The antithrombotic effect of dextran-40 in man is due to enhanced fibrinolysis in vivo

Chris I. Jones, PhD, David A. Payne, FRCS, Paul D. Hayes, MD, A. Ross Naylor, MD, Peter R. F. Bell, MD, Mathew M. Thompson, MD, and Alison H. Goodall, PhD

Leicester, United Kingdom

Background: Dextran-40 is effective in reducing postoperative Doppler-detectable embolization in patients undergoing carotid endarterectomy (CEA). Dextran-40 is thought to have antithrombotic and antiplatelet effects. The mode of action is unclear. In rats, dextran blocks uptake of tissue plasminogen activator (tPA) by mannose-binding receptors. Because this would have the effect of enhancing endogenous fibrinolysis, we explored this effect of dextran-40 on fibrinolysis in man.

Methods: Twenty patients undergoing endovascular stenting for abdominal aortic aneurysm were randomized to receive 100 mL of 10% dextran-40 or saline, over 1 hour, during their operation in addition to heparin. Blood samples were taken preoperatively, intraoperatively (immediately after operative procedure), and 24 hours postoperatively. Thrombi were formed in a Chandler loop and used to assess endogenous fibrinolysis over 24 hours, measured as the fall in thrombus weight, and the release of fluorescently labelled fibrinogen from the thrombus. Plasma samples were analyzed for markers of fibrinolysis; plasmin-antiplasmin (PAP), PAI-1, and t-PA, and for functional von Willebrand factor (vWF). Platelet response to thrombin and other agonists was measured by flow cytometry.

Results: Thrombi formed ex vivo from the intraoperative blood samples from the dextran-treated patients exhibited significantly greater fibrinolysis vs preoperative samples, seen both as a significantly greater percentage reduction in thrombus weight (from 34.7% to 70.6% reduction) and as an 175% increase in the release of fluorescence (P < .05). Fibrinolysis returned to baseline levels the next day. No change was seen in the saline-treated group. Plasma levels of PAP and PAI-1 increased significantly postoperatively in the dextran-treated group vs the saline group (P < .05). The postoperative level of functional VWF was significantly lower in the dextran-treated group vs controls. A specific reduction occurred in the platelet response to thrombin, but not to other agonists, in the intraoperative samples from the dextran-treated group (11.1% vs 37.1%; P = .022), which was not seen in the controls.

Conclusions: These data are consistent with a rise in plasmin due to dextran blockade of tPA uptake in vivo, leading to enhanced fibrinolysis, cleavage of vWF and of the platelet protease-activated receptor-1 (PAR-1) thrombin receptor. This suggests that dextran exerts a combined therapeutic effect, enhancing endogenous fibrinolysis, whilst also reducing platelet adhesion to vWF and platelet activation by thrombin. The proven antithrombotic efficacy of low-dose dextran in carotid surgery may be applicable to wider therapeutic use. (J Vasc Surg 2008;48:715-22.)

Clinical Relevance: Dextran-40 is an effective therapeutic agent in the reduction of thromboembolic events after carotid surgery, but its mechanism of action is unclear. We describe a laboratory study of the effect of dextran-40 in man that clearly demonstrates that dextran-40 enhances fibrinolysis through a mechanism that is compatible with blockade of uptake of tissue plasminogen activator in vivo. This study was conducted in patients undergoing aortic aneurysm repair for logistic reasons, but the data support the use of dextran-40 in patients undergoing carotid endarterectomy and may suggest wider applications for the use of dextran to augment fibrinolysis/thrombolysis in other thrombotic diseases.

Dextran has been used since World War II as a plasma expander. From the 1950s onwards, reports have emerged suggesting an effect of dextran on the hemostatic system, in particular in prolonging bleeding time. The exact mechanism of action has remained unknown, however.

In 1995 the Vascular Surgery Unit at the Leicester Royal Infirmary started routinely treating patients undergoing carotid endarterectomy (CEA) with low-dose dextran-40 (20 to 40 mL/h, 10% dextran-40) if transcranial Doppler monitoring revealed that significant numbers of emboli were produced after CEA. Since the introduction of this policy, 500 consecutive operations have been done without a single postoperative stroke. On administration of dextran-40, the numbers of emboli fall steadily within the first hour; however, the mechanism of action that underlies this effect is unclear.

Reports of an effect of dextran on the hemostatic system span the past 50 years and cover a range of seemingly disparate observations. Studies from the 1960s in humans and in dogs reported that dextran-70 infusions reduced platelet adhesiveness, increased bleeding time and coagulation time, and produced significant hemodilution. Greater effects were observed with high-molecular-weight dextrans. Studies in the 1970s demonstrated that these effects might be partly attributed to a reduction in von Willebrand...
factor (vWF) levels in vivo,11-13 with maximum effects seen 4 to 6 hours after infusion. Reduced levels of vWF were thought to be responsible for reduced stability of thrombi formed in a Chandler loop with blood from healthy human volunteers given 500 mL dextran-70.14 These observations and others investigating thrombus formation in vivo in rabbits15,16 also suggested that dextran may be affecting the fibrinolytic system, and a small number of studies from the 1980s and 1990s supported this hypothesis. For example, Carlin et al17 observed an increase in fibrinolytic activity in patients given 500 mL of 6% dextran-70 during the postoperative period, and similar effects have been reported in rabbits.18 More recently, Eriksson and Saldeen (1995)19 demonstrated that in patients undergoing elective surgery, an infusion of 250 mL or 500 mL of 6% dextran-70 led to a significant increase in tissue plasminogen activator (tPA) activity and antigen, with a concomitant reduction in plasminogen activator inhibitor type 1 (PAI-1). However, none of the effects seen in vivo have been reliably replicated when dextran is added to blood in vitro.

A possible explanation for the in vivo effect of dextran comes from the observation by Noorman et al20 that dextran-40 and dextran-70 given to rats blocked the uptake of tPA by the mannose receptor on rat liver endothelial cells, inhibiting clearance of tPA from the circulation. It is not clear whether similar mechanisms apply in humans and with the doses of dextran-40 (20 to 40 mL of 10% dextran-40 per hour) used to prevent postoperative thromboembolic complications in patients undergoing CEA. At these levels hemodilution is not a significant factor. We therefore explored the effect of dextran-40 in a group of patients undergoing endovascular stenting for aortic aneurysm repair, who were randomized to receive dextran-40 or saline, to enable systematic study of a variety of measures of hemostatic and fibrinolytic function.

METHODS

Subjects and study design. The study was designed as a randomized, double-blind, controlled trial in 20 consecutive men undergoing elective endovascular stenting for abdominal aortic aneurysm at the Leicester Royal Infirmary. These patients were studied in preference to patients undergoing CEA because they provided a group amenable to randomization and systemic study. Treatment of all patients undergoing CEA with dextran can result in an unacceptable risk of causing bleeding complications. The study was approved by the Ethical Practices Committee at the Leicester Royal Infirmary, and all subjects gave written informed consent.

Exclusion criteria included a history of a bleeding abnormality, cancer, renal disease, or any medication that would significantly affect fibrinolysis. Consecutive patients that met the inclusion criteria were randomized to receive either 100 mL of 10% dextran-40 (Baxter, Newbury, UK) for 1 hour during the operation or an equivalent volume of saline (0.9%). Randomization was by sealed envelopes prepared by a research nurse independent of the study, and the laboratory staff was blinded to the treatment.

All patients received unfractionated heparin infused through the arterial catheter before operation and dextran. Patients were standardized to 150 mg aspirin as the sole antiplatelet drug before surgery, and platelet aggregation in response to arachidonic acid showed all patients were responding (<20% aggregation). No differences were noted in the activated partial thromboplastin time or international normalized ratio measurements between the groups.

The two groups of men were similar in age (71.7 ± 8.6 years saline group vs 72.8 ± 4.3 years dextran group; P = .19), body weight (85.8 ± 14.9 vs 81.3 ± 10.0 kg; P = .50), disease profile, and medication (P ≥ .15 for all). Platelet and leucocyte counts for the saline group and dextran group were also similar (217 ± 46 vs 216 ± 30 × 10^9/mL, P = .93; and 8.1 ± 2.1 vs 6.8 ± 1.6 × 10^11/mL, P = .18), as was plasma fibrinogen (2.98 ± 0.81 g/L vs 2.75 ± 0.52 g/L; P = .54). Hemoglobin was significantly lower in the saline group at the start of their operation (13.15 ± 1.88 g/L vs 14.78 ± 1.04 g/L; P = .04) and remained lower at the end (10.22 ± 2.51 g/L vs 12.61 ± 1.24 g/L; P = .03), although blood loss and fluid use was similar in both groups.

Blood samples were taken at three time points: (1) preoperation, after anaesthetic but before the start of the operation, before heparin administration, and before dextran or saline infusion; (2) intraoperation, at the end of the 1-hour dextran infusion; and (3) postoperation, on the morning after the operation. Blood was collected from the arterial catheter into Vacutainer tubes (BD UK Ltd, Oxford, UK) containing either CTAD (citrate, theophylline, adenosine and dipyridamole), or tris-sodium citrate (0.105M; BD).

Generation of thrombi and measurement of fibrinolysis ex vivo. Thrombi were generated from whole blood in a Chandler loop, using a method previously described.22-24 Aliquots of 0.9 mL of citrated blood were supplemented with 50 μL of 1 mg/mL fluorescein isothiocyanate (FITC)-fibrinogen (prepared in house24), recalcified, and placed into polythene tubing 45-cm long. The ends were joined and the loops were rotated at 37 rpm for 90 minutes, resulting in an initial shear rate of 19.2 dynes/cm², rising to >500 dynes/cm² during thrombus formation. Samples were run in duplicate, and the resultant thrombus was removed, blotted, and weighed. To assess fibrinolysis the thrombi were incubated in Hanks’ buffer for 24 hours at 37°C. The supernatant was removed, and the fluorescence released from the thrombi was measured in a Spectrofluorophotometer (RF-5001PC; Shimadzu, Kyoto, Japan). Loss of fluorescence was expressed in arbitrary fluorescent units per milligram initial thrombus weight. The remaining thrombi were blotted and reweighed to calculate the loss of thrombus weight, which was the percentage fall in weight during 24 hours.
To observe the effect of dextran in vitro, 10% (v/v) dextran-40 was added to preoperation blood samples at a comparable concentration to that being infused to patients (20 μL/mL) before generation of Candler loop thrombi. In a separate experiment, recombinant tPA (rtPA; Activase, Alterplase, Genentech Inc, San Francisco, Calif) was added to the blood at final concentrations of 0.0015 to 15 μg/mL, before formation of the thrombi, to assess the effect of tPA on endogenous fibrinolysis.

Measurement of fibrinolytic factors in plasma. Blood was collected into CTAD, and plasma was immediately separated by double centrifugation of 20 minutes at 1550g and 4°C, followed by 10 minutes at 9300g. Aliquots of plasma were immediately frozen and stored at −80°C before measurement of tPA antigen, PAI-1 activity, and antigen (Actibind), plasmin-antiplasmin (PAP) complexes (all from Technoclone Ltd, Dorking, Surrey, UK), and vWF activity (Axis-Shield UK, Cambridgeshire, UK).

Measurement of the platelet response. Flow cytometric analysis of the platelet response was done in a whole blood assay, as described previously.25 Briefly, ≤10 minutes of collection, 5 μL of citrated blood was added to 50 μL of N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES)-buffered saline containing FITC-antifibrinogen antibody (Dako, Ely, UK) and either thrombin (0.16 U/mL), thrombin receptor-activating peptide (TRAP; 1 × 10−6M), or adenosine diphosphate (ADP; 1 × 10−5M; all from Sigma-Aldrich Co Ltd, Dorset, UK) and incubated for 20 minutes at room temperature. Samples containing thrombin also contained glycyl-L-prolyl-L-arginyl-L-proline (GPRP) peptide (Sigma) to block fibrin cross-linking.26 The samples were then diluted 100 times with formyl saline (0.2% v/v), and flow cytometric analysis was done using a Beckman-Coulter MCL-XL flow cytometer (Beckman-Coulter Ltd, High Wycombe, UK). Negative controls were set to 2% on samples incubated with antibody, in the presence of ethylenediaminetetraacetic acid, which prevents the binding of fibrinogen to glycoprotein (GP) IIb-IIIa. To observe the effect of dextran on the whole blood in vitro, 10% dextran-40 was also added to the preoperation blood sample at a similar concentration (20 μL/mL) to that being infused into the patients.

Platelet aggregation was measured in platelet-rich plasma ≤1 hour of sampling. Platelet-rich plasma was prepared from citrated plasma and stimulated with TRAP (2 × 10−5M), ADP (4 × 10−5M), or collagen (1.0 μg/mL; Horm collagen; Nycomed, Hart Biologicals, Hartlepool, UK). Aggregation was measured for 10 minutes in a PAP4C aggregometer (BioData Corp, Horsham, UK), setting 100% transmission against autologous platelet-poor plasma.

Statistical analysis. Two-way analysis of variance was used to analyze differences between the two treatment groups during the three time points studied. Post-test analysis for comparison between time points was performed using the Tukey multiple comparison test.

RESULTS

Effects of dextran infusion on thrombus formed in vitro. Dextran did not affect the weight of the thrombi formed ex vivo, which were similar at all time points in both the dextran- and saline-treated groups (P > .05; Fig 1, A), although a significant reduction was seen in the weight of the thrombi in the saline group (Fig 1, B and C).
the thrombus formed from the intraoperative blood samples in both groups, which was due to the effects of heparin infusion during the operation. This fall was significant in both groups, dropping from $38.0 \pm 5.9$ mg to $12.0 \pm 3.6$ mg in the saline-treated group ($P = .01$) and from $26.9 \pm 2.3$ mg to $10.5 \pm 4.2$ mg ($P = .008$) in the dextran-treated group, but not different between the two groups. Thrombus formed from blood collected in the postoperative period rose in both groups to preoperative weights.

Despite the similar initial thrombus weights, the thrombi formed from blood taken after the dextran infusion showed significantly increased fibrinolysis during a 24-hour period compared with the blood from the patients who received saline. This was seen as both a greater fall in thrombus weight, from $34.8 \pm 4.8$ to $73.0 \pm 9.0$ mg; a change of $70.6\%$, in the dextran-treated group compared with $31.3 \pm 1.6$ to $46.8 \pm 9.1$ mg; a change of $34.7\%$ in the saline-treated group ($P = .02$; Fig 1, B), and as a greater loss of fluorescently labelled fibrinogen from the thrombi ($P = .016$; Fig 1, C). Fibrinolysis in the samples collected on the morning after the operation had returned to preoperative levels in both groups. The levels of fibrinolysis seen in the samples from the dextran-treated subjects were equivalent to the effect of 6 to 7 µg/mL rtPA added to blood at the time the thrombus was formed in vitro (data not shown).

Dextran added to the preoperative blood samples in vitro had no effect on either the weight of the thrombus that formed ($36.7 \pm 8.6$ mg compared with $34.0 \pm 8.5$ mg without dextran; $P = .82$) or the rate of fibrinolysis, as measured by loss of fluorescently labelled fibrinogen ($0.69 \pm 0.44$ U/mg compared with $0.60 \pm 0.32$ U/mg without dextran; $P = .87$).

**Effects of dextran infusion of the fibrinolytic system.** Despite the increase in the rate of fibrinolysis, no significant difference was noted in the levels of tPA antigen in the blood of either group at any time point (Fig 2, A). Because the assay used measured total tPA antigen, this finding did not preclude a rise in active tPA, which normally represents only 2% to 5% of the total tPA; the remaining 95% to 98% being bound to PAI-1. We therefore looked for evidence of a change in the level of plasmin that would be altered by an increase in tPA activity.

The presence of PAP complexes in the circulation provides evidence of activation of plasminogen to plasmin by tPA in vivo.27 PAP antigen levels were significantly higher in the dextran-treated patients 24 hours after operation compared with preoperative levels ($13.59 \pm 3.3$ µg/mL vs $6.83 \pm 2.6$ µg/mL, $P = .01$).

**Fig 2.** The effect of dextran-40 infusion on plasma levels of fibrinolytic factors. Patients were randomized to 100 mL of 10% dextran-40 (n = 10; closed circles, solid line) or 100 mL of saline (n = 10; open circles, dashed line). The data are shown as mean ± standard error of the mean. A, Tissue plasminogen activator (tPA) antigen. B, Plasmin-antiplasmin (PAP) antigen. C, Plasminogen activator inhibitor type 1 (PAI-1) antigen. D, PAI-1 activity.
The effect of dextran-40 infusion on plasma levels of functionally active von Willebrand factor (vWF). Patients were randomized to 100 mL of 10% dextran-40 (n = 10; closed circles, solid line) or 100 mL of saline (n = 10; open circles, dashed line). The data are shown as mean ± standard error of the mean.

μg/mL vs 6.21 ± 2.1 μg/mL; P = .043), indicating a rise in plasmin activity that was not seen in the saline group (Fig 2, B). Levels of PAI-1 antigen and activity increased significantly in both groups during the 24 hours studied (P ≤ .02), but this rise in activity was significantly greater in the dextran group, reaching 38.16 U/mL compared with 22.75 U/mL PAI-1 activity in the saline group (P = .037; Fig 2, C and D).

Effects of dextran infusion on vWF activity. VWF was measured in an enzyme-linked immunosorbent assay (ELISA) that uses a monoclonal antibody to the GPIb binding site on vWF and which can therefore be used as an indirect assay for functionally active vWF.28 In line with previous reports we found a significant decrease in vWF activity at the intraoperative time point in the dextran group (P = .02), which was not seen in those patients receiving saline (P = .873). VWF activity increased significantly postoperatively in both groups (P = .002 and P = .024, respectively), reflecting release from damaged vascular endothelial cells and/or an acute phase response. However, the level of vWF remained significantly lower in the dextran-treated group (P = .008) compared with the saline-treated patients (Fig 3).

Effects of dextran on platelets. Incubation of whole blood with dextran in vitro had no significant effect on the platelet response to ADP, TRAP, or thrombin (Fig 4), measured as the binding of fibrinogen to activated GPIIb-IIIa. This was in marked contrast to the effect of dextran on platelets in vivo. Platelets in samples taken from the patients showed a marked reduction in the response to thrombin after infusion. Although a reduction in response was seen in both groups that could be attributed to the effects of heparin, the percentage of platelets binding fibrinogen in the dextran group was significantly lower than in the saline group (11.1% ± 2.5% vs 41.2% ± 12.3%; P = .022), indicating an effect of dextran over and above the effect of heparin in these patients (Fig 5, A).

This difference in the response to thrombin was not seen when platelets were stimulated with TRAP (Fig 5, B). Although there was a significant difference in the response to TRAP between the groups, this was seen at all time points and appears to reflect an inherent characteristic of the groups that was maintained from the preoperation time point. No significant effect of dextran infusion was seen on fibrinogen binding in response to ADP. Similarly, platelet aggregation in response to ADP, collagen, or TRAP was not significantly affected by dextran infusion (data not shown). Aggregation in response to thrombin was not measured in this study.

DISCUSSION

Clinically, dextran is used primarily as a plasma expander, yet there is clear evidence in the literature that it also has an antithrombotic effect in vivo. This has proved beneficial in the context of prevention of deep vein thrombosis29 and in carotid surgery, where it has been used to treat patients with high levels of postoperative embolization.30 By the systematic use of transcranial Doppler monitoring to identify patients who generate high numbers of emboli, targeted dextran treatment in these patients has reduced the rate of postoperative thromboembolic complications to zero.5,21,30 In those patients who were treated with a bolus of 20 mL of 10% dextran-40, followed by an infusion of 20 to 40 mL of 10% dextran-40 per hour, the rate of embolization fell rapidly within the first hour and reached levels below the clinically defined threshold of 20 emboli per hour ≤2 to 3 hours.9 However despite the clinical benefit, the antithrombotic mechanism of dextran is not well understood.
Taken together, the data from reports of the past 50 years indicated that the antithrombotic effect of dextran seen in vivo cannot be replicated in vitro. The various pieces of evidence point to a number of potential modes of action involving platelets, vWF, and the fibrinolytic system. These disparate observations were clarified by the report of Noorman et al, who demonstrated that in rats dextran binds to the mannose receptor on endothelial cells and in so doing blocks the uptake of tPA from the circulation.

That tPA is a potent fibrinolytic agent in vivo is evidenced by the therapeutic benefit of rtPA and its derivatives in restoring blood flow in occluded arteries. The level of tPA in the blood is carefully controlled in a number of ways; by regulated synthesis and release from the vascular endothelium, through inhibition by binding to PAI-1, which is present in the plasma in large excess, and by rapid clearance in the liver. Two receptors for tPA are found in the liver; the low-density lipoprotein (LDL) receptor-related protein on liver parenchymal cells, and the mannose receptor, which is found predominantly on liver sinusoidal endothelial cells.

TPA is a serine protease that activates fibrinolysis by converting plasminogen to plasmin. Small elevations in levels of active tPA in plasma would lead to increased generation of plasmin in vivo, which would have the effect of enhancing fibrinolysis though the cleavage of cross-linked fibrin by plasmin. However, plasmin has a number of other substrates, including vWF and the platelet protease-activated receptor-1 (PAR-1) thrombin receptor on human platelets, leading to desensitization of the platelet response to thrombin. Cleavage of vWF multimers and reduction of vWF-dependent shear-mediated platelet interactions have been reported after thrombolytic therapy with tPA.

The present study allowed us to explore the mechanism of dextran at the doses used to treat CEA patients and to demonstrate clearly that the effect was systemic and associated with an increase in plasmin activity in vivo. Our findings support a mechanism involving blockade of tPA uptake by mannose receptors, previously demonstrated in rats, and help to clarify the various somewhat disparate findings of the other effects of dextran in the literature. Thus, dextran infusion led to an increase in plasmin generation (evidenced by an increase in PAP complexes), resulting in increased fibrinolysis (evidenced by the increased lysis of Chandler loop thrombi) as well as degradation of vWF (as evidenced by reduced levels in the ELISA) and desensitization of the platelet response uniquely to thrombin.

Together, these effects would have an overall antithrombotic effect in vivo; higher levels of plasmin would result in more rapid breakdown of thrombus that forms on the carotid endarterectomy site, breakdown of vWF multimers would have the dual effect of a reduction of platelet adhesion to the vessel wall and loss of stability within the thrombus (as reported by Aberg and Rausing); cleavage of the platelet PAR-1 receptor would lead to a loss of response to thrombin, but not to other agonists, including TRAP, because plasmin cleaves the thrombin activation peptide from the PAR-1 receptor but not its ligand binding site.

Although we did not detect a rise in the level of tPA in the patients, this can be explained by the short half-life of both free tPA and the tPA-PAI-1 complex in vivo, which is of the order of 5 to 6 minutes. For example, in the study of Noorman et al in rats, the rise in endogenous tPA after dextran infusion had dropped to baseline levels by 45 minutes. However, there was good indirect evidence of the effects of an elevation in tPA, indicated by the rise in plasmin-antiplasmin complexes, which are a marker of a previous rise in plasmin. Because these complexes have a longer plasma half-life than tPA, they were more readily detectable. Although this observation, and the significant increased levels of PAI-1 antigen, would seem to contradict the findings of Eriksson and Saldeen, who saw a rise in tPA and a fall in PAI-1 in patients receiving dextran-70 during surgery, the timing of the samples differed from the present study. The rise in PAI-1 that we observed in the postoperative samples in the dextran- but not saline-treated
patients may be partly a reaction to previous increases in tPA. Overall, these increases in PAI-1 did not contribute to a reduction in fibrinolysis, which returned to preoperative levels in both groups in the postoperative samples.

The maximal effect of dextran has been reported to be seen at 4 to 6 hours; thus, the effect seen in the present study after an infusion of 1 hour may be submaximal. Despite this, ≤1 hour of infusion of low-dose dextran produced a 13-fold increase in ex vivo fibrinolysis, which is comparable to the effect of 6 to 7 μg/mL rtPA added to the blood before thrombus formation. It would be interesting to analyze the blood at more time points after infusion, to monitor the time course of fibrinolysis through the appearance of fibrin degradation products and D-dimer, and to look directly at the cleavage of platelet PAR-1 and vWF multimers, but multiple sampling was not possible in this patient group. A more detailed study of the effects of dextran in volunteers, in the absence of confounding clinical and therapeutic factors, may be warranted.

CONCLUSION

Our findings provide a mechanistic explanation for the beneficial effects of dextran in reducing thromboembolic events in patients undergoing CEA. They also suggest that these low doses of dextran may provide a useful, low-cost adjunct to other therapies for reducing thromboembolic complications.

AUTHOR CONTRIBUTIONS

Conception and design: CJ, DP, PH, AG
Analysis and interpretation: CJ, DP, AG
Data collection: CJ, DP, PB, MT
Writing the article: CJ, AG
Critical revision of the article: DP, PH, AN, PB, MT
Final approval of the article: CJ, DP, PH, AN, PB, MT, AG
Statistical analysis: CJ
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Overall responsibility: AG

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