Effects of Long-term Administration of High-dose Recombinant Human Antithrombin in Immunosuppressed Primate Recipients of Porcine Xenografts

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Background. Fibrin deposition is central to the acute humoral rejection process occurring in the presence of consumptive coagulopathy when pig organs are transplanted into primates.

Methods. To assess whether strategies aimed at preventing fibrin formation may extend xenograft survival, we administered high daily doses of recombinant human antithrombin (rhAT) (500 U/kg twice daily) to obtain both anticoagulant and anti-inflammatory effects in immunosuppressed primate recipients of porcine kidneys.

Results. Some degree of consumptive coagulopathy developed in both rhAT-treated (n=3) and untreated (n=3) primates. No major differences in the coagulation parameters analyzed were observed between the 2 groups. Similarly, no difference in survival was seen between rhAT-treated (20.6±4 days; range: 15–23 days) and untreated animals (17.3±11.6 days; range: 7–30 days), although the rhAT-treated primates had a higher bleeding tendency. Despite the high daily dose of rhAT, considerable fibrin deposition was observed in the graft as early as 2 weeks after transplantation.

Conclusions. These results suggest that a high daily dose of rhAT fails to influence survival or prevent fibrin formation and deposition in the graft in our pig-to-primate model. However, the potential role of rhAT administered in combination with heparins or other clotting inhibitor concentrates in this model remains to be determined.

Keywords: Recombinant antithrombin, Xenotransplantation, Coagulopathy, Humoral rejection, Pig, Primate.

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Clotting cascade activation is central to the rejection process that occurs when pig organs are transplanted into primates (*1–4*) and the concomitant disseminated intravascular coagulation (DIC) frequently observed by some researchers may represent an insurmountable obstacle to the survival of primate recipients of porcine xenografts. Recent work from our laboratory has demonstrated that various degrees of compensated consumptive coagulopathy may indeed occur in the pig-to-cynomolgus monkey model (*5*), but neither we (*5*, *6*) nor several other authors (*7–12*) were able to confirm the high prevalence of DIC reported elsewhere (*1*, *3*, *4*, *13*), even when baboons were used as the recipient species (*11*, *12*). On the other hand, there is no doubting that, irrespective of the vascularized organ transplanted or the recipient species considered, fibrin deposition is a consistent finding in organs damaged by acute humoral xenograft rejection (AHXR) (*14*). Therefore, strategies blocking fibrin formation should prevent fibrin deposition in the graft and potentially extend the survival of the transplanted organ.

Several approaches to inhibit fibrin formation have been described, one of which is represented by the use of serpin inhibitors, such as antithrombin (AT) concentrates, either derived from plasma or in their recombinant forms (*15*, *16*). These compounds are known to block several clotting cascade factors (*15*). In particular, they are able to inhibit thrombin with limited bleeding tendency, thereby offering a unique opportunity to prevent fibrin deposition in the graft.

It has also been shown that AT possesses anti-inflammatory properties (*17*, *18*). In the presence of heparin, protease inhibition by AT is increased by several orders of magnitude (*19*). *In vivo*, AT binds to glycosaminoglycans, which can be considered as heparin-equivalent molecules located on the surface of endothelial cells (*19*).

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Cowan and colleagues recently reported that daily treatment with recombinant human AT (rhAT) can considerably extend the survival of nephrectomized baboons receiving life-supporting renal xenografts (*20*). However, these studies were conducted in non-immunosuppressed xenograft recipients.

The scope of this study was to determine the potential utility of long-term administration of natural coagulation cascade inhibitors in prolonging the survival of immunosuppressed primate recipients of porcine xenografts. To this end, we have treated a group of cynomolgus monkeys and evaluated clotting parameters after exposure to long-term treatment with high-dose rhAT to obtain both anticoagulant and anti-inflammatory effects.

MATERIALS AND METHODS

Animals

All experiments and procedures were conducted in accordance with the Italian Animals Act (Law No. 116 of the 27/1/1992) and were authorized by a special Decree of the Italian Ministry of Health. Six ABO-matched Large White Landrace hDAF transgenic pigs (Imutran-Novartis) (*21*), 4–13 weeks of age and weighing between 5.2 and 16 kg were used as kidney donors. Six 4–5 year-old purpose-bred male cynomolgus monkeys (*Macaca fascicularis*) from Mauritius, weighing between 4.0 and 7.5 kg were used as recipients in the transplant studies. Three xenografted animals received rhAT (Group A), while the other three served as controls (Group B). Three additional cynomolgus monkeys from Mauritius, weighing between 3.9 and 4.5 kg, were used for the pharmacokinetic studies.

Renal Xenotransplantation

The surgical procedure and the immunosuppression used have been described in detail elsewhere (*6*, *10*). Briefly, each primate was given immunosuppression consisting of up to 4 doses of cyclophosphamide iv. perioperatively, cyclosporine A, steroids and sodium mycophenolate. All xenotransplant recipients were also pretreated with GAS914 (Novartis Pharma AG, Basel, Switzerland), an injectable polymer expressing the carbohydrate moieties Galα1-3Galβ1-4GlcNAc-R, at a dose of 1 mg/kg sc on days -3, -2, -1 and on the day of transplantation (day 0) (*22*). In animal W861 with high levels of hemolytic anti-pig antibodies (APA) notwithstanding the treatment with GAS914, the monkey's blood was perfused through an *ex-vivo* perfusion loop that included one of the donor's kidneys. After one hour reperfusion, significant reduction of APA levels was achieved and the second kidney was transplanted. Bi-monthly protocol biopsies were performed in rhAT treated animals in accordance with the requirements of the local Animal Care and Use Committee. Rejection was defined as an episode of deterioration in graft function (i.e. as an increase in serum creatinine concentration by at least 20% with or without oliguria) in the absence of any sign of technical causes of graft dysfunction and was treated with a 3–5 day course of steroids (*8*).

Pharmacokinetics and Treatment with Recombinant Human Antithrombin

Because it has been suggested that AT only confers a clinical advantage if its plasma levels are kept consistently above normal physiological levels (*23*), we performed a kinetic study on three cynomolgus monkeys with a view to designing the optimal rhAT (kindly donated by GTC Biotherapeutics, Framingham, MA) administration schedule for primates.

Two cynomolgus monkeys were administered a single dose of 500 units (U) per kg iv. while one animal received a single dose of 1000 U rhAT/kg. Blood samples were taken before infusion and after 5, 15, 30 min and 1, 2, 4, 8, 12 and 24 hr. Plasma samples were analyzed using a colorimetric *in vitro* assay (Roche Diagnostic) to determine AT activity levels. rhAT levels were also determined in urine samples collected before and then 6 and 12 hr after infusion by both colorimetric assay and by electro-immunoassay according to Laurell.

After transplantation, each animal in Group A received a dose of 250 U rhAT one hour after reperfusion. After four to six hours, the animals received additional rhAT to achieve a total dose of 500U/kg. Then 500 U/kg rhAT were administered twice daily to 2 animals in this group (animals W869 and Y222), while the third (animal W861), was administered rhAT at the same dose for the first 5 days postoperatively, then only in the event of any deterioration in graft function (i.e. on demand). No rhAT was administered to the animals in Group B.

Coagulation Studies

Blood samples were drawn and stored as previously described (*5*). Similarly, activated partial thromboplastin time (aPTT), fibrinogen, antithrombin, protein C (PC) antigen, platelet counts, D-dimer (DD), thrombin-antithrombin (TAT) complexes total and free Protein S antigen and prothrombin $F_{1+2,}$ were determined as previously reported (*5*).

Prothrombin Time

Prothrombin time (PT) was determined using Tromborel S Kit (Dade Behring, Marburg, Germany) on an automated coagulometer analyzer (Behring Coagulation Timer) following the manufacturer's instructions.

Prothrombin

Prothrombin antigen (Ag), also known as Factor II Ag, was evaluated by ELISA, as explained elsewhere (*24*). A reference curve was obtained using pooled plasma from 5 healthy cynomolgus monkeys (from Mauritius and the Philippines). Normal ranges obtained in 9 healthy cynomolgus monkeys from Mauritius were from 0.3 to 1.8 arbitrary units (AU).

Factor X

Factor X Ag was evaluated by ELISA as described for prothrombin Ag using anti-Factor X polyclonal antibodies (*25*). Normal ranges were from 86 to 156%.

Antithrombin Antigen

This assay was performed using the ELISA for Antithrombin (Affinity Biological, Ancaster, Ontario, Canada) according to the manufacturer's instructions.

Anti-Factor Xa Activity

Anti-factor Xa activity was determined using Spectrolyse heparin (Xa) (Biopool International, Umea, Sweden) on

the automated coagulometer analyzer (ACL3000, IL) following the manufacturer's instructions.

Diagnosis of Disseminated Intravascular Coagulation

Overt DIC was diagnosed on the grounds of clinical and laboratory findings as previously reported (*5*, *26*).

Histopathology and Immunohistochemistry

Tissue sections from all explanted kidneys were stained and examined as reported elsewhere (*7*). A thorough macroscopic and histopathological evaluation was also undertaken of the kidney, lung, liver, stomach, small intestine, large bowel, pancreas, heart and bladder explanted from each primate involved in the study.

RESULTS

Pharmacokinetic Studies on Cynomolgus Monkeys

Following the administration of 500 U/kg rhAT to animals Y214 and W917, AT reached its peak activity in the blood after 5 min (Fig. 1). This activity was approximately 10 times the level observed before injection. Similarly, at the 1000 U/kg dose (W861), peak AT activity levels were also observed after 5 min reaching levels approximately 20 times the baseline value. At either dose, the peak was followed by progressive decline in AT levels. The half-life of AT in the blood was approximately 4 hr, irrespective of the dose administered. At 12 hr, the AT levels were still >160% in both animals receiving 500 U/kg, and more than 300% following treatment with 1000 U/kg. In all cases, elevated AT levels were still present at 24 hr. rhAT was not detected in urine at any time point in any of the animals treated (data not shown).

Transplantation Studies

Mean survival times were 20.6±4 days (range: 15–23 days; median: 23 days) and 17.3±11.6 days (range: 7–30 days; median: 16 days) in Groups A and B, respectively. Recipient survival time, reasons for euthanasia and graft histology at autopsy are given in Table 1.

In Group A, animals W869 and Y222 had excellent initial graft function with creatinine reaching a nadir of 92 and 67 μmol/L on days 4 and 7, respectively. In contrast, animal W861 had poor initial graft function due to intra-abdominal hemorrhage requiring re-operation on day 1. After surgical repair, the animal showed a prompt clinical improvement and recovery of graft function with a progressive increase in urine output resulting in normal creatinine levels (113 μmol/L) by day 4. The creatinine levels after transplantation are shown in Figure 2A. Each of these animals had 2 or 3 rejection episodes.

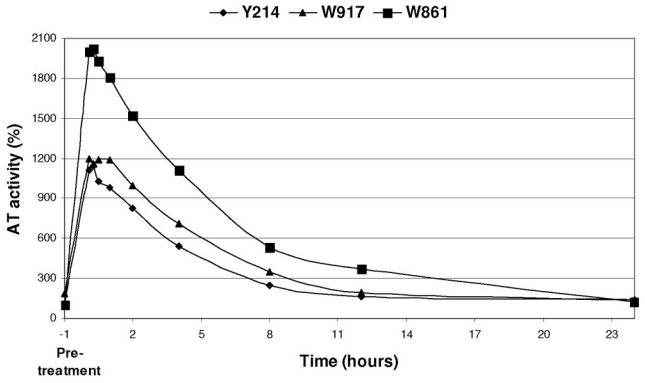

FIGURE 1. Kinetics of AT activity in the plasma following administration of a single dose of 500U (W917, Y214) or 1000U (W861) rhAT/kg in naive cynomolgus monkeys.

It is noteworthy that, during the postoperative period, all the animals in Group A had episodes of petechiae and/or hematoma, starting as early as day 5 and requiring between one and four transfusions. In addition to skin lesions, bleeding gums were observed in animal W869 from day 20 onwards. Animal Y222 also had repeated episodes of bleeding from its gums starting as early as day 2, and hematuria from day 20. This animal was sacrificed due to anuria secondary to the presence of a clot in the bladder preventing urine outflow.

At euthanasia, there was evidence of a tendency for bleeding in all Group A animals. In particular, animal W869 had melena and bilateral hemorrhagic infiltration of the psoas muscles. Animal Y222 had diffuse cutaneous and lung petechiae, various subcutaneous hemorrhagic collections, melena and a large clot above the kidney (possibly related to the previous biopsy). Animal W861 had serohemorrhagic ascitic fluid, hematoma in the middle third of the tongue, various subcutaneous hemorrhagic collections and clots in the bladder.

In Group B, animals Y217 and W926 had an excellent initial graft function with creatinine reaching a nadir of 51 and 105 μmol/L on day 4 (Fig. 2A). Animal Y071 also had a good initial graft function (creatinine: 153 μmol/L on day 2), but its creatinine levels never returned to normal. Each of these animals had one to three rejection episodes.

Unlike Group A, only limited hemorrhagic lesions were observed in two of the animals in this group during the postoperative period. In particular, petechiae were observed on the abdomen of animal Y217 from day 14 to day 17. This animal also had an episode of melena on day 30. In addition, some bruises were also apparent from day 6 onwards in animal Y071, which underwent euthanasia on day 7 in the presence of DIC or drug-related toxicity. Y217 was the only animal in this group to require a postoperative transfusion.

At euthanasia, there was little evidence of any tendency to bleed among the animals in this group. In particular, Y071 had petechiae on the face and limbs. Cutaneous hemorrhage of the foreskin was observed in animals Y071 and W926, which also had extensive scrotal edema. An enlarged and partially hemorrhagic suprarenal gland was detected in animal Y217.

All animals included in the study (rhAT-treated and controls) were euthanized due to kidney failure between 7 and 30 days after transplantation. In all cases, a picture compatible with AHXR was seen in the explanted xenograft (Fig. 3). It is worth noting that postoperative protocol biopsies in two of the rhAT-treated animals (W869 and Y222) showed histological signs compatible with AHXR (particularly fibrin deposition, IgM, IgG and C5b-9 deposits in the glomeruli) already on day 14 (Fig. 3). However, C3 deposition in the graft was only observed in one of these 2 cases (Y222) (data

TABLE 1. Survival, cause of death, and histopathological findings

Group	Animal	Survival (days)	Reason for euthanasia	Biopsy at day 14	Histology at euthanasia
A (rhAT)	W869	23	Renal failure/multisystemic bleeding	Fibrin, IgM, IgG and complement deposition	AHXR (II)
	Y222	23	Renal failure/multisystemic bleeding	Fibrin, IgM, IgG and complement deposition	AHXR (II)
	W861	15	Renal failure/ multisystemic bleeding	ND	AHXR (II)
B (Controls)	Y217	30	Renal failure/ anemia	ND	AHXR(III)
	W926	16	Renal failure	ND	AHXR (II)
	Y071	7	Renal failure/DIC or Drug-related toxicity (?)	ND	AHXR (I)

AHXR, acute humoral xenograft rejection (Grade I=damage affects <20% graft; Grade II=damage affects 20–50% graft; Grade III=damage affects >50% graft); ND, not done; DIC, disseminated intravascular coagulation.

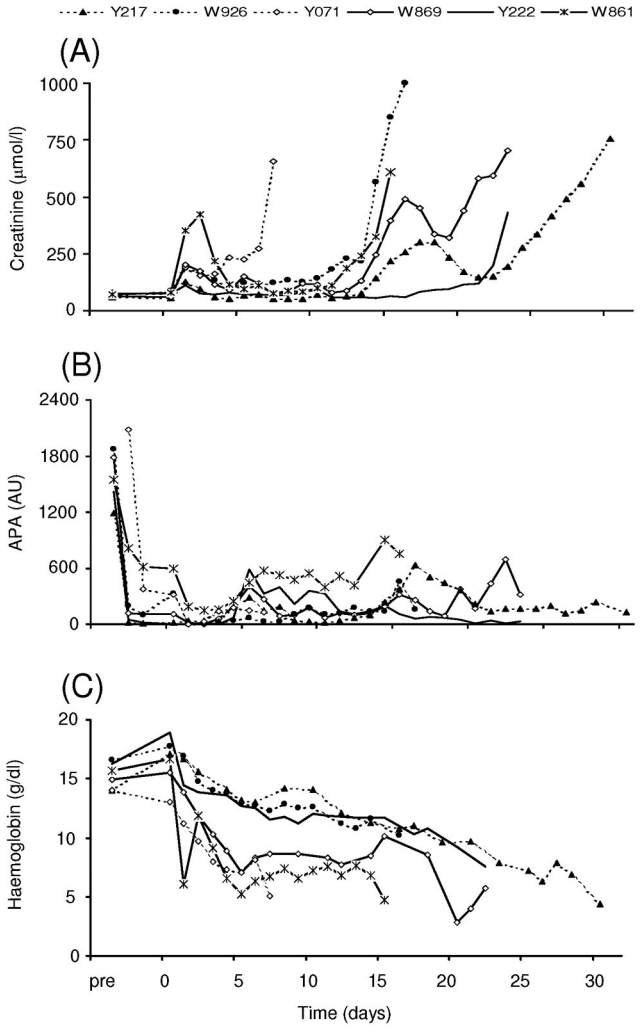

FIGURE 2. Daily levels of creatinine (A), hemolytic anti-pig antibody (APA) (B), and hemoglobin (C) in the six xenograft recipients. Solid lines represent AT treated animals whereas dotted lines refer to controls.

not shown). Clusters of platelets were observed within renal vessels of the explanted xenografts analyzed (see supporting material online).

Coagulation Studies

aPTT levels remained normal throughout the follow-up period in the Group A animals exposed to daily rhAT (Fig. 4A), whereas animal W861 (which was exposed to AT on demand) revealed a mild prolongation of aPTT in the immediate postoperative period, which became worse from day 7 onwards and deteriorated rapidly from day 13 onwards. In Group B, the two longest-surviving animals had mild aPTT prolongation despite the mean level appearing to be higher than in their treated counterpart (46.6±13.1 vs. 30.9±3.9, respectively). The third animal from this group (animal Y071) had normal aPTT levels until day 5, when an abrupt deterioration was associated with a possible DIC or treatment-related toxicity.

PT values increased in all animals after transplantation and remained generally stable (Fig. 4B). A slight prolongation of PT was seen in the last few days in animal W861, coinciding with the above-described aPTT prolongation. In the control group, PT mirrored the trend observed in the AT-treated subjects. In one case (animal Y071), PT deterioration was associated with a marked increase in aPTT.

As for Factor X Ag, five animals (two treated and three controls) had substantially similar profiles throughout the postoperative follow-up, with levels slightly reduced compared to pretransplant values (Fig. 4C). In contrast, high levels of Factor X Ag were observed throughout the postoperative period in the AT-treated animal that had the highest pretransplant values (W869).

Factor II Ag measurements remained substantially within normal pretransplant levels and no clear-cut difference was detected between treated animals and controls (Fig. 4D).

After an initial perioperative rise, fibrinogen levels usually returned to within normal ranges by the end of the second week (Fig. 4E). However, a rapid and irreversible drop was observable in the untreated animal that underwent euthanasia in the presence of DIC or drug-related toxicity (animal Y071).

Platelet counts showed a similar trend in both treated and control animals (Fig. 4F). Animal Y071's platelet counts dropped rapidly and reached a nadir of 13,000 cells/mm^3 by day 7.

With regard to anti-factor Xa activity, a sharp increase

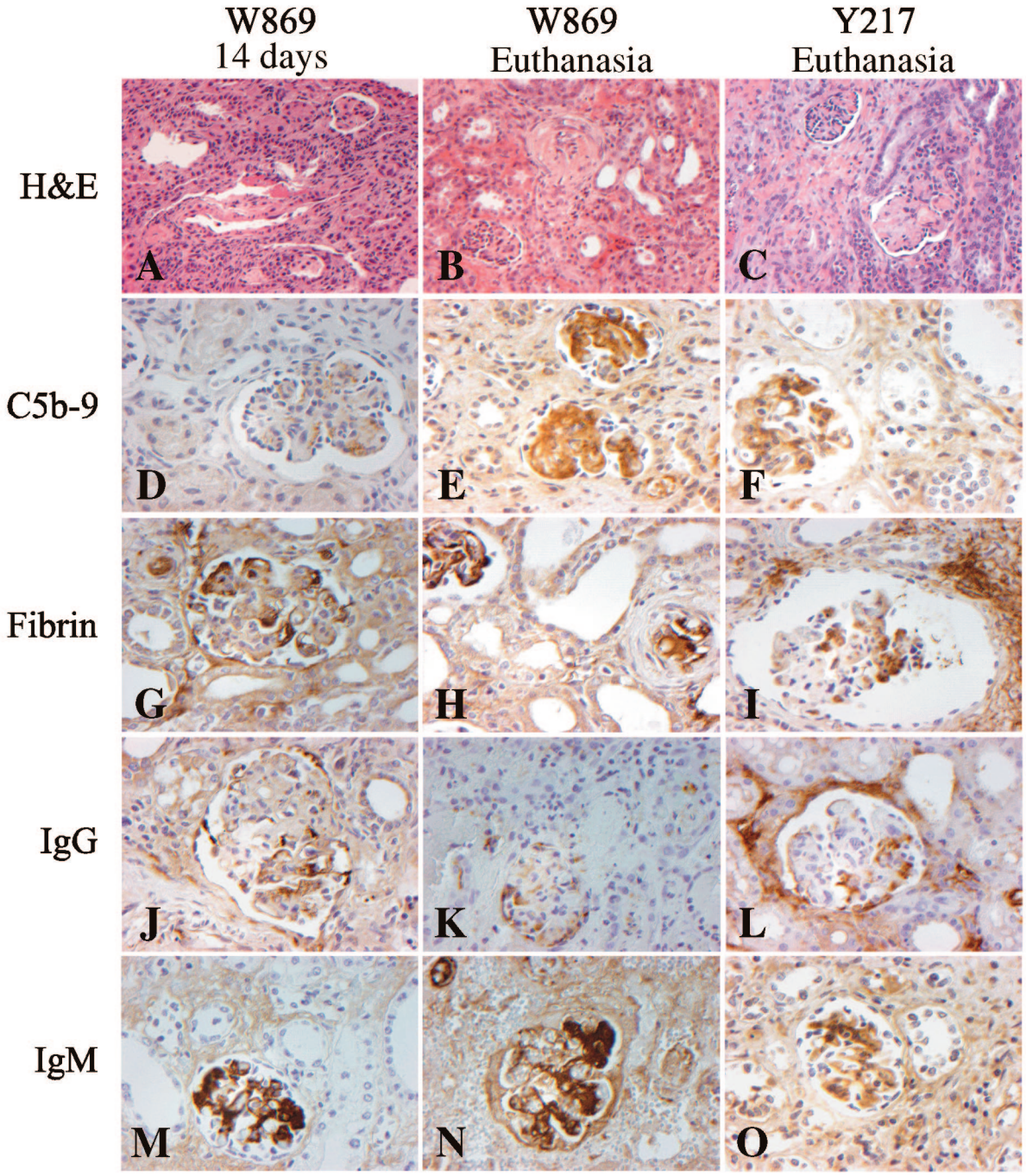

FIGURE 3. Hematoxylin and eosin (H&E) stains (A–C; original magnification, 50×) and immunopathology (D–O; original magnification, 100×) of hDAF kidneys from representative rhAT–treated (W869) and control (Y217) animals. Significant deposition of complement component C5b-9 (D–F), fibrin (G–I), IgG (J–L), and IgM (M–O) could be seen in both xenografts when explanted with AHXR. Such findings were already visible in the biopsy taken in rhAT-treated animals as early as 14 days after transplantation.

was seen in all cases perioperatively, coinciding with the intraoperative administration of heparin (Fig. 4G). Soon after xenotransplantation, variations in anti-factor Xa activity may be related to the early clinical management of the transplanted animals (infusions and flushing of iv. lines). After the first week, anti-factor Xa activity remained consistently higher in the AT-treated animals than in the controls. In one treated animal (animal W861), high levels of anti-factor Xa activity (0.5 U/ml) were recorded in the last few days before euthanasia; this finding was concomitant with a deterioration in other coagulation parameters, as described above.

Anti-factor Xa activity levels were also evaluated in animals Y222 and W869 before and then at 1 hr (in animal Y222 only), 3 and 6 hr after the morning administration of AT on

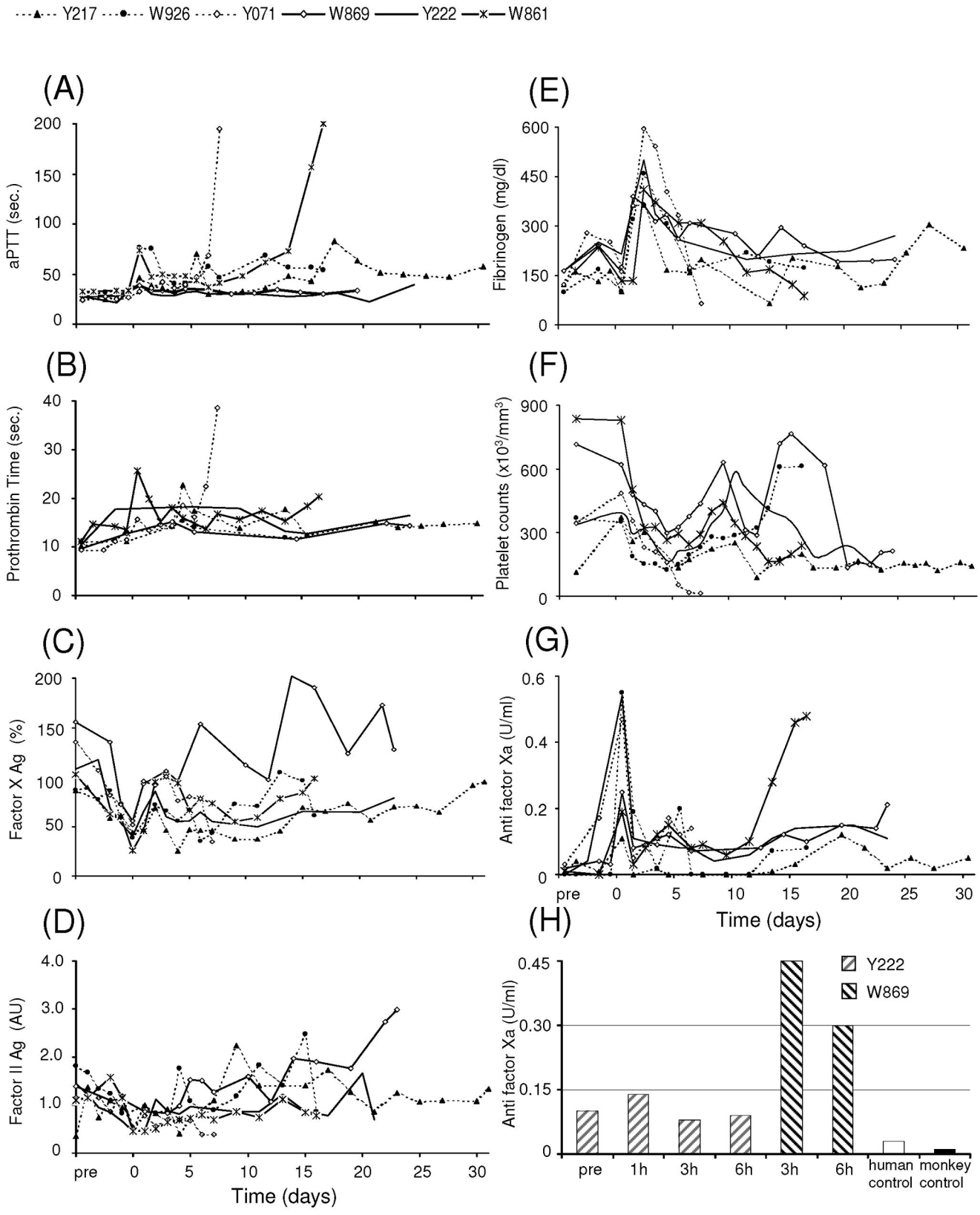

FIGURE 4. Daily aPTT (A), prothrombin time (B), factor X Ag (C), factor II Ag (D), fibrinogen levels (E), platelet counts (F) and anti-factor Xa activity (G) in the six xenograft recipients. Levels of antifactor Xa activity at 1 hr (in animal Y222), and at 3 and 6 hours in animals Y222 and W869 following the administration of AT is also presented (H). Pooled plasma from healthy monkeys (n=6) and humans (n=20) were used as controls. Solid lines represent AT treated animals whilst dotted lines refer to controls.

days 7 (Y222) or 8 (W869). As shown in Figure 4H, no significant changes in anti-factor Xa activity were seen in animal Y222, whilst levels up to 0.4 U/ml and 0.3 U/ml were seen 3 and 6 hr after rhAT administration, respectively, in the case of animal W869.

Figure 5A shows that activity AT levels in the animals in Group A were consistently above 150%, except in animal W861 where a different AT administration protocol was used. It should be noted that no rhAT was administered to this animal from day 6 to day 11. AT Ag substantially mirrored the AT activity levels in both treated and control animals (Fig. 5B).

Protein C Ag levels dropped significantly immediately after transplantation and either remained stable or recovered moderately during the follow-up (Fig. 5C). No clear difference was detectable for this parameter between the treated and control animals, nor was there any clear consumption in either group. The same was true for total Protein S (Fig. 5D). Free Protein S Ag remained within the normal range for the

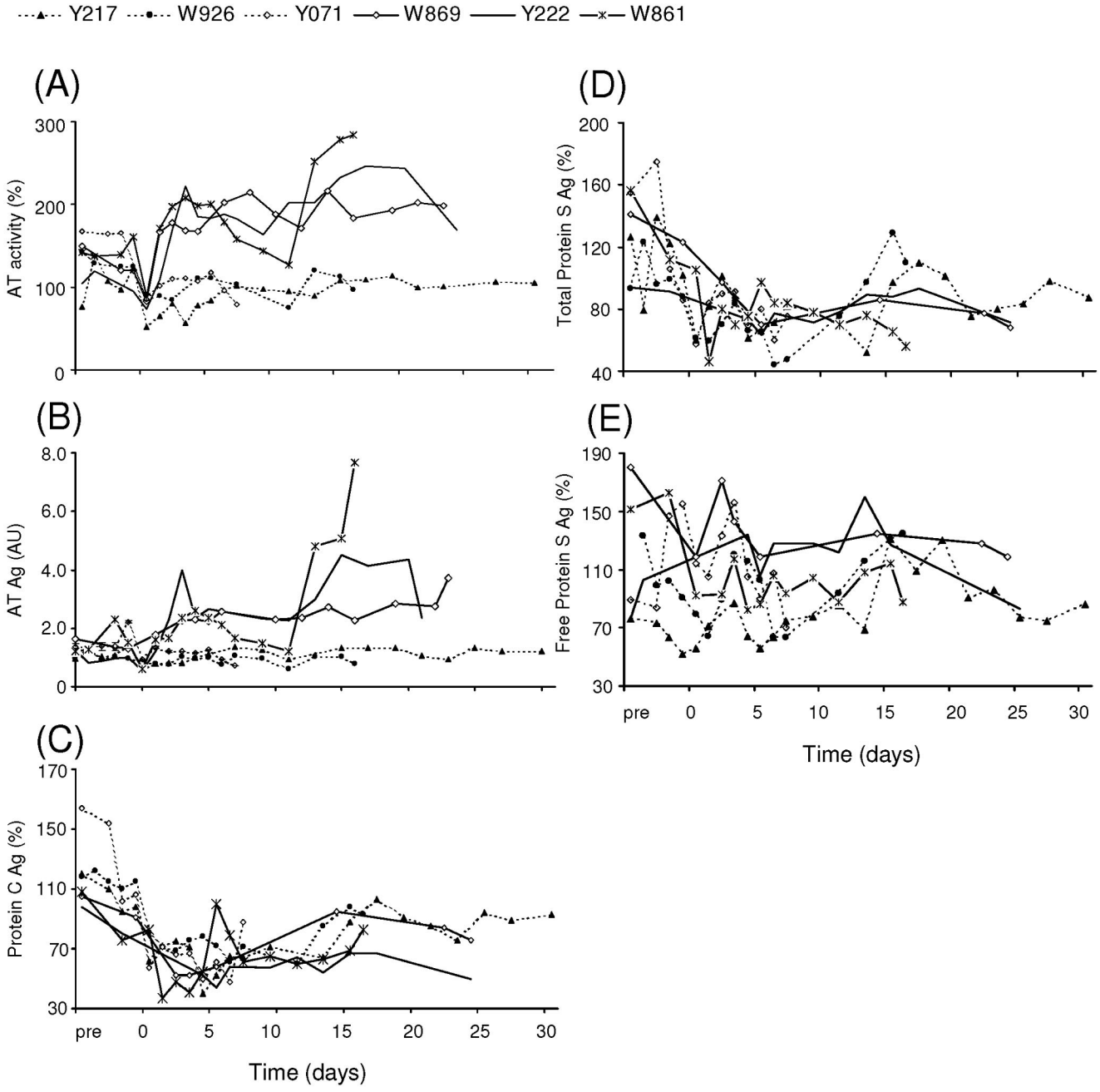

FIGURE 5. Daily levels of physiological clotting inhibitors in the six xenograft recipients: AT activity (A), AT Ag (B), protein C antigen (C), total protein S antigen (D) and free protein S antigen (E). Solid lines represent AT treated animals, whereas dotted lines refer to controls.

duration of the study, although fluctuations were observed (Fig. 5E).

As expected, DD levels increased rapidly immediately after transplantation and remained consistently high (up to >4000 ng/ml) throughout the follow-up, irrespective of AT administration and clinical outcome (Fig. 6A).

TAT levels tended to rise during the follow-up, with no apparent difference between treated and control animals (Fig. 6B). F_{1+2} increased after transplantation in both groups and remained higher than pretransplant values throughout the study (Fig. 6C). In the two weeks preceding euthanasia, animal W869 had high F_{1+2} levels.

Figure 2B shows the APA titers for the six animals. A marked reduction in APA levels followed the administration of GAS914 in each case. In one case (animal W861), however, the persistence of high levels of APA (greater than 500 AU) even after GAS914 treatment, led us to undertake *ex-vivo* antibody removal, as described above. No clear correlation emerged between APA titers and graft function in the postoperative period. Postoperative hemoglobin levels are given in Figure 2C. A rapid drop in hemoglobin levels was seen in two treated and one untreated animal. In one case (W861) this was due to a surgical complication.

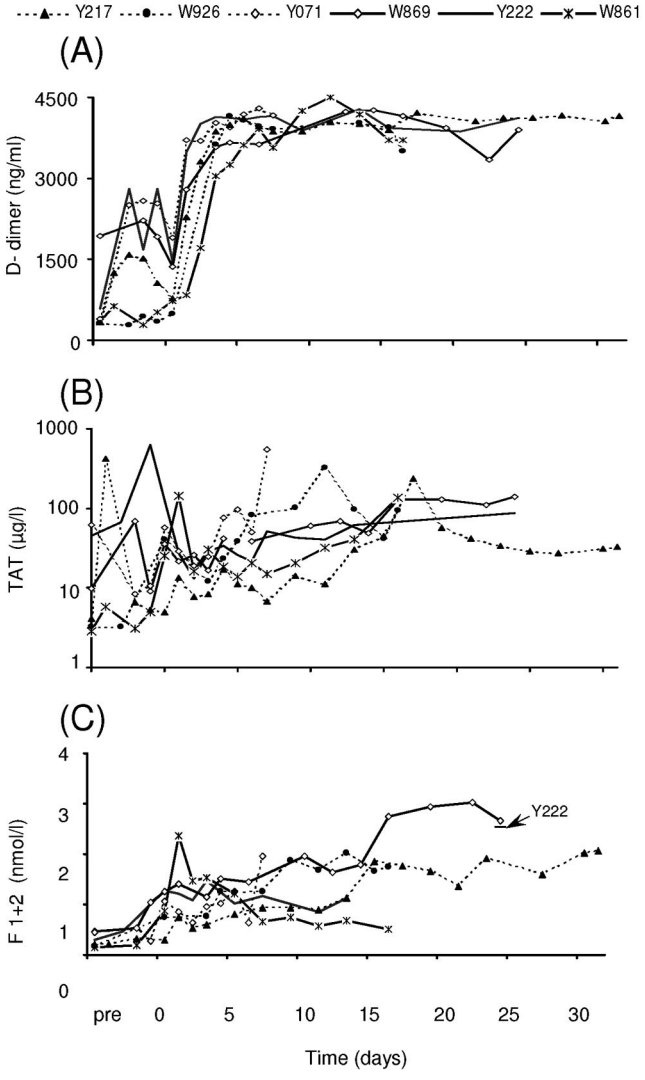

FIGURE 6. Daily levels of D-dimer (A), TAT (B) and F_{1+2} (C) in the six xenograft recipients. Solid lines represent AT treated animals, whereas dotted lines refer to controls.

DISCUSSION

Preventing fibrin formation appears to be crucial to avoid the occurrence of AHXR and ensure long-term survival of porcine xenografts transplanted into primates. High doses of rhAT proved remarkably effective in significantly prolonging the survival of non-immunosuppressed primate recipients of life-supporting pig organs, possibly by preventing systemic coagulopathy (*20*). In the present study, high-dose rhAT was administered daily to the immunosuppressed primate recipients of porcine xenografts in our experimental model. We assessed the capacity of rhAT to prevent fibrin deposition in the transplanted organ, and we explored its effects on the possible onset of coagulopathies and on the xenografted animals' survival.

Our results indicate that rhAT fails to prolong survival in our renal pig-to-cynomolgus monkey xenotransplantation model. Of the 3 rhAT-treated animals, the 2 given a twice-daily administration of the compound survived longer than the animal given rhAT on demand. Neither of the rhAT regimens used was associated with a coagulopathy consistent with overt DIC, but several animals in both the rhAT-treated and untreated groups had some degree of consumptive coagulopathy. A consumptive coagulopathy possibly associated with severe drug toxicity was observed in one animal belonging to the control group. Thus, unlike the pig-to-baboon model, where DIC has frequently been reported (*1–4*), there was no evidence of any real advantage of using rhAT to prevent consumptive coagulopathy or overt DIC in our pig-to-cynomolgus monkey model. Indeed, the majority of the coagulation parameters we explored did not differ substantially between the two groups, with the possible exception of aPTT.

Although survival was no different between the treated and control animals, clinical signs of a tendency for bleeding were more commonly observed in the treated animals. Accordingly, the rhAT-treated animals required more blood transfusions than controls. After the first postoperative week, anti-factor Xa activity was higher in the rhAT-treated group, although the levels were only slightly higher in the longest-surviving animals. These levels could reflect the effects of a small dose of heparin administered together with rhAT to keep the iv. lines patent. Increased anti-factor Xa activity was also seen in the controls where a similar clinical management protocol was applied. It is worth noting that the absolute anti-factor Xa activity levels in the rhAT-treated animals were, at most, as high as the lower limit considered therapeutic in humans exposed to standard or low-molecular-weight heparin, so excess anti-factor Xa activity could not, per se, account for the bleeding complications in the rhAT-treated animals.

There was no relationship between anti-Xa activity in rhAT-treated animals and the tendency to bleed, a finding consistent with the lack of excessive anticoagulation in the animals treated with high-dose rhAT. As a consequence, the bleeding complications in the rhAT-treated animals cannot be explained exclusively on the basis of the anticoagulant effects of rhAT.

It is noteworthy that both aPTT and PT were substantially normal throughout the study in the two animals exposed to continuous rhAT treatment and neither parameter enabled us to predict the impending hemorrhage. Bleeding tendency has been reported in humans receiving high-dose rhAT to treat DIC, possibly in relation to the concomitant administration of low-dose heparin that also prevents the anti-inflammatory properties of AT (*19*, *27–29*). For strategies requiring the prolonged administration of high daily doses of AT to be considered for potential clinical applications, the bleeding risk should be borne in mind and carefully evaluated as a potential drawback of such an approach.

It should be noted that our considerations apply only to the prolonged administration of very high doses of rhAT in the species considered, while no definite conclusions can be drawn on the possible bleeding tendency that may be associated with the use of rhAT at lower doses in humans.

The main purpose of the high doses of rhAT used in our study was to prevent fibrin formation and deposition in the porcine xenografts transplanted into the primates and to take advantage of the anti-inflammatory effects reported for rhAT (*18*).

Going against our expectations in the light of the known mechanism of action of AT, high daily doses of rhAT were unable to prevent fibrin formation and deposition in the graft. It surprised us to see that both the rhAT-treated animals undergoing protocol biopsies presented severe graft damage as early as day 14 (despite a normal graft function in one case). In particular, the biopsy revealed a considerable amount of fibrin deposition substantially similar to the situation found in the explanted graft. The inability to prevent fibrin deposition in our model using rhAT cannot be attributed to inadequate plasma levels after its administration. Our kinetic studies demonstrate that the quantity of rhAT infused was sufficient to maintain rhAT activity levels in the plasma consistently above 150% even 12 hr after administration, under which conditions both anti-inflammatory and antithrombotic effects were expected (*18*). On the other hand, the rhAT used in this study might not be as efficient in the primate species considered as in humans. Nonetheless, we could foresee no potential benefit from modifying the dosage in our model: a higher dose could exacerbate the bleeding complications, a lower one would probably be even less effective in preventing fibrin formation.

How can we explain the discrepancy between the findings reported by Cowan and colleagues in nonimmunosuppressed primates (*20*) and our data? Survival in nonimmunosuppressed animals probably depends primarily on the severity and rapidity of onset of a consumptive coagulopathy in the baboon species (*20*). The large amount of thrombin formed in the baboon model may be partially balanced by the inhibiting effect of high-dose rhAT, resulting in a milder coagulopathy and longer survival. In our model, where immunosuppression results in longer survival and we observe a less pronounced coagulopathy that only rarely progresses to overt DIC (*5*, *6*, *8–10*), the advantage of administering rhAT to prevent thrombin formation and progressive coagulopathy is probably lost.

Taken together, our data suggest that high-dose daily rhAT has no effect on AHXR prevention. Since AT activity requires interaction with the endothelial cell through glycosaminoglycans, we cannot yet rule out the possibility of endothelial surface damage due to xeno-directed immunological events being responsible for a reduced AT activity.

In conclusion, our studies indicate that high daily doses of rhAT do not prevent fibrin formation in our pig-to-primate xenotransplantation model. The lack of effect on survival is apparently unrelated to any bleeding complications, which were nonetheless more pronounced in rhAT-treated animals. The potential role of rhAT, in combination with heparins or other clotting inhibitor concentrates, in controlling the coagulopathies associated with xenotransplantation and preventing fibrin deposition in a xenograft remains to be determined.

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