss reported in our manuscript depicts the data obtained in an established rabbit model of ear blood loss [6]. On a gravimetric basis recombinant hirudin exhibited much lesser hemorrhagic effects than heparin. We agree with Dr. Nowak's statement that blood levels > 50 $\mu\text{g/ml}$ may cause hemorrhagic effects. This is consistent with the animal model data where significantly higher bleeding was noted only at dosages > 2.5 mg/kg. We do not understand any difference of opinion in this regard.

Under no circumstances do we claim that these data are directly relevant to clinical settings. For the intended clinical usages well-defined statistically valid clinical trials are mandatory. It must be

stressed that animal experimental data may or may not have any bearing on the clinical outcome. Thus, the only way to determine proper dosages is to carry out well-defined dose response studies in clinical settings.

We have listed the detection limits of various methods studied in our laboratory for recombinant hirudin after extensive experimental trials. We do not agree with Dr. Nowak that the sensitivity should be 50 times higher. In our hands, this is not true. Moreover, the sensitivity limits of each test are independent of one another, and different instrumentation will effect the detection limits of each assay. At the present time, a dedicated method for hirudin is not available. The methods

described are those developed for heparin. Thus, the overall validity of these assays for use with hirudin is questionable.

We hope that our clarifications are adequate for resolving these uncalled-for controversies.

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