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New TPO treatment schedules of increased safety and efficacy: pre-clinical validation of a thrombopoiesis simulation model

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Summary. Thrombopoietin (TPO) immunogenicity hampers its development as a therapeutic agent for attenuating thrombocytopenia and improving platelet harvest in donors. This work was aimed at validating, in mouse and in monkey experiments, a thrombopoiesis computer-model prediction that platelet counts, similar to those obtained with accepted TPO dose scheduling, can also be achieved by new and safer schedules of significantly reduced doses. To this end we compared, in a two-arm mouse experiment, platelet increases obtained with a single intraperitoneal dosing of recombinant mouse TPO (17·5 μg/kg), with those obtained by the model-suggested protocol of a significantly reduced dose (2 μg/kg on 4 consecutive days). The two TPO regimens generated similar platelet profiles, peaking at *ca.* 2700×10^9/l platelets. In rhesus monkeys, treated by rhesus monkey recombinant TPO (5 μg/kg on 4 consecutive days), the suggested protocol yielded effective platelet stimulation with significantly reduced immunogenicity. The model's ability to predict individual monkey responses to several new TPO administration protocols was further validated, proving sufficient robustness in providing good predictions with limited input data. The simulation tool could be used for testing the effects of different therapeutic agents on thrombopoiesis. Human trials are warranted for testing the suggested improved TPO protocol, possibly in conjunction with chemotherapy.

Keywords: thrombopoiesis, immunogenicity, thrombopoietin, modelling, treatment schedule.

Thrombocytopenia poses a major clinical problem in the management of haematology and oncology patients and contributes significantly to morbidity and mortality in cancer patients (Demetri, 2001). In addition, it limits cancer therapy by preventing the administration of drugs at optimal doses and schedules (Demetri, 2001; Elting *et al*, 2001). Repeated transfusions may aid in preventing bleeding, but increase the risk of pathogen transmission and transfusion reactions, as well as health care costs and inconvenience to patients. For these reasons, an agent that stimulates platelet production is greatly significant in preventing or attenuating thrombocytopenia, as well as in improving the efficacy of platelet harvesting in donors (Haznedaroglu *et al*, 2002).

Several recombinant or synthetic thrombopoiesis-stimulating agents currently exist. These agents are derived from the naturally occurring cytokine with thrombopoietic activity, thrombopoietin (TPO) (Vadhan-Raj, 1998; Harker, 1999). However, an efficient method for defining drug schedules, which maximizes these agents' therapeutic benefits and minimizes adverse complications, is yet to be established (Neelis *et al*, 1998).

Recently, a detailed mathematical model of thrombopoiesis has been put forward (Skomorovski & Agur, 2001). The model breaks down thrombopoiesis into 11 developmental compartments from stem cells to peripheral blood platelets. It takes into account the intrinsic cellular dynamics in each compartment, as well as the detailed kinetic effects of endogenous and exogenous TPO. The model is integrated in a computer tool to simulate momentary *in vivo* effects of drug administration schedules on the peripheral platelet levels and on cell counts in each of the different bone marrow compartments. Using published data (Vadhan-Raj *et al*, 1997; Miyazaki *et al*, 1999), the model's ability to retrieve post-TPO-treatment platelet profiles in patients has been retrospectively validated (Skomorovski & Agur, 2001).

The use of recombinant human TPO as a therapeutic agent has been stalled by safety and efficacy complications

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caused by the immunogenicity of a recombinant variant, megakaryocyte growth and development factor (MGDF) (Basser, 2002). Therefore, the present work was aimed, first and foremost, at preclinically validating the model-suggested new TPO regimen of improved efficacy and reduced immunogenicity. In addition we wished to validate, under various levels of prior empirical information, the thrombopoiesis model's ability to accurately predict an individual's response to different drug schedules.

MATERIALS AND METHODS

Mouse experiments

Animals. BALB/c male mice, approximately 12 weeks of age and weighing about 25 g at the time of the study initiation, were acclimatized for at least 5 d before the study initiation. Eight groups of five mice each were used. All test animals were kept under environmentally controlled housing conditions throughout the entire study period.

Test agents. Recombinant mouse TPO (rmTPO) was kindly supplied by Genentech Inc., South San Francisco, CA, USA. Full-length TPO was produced by Chinese hamster ovary (CHO) cells, dissolved in phosphate-buffered saline (PBS)/0·01% Tween 20 (denoted vehicle) at a concentration of 0·375 mg/ml and stored in 1 ml vials at 4°C. The purity was >99%. Before *in vivo* administration, the drug was diluted in PBS/0·01% Tween 20 to the appropriate concentration. PBS/0·01% Tween 20 was used as placebo. Both the test material (TPO) and the vehicle were administered by intraperitoneal injection. All treatments were administered at the same hour, and the volume of single intraperitoneal injections was 20 ml/kg.

Blood sampling. Blood samples were collected at a similar daily hour by puncture of the retro-orbital plexus using a micro-haematocrit capillary tube. Blood was transferred to tubes with ethylene diamine tetra-acetic acid (EDTA), and delivered at room temperature (RT) to the laboratory. Complete blood cell counts were measured using a Sysmex F-800 haematology analyzer (Toa Medical Electronics Co., Ltd, Kobe, Japan). The volume of individual blood samples collected from test animals did not exceed 150 μl per sample.

Serial observations. Observations for any adverse effects of the treatment were carried out at least once daily throughout the entire study period.

Study design. Mice were randomly assigned to two arms. Mice in arm A received a single bolus intraperitoneal injection of 17·5 μg/kg rmTPO. Mice in arm B received a total dose of 8 μg/kg, divided over four daily intraperitoneal injections of 2 μg/kg rmTPO. Animals in control group received vehicle only or no treatment at all. To enable blood sampling on days 5, 6 and 7 (days of suggested platelet peak), and on days 10 and 11 (days of suggested return to baseline), each arm was subdivided into three groups, which were sampled for subsequent haematological analysis as follows: one group from each arm was sampled on days 5 and 10, another on days 6 and 11, and the third on day 7. The placebo group was sampled on days 5 and 11. The group of no treatment was sampled on days 0 and 11 in order to determine the baseline platelet counts.

Statistics. Final evaluation of the results was primarily based on determining the comparable changes in the level of thrombocytes in animals of the treatment groups versus that of the vehicle control group. Standard deviations were calculated assuming a Gaussian distribution. The Wilcoxon Two-Sample Rank test was performed to determine the similarity of the platelet peak values.

Rhesus monkey experiments

Animals. Purpose-bred male rhesus monkeys (*Macaca mulatta*), weighing 2·5–4·0 kg and aged 2–3 years were used. The monkeys were housed in groups of four to six in stainless steel cages in rooms equipped with a reverse filtered air barrier, normal day light rhythm and conditioned to 20°C with a relative humidity of 70%. Animals were fed *ad libitum* with commercial primate chow and fresh fruits, and received acidified drinking water. All animals were free of intestinal parasites and seronegative for herpes B, hepatitis B, simian T-lymphotropic viruses and simian immunodeficiency virus. Housing, experiments and all other conditions were approved by an ethical committee in accordance with the legal regulations in The Netherlands.

TPO administration. Vials of 1 ml, containing 0·930 mg/ml recombinant full-length rhesus monkey TPO produced by CHO cells were supplied by Genentech Inc. A dose of 5 μg/kg was diluted to a volume of 1 ml with PBS/0·01% Tween 20 before administration. TPO was administered subcutaneously. Because of previously demonstrated iron deficiency after TPO administration (Neelis *et al*, 1997a), the monkeys received iron supplementation; 0·5 ml Imferon intramuscularly [Fe(III) 50 mg/ml; Fisons Pharmaceuticals, Loughborough, England] alternately in the left or right thigh, simultaneously with the growth factor administration.

Haematological examinations. Complete blood cell counts were measured in EDTA-anticoagulated blood between 8 and 9 AM using a Sysmex F-800 haematology analyzer (Toa Medical Electronics Co.). Serum was collected weekly and frozen at −20°C.

Detection of TPO antibodies (Abs). The concentration of anti-TPO Abs was measured by using a sensitive enzyme-linked immunosorbent assay (ELISA) with a detection limit of 10 pg/ml. Microtitre Covalink 96 wells plates (Nunc, Rockslide, Denmark) were activated by treatment with a dithiobis (succinimidyl proprionate) (Pierce, Rockford, Illinois, USA) solution in methanol, for 30 min at RT. Afterwards, wells were incubated with 50 μl (1 μg/ml) recombinant full-length rhesus monkey TPO produced by CHO cells (Genentech Inc.) in PBS (pH 7·4) for 2 h at RT. Non-specific binding sites were blocked with a PBS–albumin solution (1% bovine serum albumin; A7030, Sigma, Zwijndrecht, The Netherlands), 250 μl per well, and plates were stored overnight at 4°C. Before use, plates were washed six times with 200 μl PBS/Tween-20 (0·05%) solution at RT. Subsequently, 50 μl serum 1/100 diluted in PBS–Tween-1% BSA was added to the wells and the plates were incubated for 1 h at RT. After six washes, rabbit-anti-rhesus-Ig-PO (A2054, Sigma) diluted 1/35 000

according to the manufacturer's instructions, was added, followed by an incubation of 1 h at RT. After incubation, the wells were washed six times with 200 μl PBS/Tween solution and 100 μl 3,3′,5,5′-tetramethylbenzidine- substrate (Kirkegaard and Perry Laboratories, Maryland) was added. All incubations were performed while shaking the plates gently using an INNOVA 2100 platform shaker (New Brunswick Scientific, Nijmegen, the Netherlands) set at 75 r.p.m. The colorimetric reaction was stopped by addition of 100 μl 1 mol/l ortho-phosphoric acid (Merck, Darmstadt, Germany) and the absorbance at 450 nm was measured in a microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA). For comparison, a dose response curve was incorporated in each experiment by coating a set of wells with rHuman TPO and one with rRhesus TPO, incubating with dilutions of mouse monoclonal anti-Human-TPO (R & D systems, Minneapolis, MN, USA) and detected with a 1/10 000 dilution of GAM-PO (A8924, Sigma). Samples were measured in quadruplicate. The results are expressed as optical density (OD).

Study design. In the first stage of this experiment, each of the five monkeys received a different individual TPO schedule (Fig 1A). Blood samples were drawn for blood counts and serum Abs against TPO. In the second stage, two other monkeys were each given a schedule of four daily doses of TPO (5 μg/kg per dose), implementing the same regimen as in mice (Fig 1B).

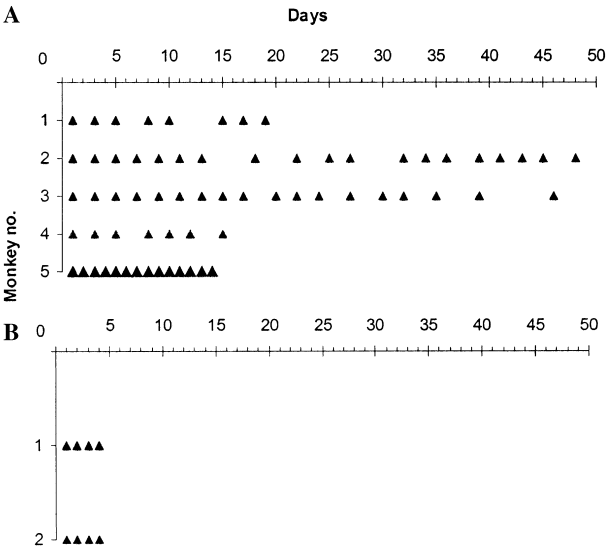

Fig 1. Thrombopoietin (TPO) administration schedules to monkeys. Each triangle represents a dose of 5 μg/kg recombinant full length rhesus monkey TPO. (A) Five different protocols of the five monkeys studied. (B) Identical protocols of the two monkeys of the second group. Each of these monkeys was subsequently treated with the same schedule for two additional times with similar platelet responses, and did not develop neutralizing antibodies (Abs) (data not shown, unpublished observations).

Thrombopoiesis computer model

The computer model employed in this work (Skomorovski & Agur, 2001) consists of a comprehensive and very detailed mathematical description of thrombopoiesis, from the level of stem cells, to the level of platelets in the blood. The precise description of the model's many equations is beyond the scope of this report, but its general structure is represented in Fig 2 and its basic assumptions are detailed hereafter.

As shown in Fig 2, the model comprises 11 compartments comprehensively describing thrombopoiesis. The first nine represent the different stages of bone marrow cell progression. The 10th compartment represents the mature platelets in peripheral blood. Each of these 10 compartments is further sub-divided according to the maturation stage (age) of the cells in it. An additional 11th compartment represents TPO concentration in the blood.

Mathematically, the thrombopoiesis model is discrete in time and is formulated through delay difference equations (DDEs). The exact state of the system at any time-step is represented through the momentary distributions of cell numbers in each compartment, so that each cell in each subpopulation within a given compartment is characterized by two indices: calendar time and age. In addition, the model includes functions describing the kinetic characteristics of cells in the different compartments, such as compartmental transit time, the rate of cell flow from one compartment to the next, the duration of megakaryocytes' endomitosis, and platelet release rate from mature megakaryocytes.

The model assumes that blood platelets are eliminated in two ways: age-independent, through normal consumption by the body (e.g. clotting), and age-dependent, kinetically characterized by a 'transit time' in the blood. The model includes TPO pharmacokinetics (PK), consisting of production by the body, absorption of exogenous TPO, consumption by cells, and other mechanisms of clearance. It also includes TPO pharmacodynamics (PD) characteristics, namely, the effects of TPO on the kinetic parameters of cells in the above mentioned cellular compartments.

Parameter adjustment module

Iterative adjustment of biological and PK/PD parameters was performed within the range of potential real-life values, so as to most accurately retrieve the experimental results. The module used the thrombopoiesis simulation model (described above), an empirical platelet profile of one rhesus monkey (monkey no. 5) treated by TPO (see Fig 1 for protocol details), and an efficient search algorithm (e.g. modified Powell's conjugate directions method) (Brent, 1973; Press *et al*, 1993; unpublished observations).

Calibration of the thrombopoiesis computer model

Parameter calibration was performed in order to fine-tune the model to thrombopoiesis of the species in question. It involved definition of a biologically realistic range of values for each parameter in murine thrombopoiesis (Ebbe & Stohlman, 1965; Schermer, 1967; Siegers *et al*, 1979; Kaushansky *et al*, 1996; Arnold *et al*, 1997; Ulich *et al*, 1999; Luoh *et al*, 2000) and simian thrombopoiesis (Farese *et al*, 1995; Harker *et al*, 1996a,b; Neelis *et al*, 1997b; Wagemaker *et al*, 1998; De Serres *et al*, 1999; Sola *et al*, 2000), which were then entered as the initial input for the

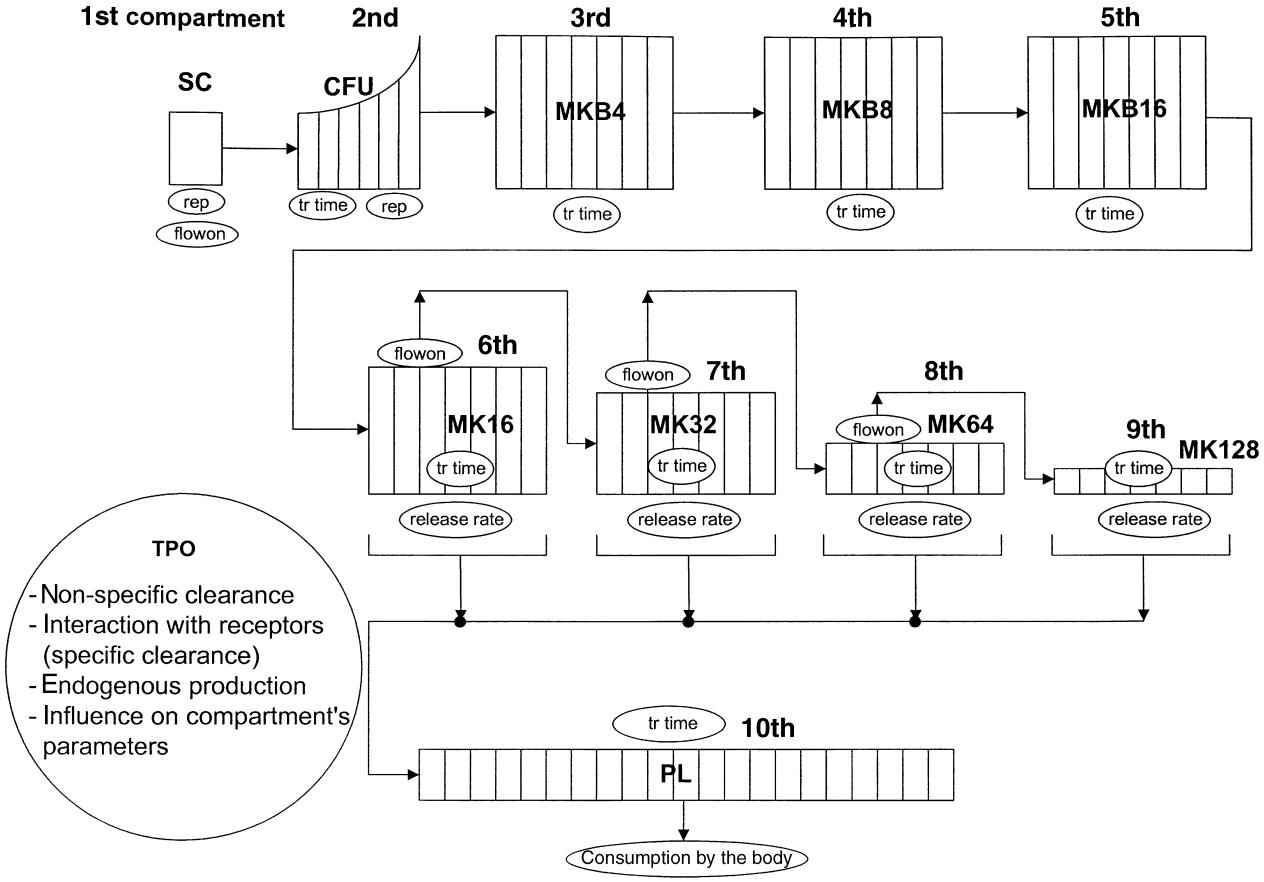

Fig 2. A scheme of the thrombopoiesis computer model. The first 10 compartments are: SC, stem cells; CFU, colony forming units; MKB (4, 8 and 16), megakaryoblasts (of ploidies 4 N, 8 N and 16 N respectively); MK (16, 32, 64 and 128), megakaryocytes (of ploidy 16 N, 32 N, 64 N and 128 N respectively); and PL, platelets. The 11th compartment is indicated by the letters 'TPO'. Relevant key parameters included in the kinetic calculations of the different compartments are denoted as follows: rep, amplification rate; flowon, flow-on fraction; tr time, transit time; and release rate, rate of platelet release. The perpendicular compartmental subdivisions represent cell-age transition. The arrows indicate the direction of inter-compartmental cell flow.

fine-tuning process. Thus, the set of model parameters, best fitting the available empirical results of the species in question was identified by multiple curves fitting within the above ranges.

RESULTS

Similar platelet peaks can be obtained under significantly varying TPO schedules – validation in mice

We checked in mice the model's prediction that platelet counts, similar to those achieved with the accepted protocol, can be also generated under different schedules, of appreciably reduced TPO doses. Hence, two different administration schedules were applied to mice of the two experiment arms and their subsequent responses were compared. As this experiment was aimed at demonstrating improved efficacy (i.e. higher platelet yield per TPO unit) of a newly suggested TPO schedule, it was imperative that similarity in platelet yields reflect actual similarity between the dose schedules effect on platelet count, rather than both schedules merely driving platelet counts to saturation.

In mice, a maximum elevation in platelet blood count, in response to a single dosing, of about five- to six-fold above baseline was observed following the administration of 250 μg/kg pegylated recombinant murine MGDF (PEG-rmMGDF), equivalent to *ca.* 2500 μg/kg non-PEG-rmMGDF (Daw *et al*, 1998). Based on this information, mice in the first arm received a single injection of 17·5 μg/kg rmTPO, well below the above reported saturating level. The second arm tested the model-generated proposition that the same platelet yields can be obtained in mice receiving a total dose of 8 μg/kg rmTPO, divided over four equal daily injections, whereas the null hypothesis was that a significantly smaller total dose of rmTPO would be less efficient in elevating the platelet counts.

The platelet profile of arm A, in which the mice received a single dose of 17·5 μg/kg rmTPO, was similar to the profile of arm B, in which the mice received a total dose of 8 μg/kg, divided over four equal daily fractions (Fig 3A). The differences were not statistically significant (Student's *t*-test). The average profiles of both arm A and B peaked at approximately the same time (day 5 vs. day 6 respect-

ively) and at similar mean platelet counts of $2741 \pm 193 \times 10^9$/l and $2685 \pm 164 \times 10^9$/l respectively. The latter schedule resulted in slightly extended thrombocytosis. Mean platelet volume (MPV), which is known to decrease in thrombocytosis induced by low-dose TPO in mice (Kabaya *et al*, 1996), was also compared between arm A and arm B (Fig 3B). Results show that the familiar phenomenon of MPV decrease also occurred to a similar extent in both groups. Again, arm A preceded arm B in reaching its nadir by about 24 h (6·76 fL on day 6 vs. 6·74 on day 7 respectively). Specific adverse effects were not observed following either treatment.

Testing the predictions of the thrombopoiesis model in monkeys

In order to evaluate the efficacy of the treatment regimen in monkeys, already validated in mice, we first checked the ability of the thrombopoiesis simulation model to predict individual monkey responses to different TPO treatments. Subsequently, we simulated monkey responses to the model-suggested protocol and compared our prediction with the platelet counts obtained *in vivo*.

To check the simulation model's ability to predict *individual* responses to different drug schedules, the model's parameters were customized to accurately retrieve the platelet profile of one specific individual from the monkey experimental group. Next, the model was applied to predict the responses of other monkeys in the same group to new dosing regimens. The underlying assumption is that members of a subpopulation, similar in genetic background as well as in environmental conditions, will also have a similar response to TPO treatment.

First we fine-tuned the model's parameters to fit one monkey empirical platelet profile. The quantitatively adequate simulation of thrombopoiesis response to treatment was apparent from its resemblance to the empirical results (Fig 4). This calibrated model was further applied to predict the profiles of the other four monkeys, which had each received a different drug protocol (see protocols in Fig 1A). The model's predictions for monkeys no. 1, 2 and 4 were remarkably close to the empirical data-points (Fig 5). Note that, as the platelet counts were monitored in these monkeys only once weekly, an *a priori* mismatch is introduced into the comparison between the empirical results and the daily predicted counts. Therefore, there is no reason to assume that the peaks in the predicted responses on days that were not tested empirically, did not actually take place (days 11 and 25 in monkey no. 1, days 15 and 32 in monkey no. 2, and days 11 and 21 in monkey no. 4). In contrast, the large discrepancy between the predicted and the empirical results of monkey no. 3 (Fig 6) cannot be accounted for by the resolution difference between real-life monitoring and the simulations. This monkey became rapidly refractory to TPO treatment, as a result of undue formation of neutralizing Abs. Monkeys no. 1 and 2 also developed an antibody response that neutralized endogenous TPO upon a subsequent boost with exogenous TPO (unpublished observations), however not within the time frame displayed in Fig 1A. The model's predictive ability was further challenged when the present calibration was employed for predicting responses to TPO of another group of monkeys.

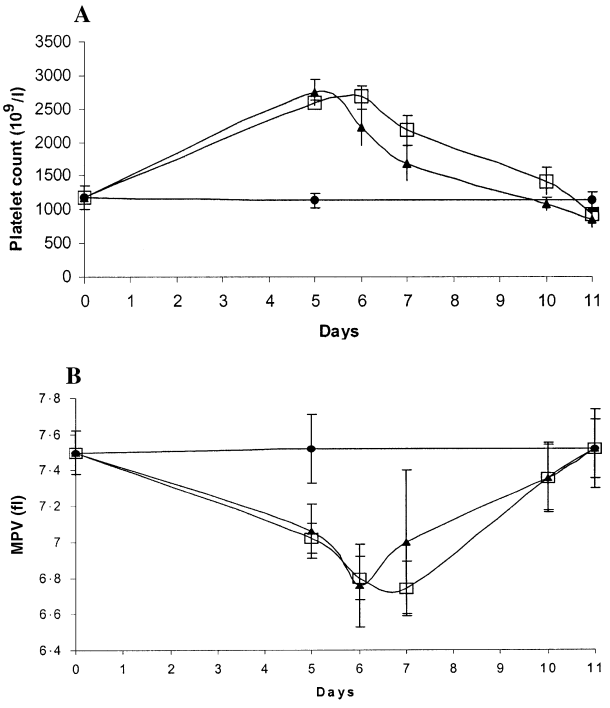

Fig 3. (A) Daily platelet counts and (B) MPV measurements, of mice treated with thrombopoietin (TPO). Comparison is between a 'conventional' single dose treatment (arm A, solid triangles) and a model-suggested reduced dose treatment (arm B, open squares) and a control group (solid circles); average ± SD of five mice is shown, per each entry. Day 0 is the day of TPO treatment initiation. Mice in arm A received a single dose of 17·5 μg/kg TPO; mice in arm B received 4 daily doses of 2 μg/kg TPO each, a total of 8 μg/kg.

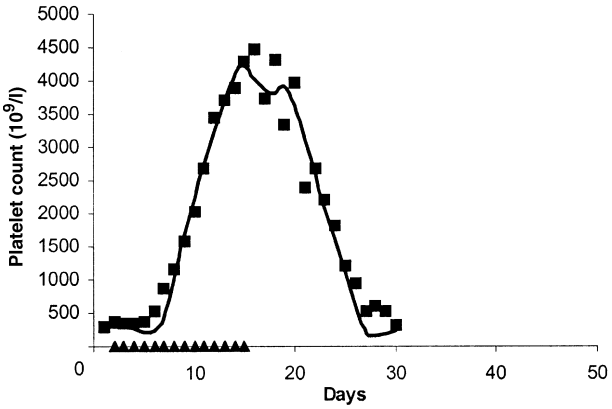

Fig 4. Empirical versus simulated platelet counts of monkey no. 5 in response to thrombopoietin (TPO) treatment. Fourteen daily doses of 5 μg/kg TPO were administered (administration days marked by triangles). Measurements were performed daily (rectangles), simulation results are indicated by a solid line.

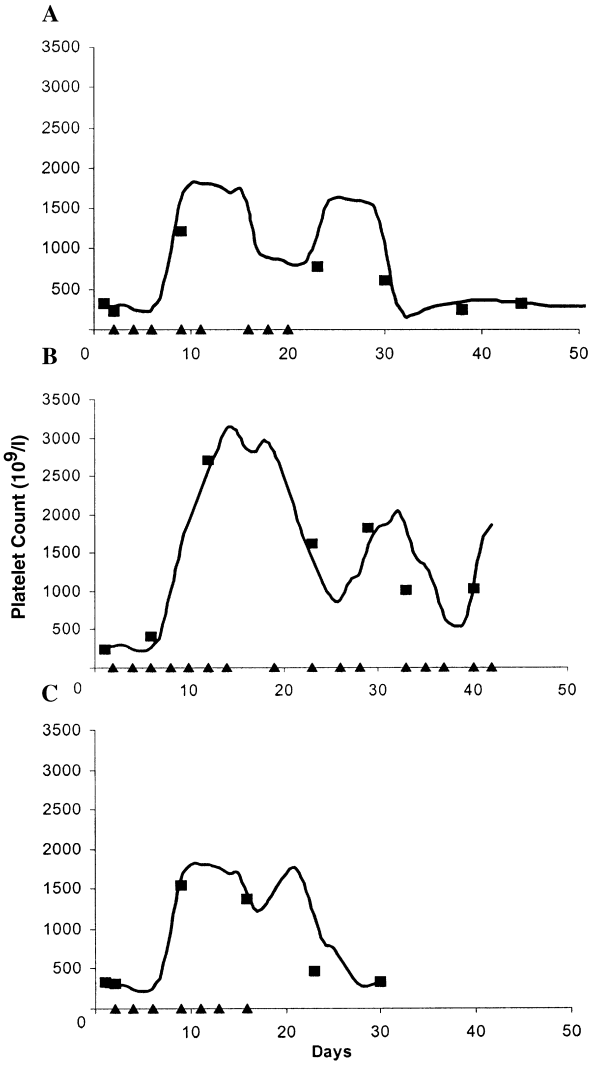

Fig 5. Empirical versus simulated platelet counts. Platelet counts of monkeys no. 1, no. 2 and no. 4 (A, B and C respectively) in response to multiple dosing treatments by 5 μg/kg thrombopoietin doses (administration day marked by a triangle). Measurements were performed weekly or biweekly (rectangles); simulation results are indicated by a solid line.

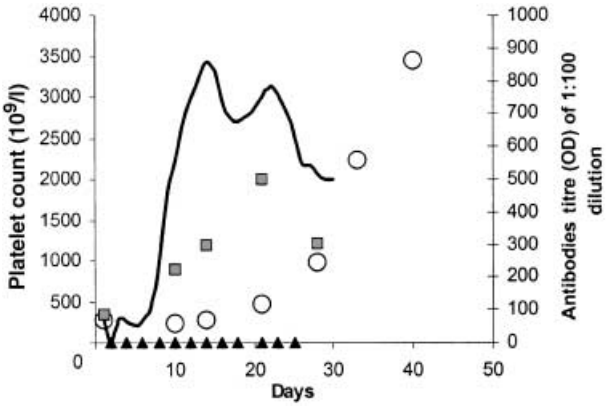

Fig 6. Platelet counts of monkey no. 3 in response to multiple dosing treatment with 5 μg/kg thrombopoietin doses (administration days marked by triangles). Measurements were performed about once weekly (rectangles); simulation results are marked by a solid line. Antibody titre readings are shown as open circles.

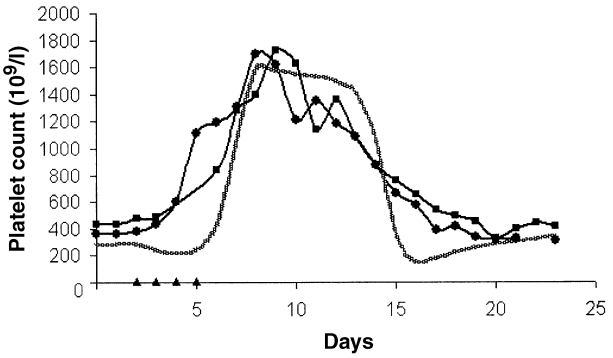

Fig 7. Comparison between predicted and empirical responses of monkeys to the suggested thrombopoietin (TPO) treatment. The model predictions (line only) and empirical platelet profiles of monkeys A and B (solid squares and circles respectively) are presented. TPO was administered as four daily doses of 5 μg/kg each (administration days marked by triangles). The model's predictions were generated under the assumption of a baseline level of 280×10^9 platelets/l in the simulated monkeys (see also text).

A safer TPO schedule – model validation in monkeys

The model-based regimen validated in mice was then evaluated for efficacy and safety in two monkeys, each undergoing three treatment cycles (see protocol in Fig 1B). The resulting elevation in platelet counts peaked at $1700–2400 \times 10^9$/l following each treatment cycle. The results of the second cycle were similar to those of the first in showing no neutralizing Abs. Only when the dose schedule was applied for the third time, were very low Ab titres detectable, which did not jeopardize TPOs efficacy and did not result in decreased platelet counts (unpublished observation). The individual empirical responses of the two monkeys to the first treatment cycle are shown in Fig 7, as compared with the model predicted response. The empirical onset of platelet count elevation preceded the predicted one by 2 d. The platelet count peak was around day 8 in both observed and predicted results, with similar measured peaks of *ca.* 1700×10^9 platelets/l, compared with a predicted peak of *ca.* 1600×10^9 platelets/l. However, it is important to note that the baseline counts of these two monkeys (about 420×10^9 platelets/l) were not taken into account: The present model calibration was performed based on the previous baseline count (about 280×10^9 platelets/l; Figs 4–6). Hence, the predictions of these two monkeys were biased towards lower expected platelet counts. Abs to TPO were not detected in the monkeys treated in this experiment. A significant descent began on

day 13 in the predicted result with a return to baseline counts on day 20 in both, yet it was more gradual in the empirical results.

DISCUSSION

The main purpose of this work was to validate the model-generated prediction that a fractionated regimen of reduced total TPO dose and an optimally spaced administration will maintain TPO efficacy and eliminate its immunogenic side effects. We showed here for the first time that it is possible to retrieve quantitative biomathematical model predictions, enabling suggestion of improved clinical treatments.

For the purpose of this validation we first showed in a mouse experiment that two schedules of TPO administration, which differed significantly in total dose, did not differ in efficacy. The empirical results (Fig 3A) exhibited highly significant similarity between the platelet profiles of mice in the two treatment groups. As the change in MPV, an indicator of platelet production kinetics, was also similar in both arms (Fig 3B), we infer that very similar stimuli were received by the thrombopoietic system. Thus, the use of smaller doses with an interval of 24 h, suggested by the model predictions, proved equal in effect to the more than double dose, administered as a bolus injection. This experiment joins previous *in vivo* experiments with other drugs in verifying a mathematical theory, which essentially suggests that treatment efficacy can be decisively influenced by modulating the inter-dosing interval according to the internal cell kinetics (Agur *et al*, 1988; Agur *et al*, 1991; Ubezio *et al*, 1994).

The monkey experiment supports the model prediction of an equally efficient and non-immunogenic treatment, if dose is fractionated by an appropriate dosing interval. The model-suggested protocol has been administered to monkeys in three cycles at 3- to 4-month intervals. The treatment showed high efficacy with no effective neutralizing antibody response. Note that an equal total dose, delivered as a single bolus injection of 20 μg/kg, may be similarly effective in avoiding immunogenicity. Nevertheless, based on the mouse experiment, this one bolus protocol is expected to be less effective. Therefore, although, *a priori*, from a clinical point of view, it may not seem beneficial to the patient to replace a single injection with four, it may be important for safety considerations. A current modelling effort quantifies antibody formation as a result of TPO administration. This could enable the design of effective drug administration protocols with minimized immunogenicity.

In addition to validating the model-suggested improved TPO protocol, we also validated the monkey thrombopoiesis model and its predictions of individual responses to different TPO regimens. These validations were performed assuming that a genetically homogeneous group of animals that are subject to the same laboratory conditions will show similar responses. However, such a group is prone to expected variability as a result of extrinsic factors as well as intra-group variability. In the case of the monkeys, there was some variation in age and weight (data not shown). Nevertheless, the results of the predicted versus the measured response to TPO in monkeys no. 1, no. 2 and no. 4, which were not compromised by a rapid antibody response, showed a high accuracy of the model's predictions (Fig 5A–C), and suggest that the contribution of intra-group variation to the overall TPO effect on platelet counts is of relatively minor significance.

In order to predict the results of an additional group of monkeys that were subject to a new TPO protocol (Fig 7), the rhesus monkey tool, calibrated to simulate the above group of five monkeys, was implemented. In the case of this additional group, the tool's predictive ability was more accurately verified, as it was challenged by the additional inter-group variability as well as by the daily-monitored blood counts leading to higher empirical precision. Nevertheless, the predicted response was very similar to the empirical one in time and magnitude of peak platelet levels.

It is of note that in the latter simulations we used the original baseline setting of the first monkey validation (280×10^9/l) instead of the actually measured baseline levels (420×10^9/l). As the model correctly predicted time and magnitude of the response, it turned out to be sufficiently robust to provide predictions with limited input data (in this case, data of only a single monkey, no. 5, for which daily measurements were available).

Kinetic differences were observed between the monkeys' experimental results and the model's predictions (Fig 7). The observed results showed an earlier onset of platelet count elevation, an earlier decline from the observed peaks and a less abrupt return to normal levels. Such differences may result from inaccurate estimates of a few, or even of a single kinetic parameter among the many implemented in the model (e.g. transit time, platelet elimination rate, etc.), as fine-tuning of the full process kinetics was based on the kinetics of only one monkey (no. 5). It is anticipated that input data from more monkeys will further improve the model adjustment, to the extent that these minor deviations will also be resolved.

Human validation of the thrombopoiesis computer tool incorporating the effects of chemotherapeutic drugs is underway. Once adjusted, this tool is expected to aid in suggesting improved drug protocols for the individual or for a population of patients. Human trials are warranted for testing the suggested improved TPO protocol, possibly in conjunction with chemotherapy.

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