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Renal and Hepatic Handling of Endogenous Tissue-Type Plasminogen Activator (t-PA) and Its Inhibitor in Man

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Key words

t-PA – PAI – DDAVP – Clearance

Summary

In eight male patients with normal liver and kidney function fibrinolytic components were measured in arterial blood and in renal and hepatic vein blood, obtained during catheterization for analysis of hypertension. Blood samples were collected simultaneously from veins und corresponding arteries before and 5 minutes after the completion of intravenous injection of desmopressin (DDAVP), 0.4 µg/kg body weight over a 10 minute period.

DDAVP induced a rise in t-PA antigen and activity, and in von Willebrand factor, accompanied by a decrease in free PA-inhibitor level. We failed to detect a significant rise in plasma urokinase activity. The concentrations of fibrinogen, plasminogen, α_2-antiplasmin, antithrombin III and coeruloplasmin did not change either.

Renal production of t-PA under basal conditions was inferred from a negative arterio-venous (A-V) difference in t-PA-activity and in t-PA-antigen levels but this could not be confirmed by orthogonal regression analysis of the same data. A-V differences of other fibrinolytic factors were negligible.

In the hepatic vessels a significant positive A-V difference of t-PA-activity and of t-PA-antigen levels was a uniform finding. After DDAVP, when plasma levels were elevated, the mean A-V difference was proportionally higher, consistent with a constant fractional elimination rate. Free PA-inhibitor was virtually absent from arterial blood after DDAVP, but appeared in hepatic vein blood, indicating either production of the inhibitor by the liver or dissociation of a circulating complex of t-PA and its inhibitor in the liver. The blood levels of the other investigated components did not show any change upon passage through the liver. Plotting of individual venous versus arterial levels of t-PA activity and of t-PA-antigen both before and after DDAVP injection yielded straight lines which in orthogonal regression analysis showed a slope significantly lower than unity; this was interpreted as net clearance of the activator. Clearance rate was not changed by DDAVP. Assuming a normal blood volume and hepatic blood flow in these subjects, the t½ of the disappearance of endogenous plasminogen activator over the liver can be calculated: t-PA-activity: 3.1 min, t-PA-antigen: 4.1 min. Since these figures are close to those found by others for the half-disappearance time of t-PA from the entire circulation, it is concluded that t-PA is eliminated from the blood mainly by the liver. The t½ of disappearance of endogenous, free PA-inhibitor is about 3 times longer, in our example 10.4 minutes.

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Introduction

The liver plays a fundamental role in the regulation of the fibrinolytic properties of the blood. On the one hand, several factors of the fibrinolytic system, for instance plasminogen and α_2-antiplasmin, are produced by the liver (1, 2). On the other hand, the liver has the capacity to clear the blood from fibrinolytic enzymes, among which tissue-type plasminogen activator (t-PA) as has been shown in animals (3–6).

The function of the kidney with regard to the homeostasis of fibrinolysis is less clear. Urokinase, the main fibrinolytic activator in urine, is produced in the kidney, but the functional level of pro-urokinase (u-PA) in blood is normal in anephric patients (7). Temporary sequestration of the kidneys from the circulation lowers the blood fibrinolytic activity, whether t-PA or u-PA, in rabbits (8) and in rats (9). In rabbits (8), in dogs (10), and in man (11–14) total fibrinolytic activity has been reported to be higher in renal vein blood than in arterial blood. This has been interpreted as evidence of renal contribution to the blood content of plasminogen activators. Apart from that, the kidney might well have the capacity of regulating the plasma level of low molecular weight PA-inhibitors (15), which might influence the measured fibrinolytic activity of the blood.

The aim of this study was to assess the renal and hepatic contributions to the synthesis and clearance of endogenous fibrinolytic activators in man. Previously, information on the clearancee had to be inferred from measurements of the apparent half-life of the activators in the circulation. This was carried out by artificially increasing the plasma level, either by applying stimuli for release of endogenous activators, e.g. by exercise, or by injecting the purified and radiolabeled substance into the circulating blood, and – in both cases – by subsequent measuring the time-dependent decay of the concentration or label in venous blood. Determination of the half-life by injection of a substance is complicated by degradation of the substance and reappearance of labeled metabolites. Moreover, the measured half-life is an overall estimate of several factors, viz. distribution over an extravascular compartment, binding to inhibitors and specific clearance of the blood in possibly more than one organ. After exercise and other stimuli of release of endogenous t-PA, the decay of the increased blood level may be influenced by prolonged action of a mediator or even by reactive synthesis. We circumvened most of these problems by measuring directly the concentration gradient of endogenous fibrinolytic activators that are maintained over the kidney and the liver. We had the opportunity of performing fibrinolytic assays on blood samples obtained from the hepatic and renal veins and from the corresponding arteries of patients subjected to routine catheterization for analysis of hypertension. The results provided a possibility to calculate the elimination rate of the fibrinolytic activators over the separate organs and thereby allowed conclusions on the relative contribution of these organs to the total elimination rate of the substances from the circulation.

Patients, Materials and Methods

Patients

The study was performed on eight male patients, aged 25–63 years, with hypertension. The blood pressure ranged from 167–195 mmHg (systolic), and 103–125 mmHg (diastolic); the highest creatinine level was 123 µmol/l. A complete routine check-up had not revealed a cause of the hypertension in these patients. Therefore, a catheterization was done with the aim of studying the renin/angiotensin system before and after intravenous injection of desmopressin acetate (DDAVP), a vasopressin-derivative which is known to stimulate the renin-angiotensin system (16, 17). In the blood collected during this procedure for measurement of renin concentration, fibrinolytic parameters were measured. Routine renal and liver function tests were normal in each case. In all of them the hypertension was eventually classified as essential. The patients gave their informed consent to the procedure.

Catheterization Procedure and Blood Sampling

On the 8th day of a standardized regime (3 g NaCl/day) within the hospital and after a period of at least 14 days free from medication, the patients were subjected to the catheterization procedure after an overnight fast. Catheters were flushed with heparin, 5 IU/ml, before insertion and left in place for 30 minutes before collection of the first blood samples in order to avoid a stress reaction. After rinsing the catheter with sterile saline, venous blood was collected from the right and left renal vein and from a hepatic vein; arterial blood was sampled from the aorta at the site of the branching-off of the renal arteries and the coeliac artery, respectively (Fig. 1). Sampling of venous and the corresponding arterial blood was done by two people at exactly the same time. The sampling was repeated in the same sequence, precisely 15 minutes later, i.e. 5 minutes after completion of intravenous injection of desmopressin (DDAVP, 1-desamino-8-D-arginine-vasopressin, Ferring, Malmö, Sweden, 4 µg/ml), 0.4 µg/kg body weight, undiluted into an arm vein in the space of 10 minutes. Blood was collected in 0.11 M sodium-citrate (9:1); platelet-poor plasma was prepared immediately by centrifugation in a refrigerated centrifuge at 4° C for 20 minutes at 2000 g and stored at −70° C.

t-PA-antigen was measured with an enzyme immuno-assay (Rijken et al. 18).

t-PA-activity was measured by application of redissolved euglobulin fractions on plasminogen-rich bovine fibrin plates in the presence of C1 inactivator (19); results are expressed in square millimeters of lyzed area.

PA-inhibitor was determined by titration of 1:20 diluted plasma with purified t-PA and spectrophotometric assay of the resulting activity as described by Verheijen et al. (20). The inhibition level was extrapolated from the activity curve and expressed as percentage of the inhibiting capacity of a pool of 10 normal donors (21).

Plasma-urokinase (u-PA). activity was estimated indirectly on plasminogen-rich bovine fibrin plates by the quenching effect of anti-urinary urokinase antibodies on the fibrinolytic activity of euglobulin fractions prepared in the presence of dextran sulphate (22). Results are expressed in arbitrary units, read from a standard curve of lysis zones obtained with dilution of a similar euglobulin fraction prepared from pooled normal plasma (23).

Plasminogen was measured immunologically on Partigen plates (Behringwerke, Marburg, W. Germany) and functionally after addition of streptokinase (Kabikinase, Kabi, Stockholm, Sweden) with a chromogenic substrate (S-2251, Kabi, Stockholm, Sweden), according to the manufacturer's instruction.

α_2-antiplasmin and *antithrombin III* were measured in an amidolytic assay using KABI-kits (Kabi, Stockholm, Sweden), according to the manufacturer's instruction.

Fibrinogen was estimated immunologically by radial immunodiffusion on NOR-Partigen plates (Behringwerke, Marburg, W. Germany).

Factor VIII-related antigen (VIIIR:Ag) was measured by rocket immunoelectrophoresis as described earlier (22), using Clot-immun AHG, associated protein antibody (Behringwerke, Marburg, W. Germany).

α_2-macroglobulin and *coeruloplasmin* were measured immunologically by radial immunodiffusion on NOR-Partigen plates (Behringwerke, Marburg, W. Germany).

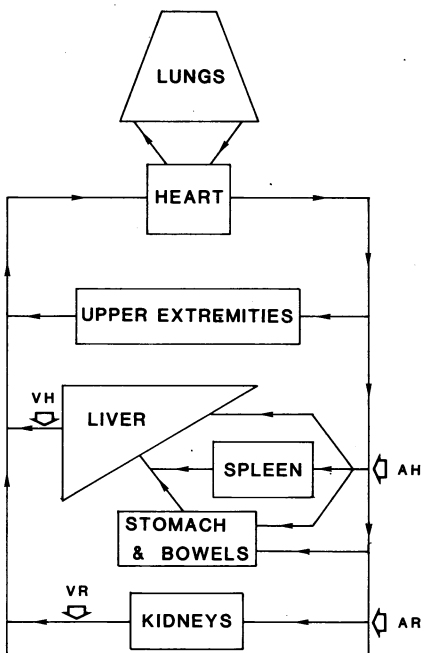

Fig. 1 Diagram of the circulation with indication of the sampling sites: AH = hepatic artery; VH = hepatic vein; AR = renal artery; VR = renal vein

Statistics

Results from measurements in blood obtained from left and right renal arteries and veins, each determined in triplo, were compared by Student's t-test on paired observations. As there were no significant differences ($\alpha < 0.05$) the values of right and left vessels were taken together for further calculations.

The results of the assays of fibrinolytic components in arterial and in venous blood were analysed in two different ways:

I. Arterio-venous (A-V) differences of each individual were obtained by subtraction of the values measured in simultaneously collected arterial and venous blood samples. Whether the average A-V differences differed from zero was tested by parametric (Student's t-test) and by non-parametric methods (Wilcoxon's rank test). A positive difference indicates net clearance, a negative suggests net production of the substance in the organ. However, since the liver is constituted of different types of cells, some of which may remove a substance from the blood stream while others at the same time may give off the substance, due to synthesis or release, the values obtained by this way of analysis, i.e. the net results, obscure the separate contributions.

II. The wide range of concentrations and enzyme activities in arterial and corresponding venous blood samples encountered in our subjects also allowed regression analysis. Since both arterial (C_A) and venous (C_V) levels are to be regarded as stochastic variables, orthogonal regression analysis was carried out (25). On theoretical grounds it is expected that the concentration gradient that is maintained over the organs, obeys the relation:

$$C_{V_i} = a \cdot C_{A_i} + b \qquad (1)$$

in which the subscript i indicates the individual levels. The slope (a) stands for the average fraction of C_A which has passed through the organ, irrespective of concomitant synthesis ($0 \leq a \leq 1$), and the intercept (b) for the average concentration increment by synthesis, irrespective of concomitant clearance ($b \geq 0$).

Calculation of $t^1/_2$ of Disappearance over Separate Organs

The elimination rate of a substance in an organ can be calculated according to Fick's principle which states that the amount of a substance taken up by an organ per unit of time is equal to the arterial level of the substance minus the venous level (A-V difference) times the blood flow (26). In a formula:

$$\text{elimination rate}_i = (C_{A_i} - C_{V_i}) \cdot v \qquad (2),$$

Table 1 Levels of fibrinolytic factors, inhibitors of fibrinolysis and coagulation, and levels of some other proteins and renin activity in arterial blood (mean of three samplings from both renal arteries and the coeliac artery) just before and 5 minutes after the completion of intravenous injection of DDAVP 0.4 μg/kg body weight in 10 minutes (means ± S.D.)

	before DDAVP	after DDAVP	p[1])
t-PA activity (mm^2)	39 ± 8	232 ± 23	<0.001
t-PA antigen (ng/ml)	14 ± 2	28 ± 3	<0.001
PA-inhibitor (%)	74 ± 11	5 ± 3[2])	<0.001
u-PA activity (arb units)	28 ± 2	28 ± 3	NS
α_2-antiplasmin (%)	92 ± 4	86 ± 4	NS
antithrombin III (%)	96 ± 4	88 ± 3	<0.005
plasminogen (mg/l)	98 ± 3	96 ± 2	NS
fibrinogen (g/l)	4.2 ± 0.3	4.3 ± 0.2	NS
coeruloplasmin (g/l)	0.31 ± 0.05	0.30 ± 0.05	NS
factor VIII R:Ag (%)	111 ± 21	181 ± 20	<0.001
renin (μU/ml)	19 ± 8	50 ± 27	<0.001

[1]) Probability according to paired t-test, NS = not significant ($p < 0.05$).
[2]) free t-PA-inhibitor was nil in 6 patients.

in which C_{A_i} and C_{V_i} stand for the arterial- and venous concentrations of the individual, respectively, and v for the blood volume passing per unit time through the organ. In the event that in each individual, apart from clearance, also synthesis by the same organ occurs, formula (2) gives the net result, i.e. the net elimination rate (see also above: statistical analysis I). If the elimination constant is defined as

$$k_{e_i} = \frac{\text{elimination rate}_i}{V \cdot C_{A_i}} \quad (3),$$

in which V stands for the circulating blood volume, then it follows from (2) and (3) that

$$k_{e_i} = \frac{v}{V} \cdot \left(1 - \frac{C_{V_i}}{C_{A_i}}\right) \quad (4).$$

From formula (1) (v.s.: regression analysis) it follows that if there were no synthesis (b = 0),

$$C_{V_i} = a \cdot C_{A_i} \text{ or } \frac{C_{V_i}}{C_{A_i}} = a \quad (5).$$

Combination of formula's (4) and (5) leads to

$$k_e = \frac{v}{V} \cdot (1 - a) \quad (6),$$

which represents the average elimination constant, irrespective of concomitant synthesis by the same organ. The t½ of disappearance can be calculated from the relation:

$$t\tfrac{1}{2} = \frac{\ln 2}{k_e} \quad (7).$$

Results

1. Effect of DDAVP (Table 1)

A rise in t-PA-activity and in t-PA-antigen content of arterial blood was found in each subject after DDAVP injection. The free PA-inhibitor level decreased to zero in most subjects; in only two of them did small amounts of PA-inhibitor remain detectable. Arterial plasma-urokinase levels remained unchanged. Factor VIII-antigen rose by about 70%, and the concentration of plasminogen, antiplasmin and antithrombin III decreased slightly, as described previously (24). Fibrinogen and coeruloplasmin concentrations did not change appreciably.

2. Comparison of Arterial and Renal Vein Blood

Both parametric and non-parametric statistical analysis indicated a slightly but significantly higher t-PA activity and a higher t-PA-antigen level in renal venous blood than in arterial blood, but only before DDAVP-injection (Table 2). Plotting of the individual levels of t-PA-activity (Fig. 2) and t-PA antigen in renal vein versus arterial blood, both before and after DDAVP, showed a straight line. Regression analysis reveals that the slope is not significantly different from unity and that it runs almost through the origin (Table 4). None of the other parameters changed significantly upon passage of the blood through the kidneys.

3. Comparison of Arterial and Hepatic Vein Blood (Table 3)

In each case, the t-PA activity in liver vein blood was substantially lower than in arterial blood. This was especially prominent after induction of a high t-PA level by stimulation with DDAVP. The orthogonal regression analysis of venous versus arterial levels (Table 4) shows a slope of 0.45 before, and 0.29 after DDAVP. The slopes of these two lines do not differ significantly ($F[2; 12] = 0.46$, $p = 0.72$). Based on these data it cannot be concluded that these lines are different. When both groups of data are taken together, a line is found with a slope of 0.34; the intercept of this line is not significantly different from zero (see also Fig. 3). The regression analysis of these data should be interpreted as an indication of clearance of the blood from active t-PA in the liver without a substantial synthesis of t-PA by this organ. The coincidence of the lines representing baseline levels and levels obtained after DDAVP strongly suggests that this drug does not influence the rate of clearance.

The mean t-PA-antigen levels in hepatic vein blood were approximately 30% lower than in arterial blood, both before and after DDAVP (Table 4). Orthogonal regression analysis (Table 4) shows positive but statistically insignificant intercepts of

Table 2 Levels of factors in renal arterial and venous blood (means ± S.D. of left and right vessels), before and after DDAVP injection

	before DDAVP			after DDAVP		
	arterial	venous	p[1])	arterial	venous	p[1])
t-PA activity (mm^2)	38 ± 7	43 ± 8	<0.05	238 ± 24	240 ± 25	NS
t-PA-antigen (ng/ml)	13.6 ± 1.3	14.2 ± 1.4	<0.05	27 ± 2.3	27 ± 2.5	NS
PA-inhibitor (%)	74 ± 10	73 ± 12	NS	4.8[2])	4.6[2])	NS
u-PA activity (arb units)	28 ± 2	28 ± 2	NS	28 ± 3	29 ± 3	NS
Factor VIII R:Ag (%)	110 ± 23	112 ± 22	NS	179 ± 20	180 ± 21	NS
Plasminogen (mg/l)	99 ± 3	98 ± 3	NS	97 ± 3	96 ± 2	NS
α_2-antiplasmin (%) 78.8 = 100%	91 ± 4	92 ± 4	NS	87 ± 4	86 ± 4	NS
Antithrombin III (%) 81.5 = 100%	94 ± 4	96 ± 4	NS	88 ± 2	93 ± 4	NS

[1]) probability according to paired t-test, NS = not significant ($p > 0.05$).
[2]) free t-PA-inhibitor was 0 in 6 patients.

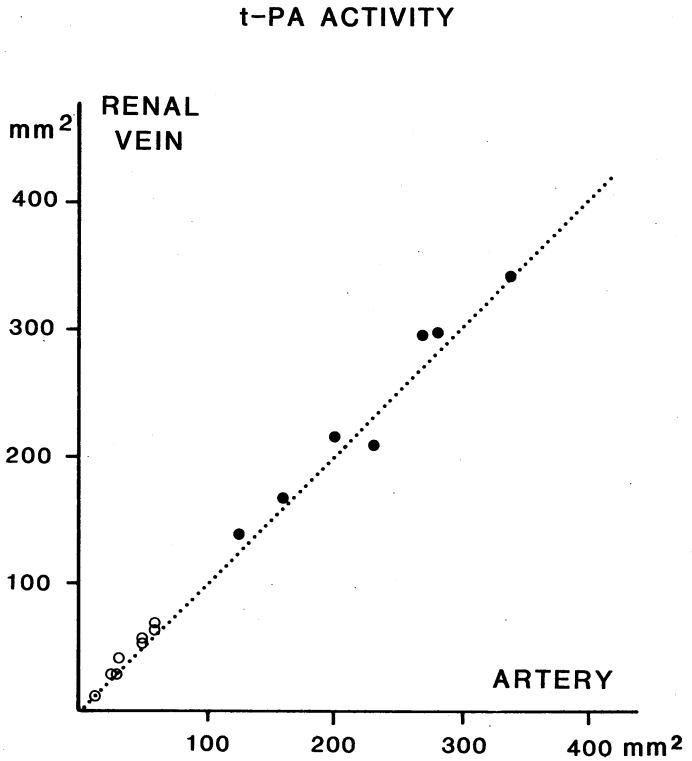

Fig. 2 Orthogonal regression analysis of t-PA activity (mm^2 of lysis area) in renal artery and vein. Open symbols = before DDAVP; closed symbols = after DDAVP. The dotted line represents the hypothetical regression curve of equal arterial and venous levels

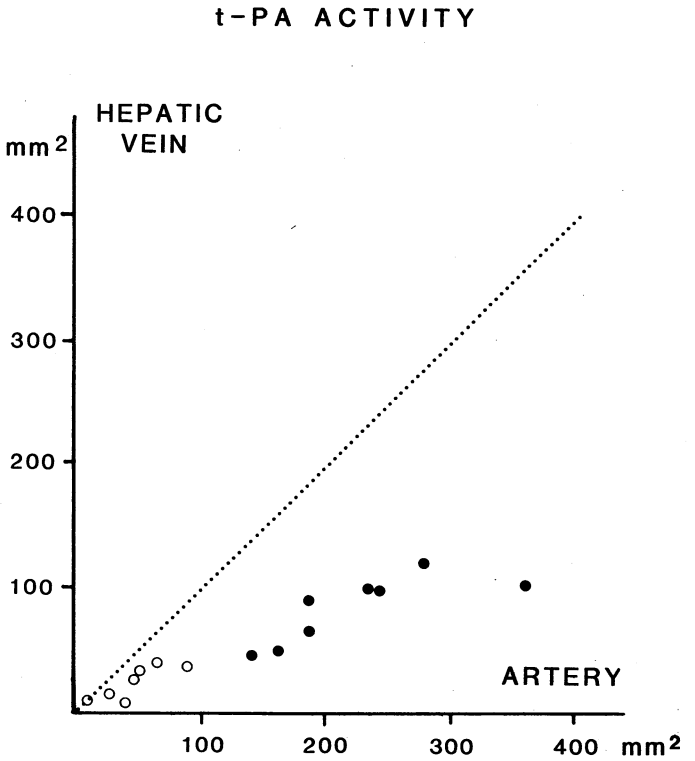

Fig. 3 Orthogonal regression analysis of t-PA activity in hepatic artery and vein, expressed in square mm lysis area. Open symbols = before DDAVP; closed symbols = after DDAVP

the ordinate both before and after DDAVP. As these lines can be regarded as coinciding ($F[2; 12] = 0.29$, $p = 0.50$) they were taken together. The slope of the common line is 0.50 and the intercept is significantly different from zero (Fig. 4, Table 4). This suggests clearance of t-PA-antigen by the liver and concomitant production.

The difference between arterial and venous blood levels of free PA-inhibitor was not significant before DDAVP. After DDAVP, however, arterial blood of most subjects never contained any free PA-inhibitor, whereas in all but one liver vein sample, a substantial amount of PA-inhibitor was found (see Fig. 5). Only pre-DDAVP levels are used for regression analysis. The slope of 0.80 indicates clearance in the liver, the positive intercept suggests production (Table 4).

No significant difference was found between levels of other fibrinolytic factors and proteins in arterial and venous blood. Likewise, regression analysis failed to show a consistent change in the activity of the blood on passage through the liver.

4. t½ of Disappearance of t-PA

When a normal average blood volume and hepatic blood flow in our subjects are assumed, the data provided by this study allow calculation of the elimination rate of the various substances by the liver. When the plasma volume (V) is assumed to be 2400 ml and the plasma flow (v) through the liver about 800 ml per minute (26), the t½ of disappearance of t-PA activity, t-PA antigen and free PA-inhibitor can be calculated from the slopes (a) in Table 4

Table 3 Comparison of arterial and hepatic vein blood, before and after DDAVP injection (means ± S.D.)

	before DDAVP			after DDAVP		
	arterial	venous	p[1])	arterial	venous	p[1])
t-PA activity (mm^2)	42 ± 10	23 ± 5	<0.2	222 ± 25	86 ± 9	<0.001
t-PA antigen (ng/ml)	15.5 ± 1.8	12 ± 1.2	<0.02	29.8 ± 3.3	18.9 ± 1.7	<0.001
PA-inhibitor (%)	73 ± 12	78 ± 10	NS	6.1[2])	33.5 ± 12	<0.01
u-PA activity (arb units)	28 ± 3	26 ± 1	NS	27 ± 3	28 ± 2	NS
factor VIII R:Ag (%)	111 ± 20	109 ± 22	NS	181 ± 20	180 ± 21	NS
plasminogen (mg/l)	98 ± 3	100 ± 2	NS	96 ± 3	95 ± 3	NS
α_2-antiplasmin (%)	94 ± 3	94 ± 4	NS	87 ± 4	90 ± 3	NS
antithrombin III (%)	96 ± 3	92 ± 3	NS	87 ± 3	90 ± 2	NS
fibrinogen (g/l)	4.2 ± 0.3	4.3 ± 0.3	NS	4.3 ± 0.2	4.2 ± 0.3	NS
coeruloplasmin (g/l)	0.31 ± 0.05	0.32 ± 0.05	NS	0.30 ± 0.05	0.31 ± 0.05	NS
α_2-macroglobulin (mg/l)	22.8 ± 0.3	22.4 ± 0.3	NS	21.7 ± 0.2	22.1 ± 0.2	NS

[1]) probability according to paired t-test, NS = not significant ($p > 0.05$).
[2]) free t-PA-inhibitor was 0 in 6 patients.

Table 4 Slope a and intercept b obtained by orthogonal regression equation of in- and out-flowing blood from liver and kidneys, according to the formula $C_{V_i} = a \cdot C_{A_i} + b$ (see Methods)

	Liver			Kidney		
	before DDAVP	after DDAVP	all	before DDAVP	after DDAVP	all
t-PA-activity						
slope	0.45*	0.29*	0.34*	1.13*	1.03	1.03
SD	0.10	0.09	0.03	0.05	0.09	0.03
r	0.89	0.98	0.95	0.99	0.98	0.99
intercept	4.21	21.22	9.39	0.73	4.30	4.26
SD	4.59	21.04	5.00	2.27	21.39	4.40
t-PA-antigen						
slope	0.61*	0.49*	0.50*	1.00	1.10	0.99
SD	0.16	0.11	0.05	0.06	0.14	0.05
r	0.84	0.89	0.93	0.99	0.96	0.99
intercept	2.64	4.38	4.23*	0.47	–2.97	0.33
SD	2.62	3.24	1.31	0.86	3.75	1.03
PA-inhibitor						
slope	0.80*	–	–	1.39*	–	–
SD	0.10	–	–	0.20	–	–
r	0.96	–	–	0.95	–	–
intercept	20.00*	–	–	–26.51	–	–
SD	7.84	–	–	14.56	–	–

* Significant with a significance level of 5% for the hypotheses that the slope differes from unity or the intercepts differs from zero.

through formula's (6) and (7). The results, compiled in Table 5, show that t-PA activity is removed most rapidly (t½ 3.1 min), followed by t-PA antigen (t½ 4.1 min) and free PA-inhibitor (t½ 10.4 min).

Discussion

The unique opportunity to measure fibrinolytic components in blood collected in the renal and hepatic veins and in the arteries supplying these organs with blood, created the possibility to calculate the production and elimination rates of endogenous, autologous, substances per organ and to compute their half-life. This approach is fundamentally different from the tracing of (labeled) purified t-PA preparations after intravenous injection and it lacks the disadvantages of the pharmacological method, as outlined above.

In our approach, the statistical analysis can be performed in two different ways, either via the paired t-test, or by regression analysis. Both methods are followed in this study, but eventually we choose for the latter to calculate the t½ of disappearance, mainly for two reasons. First, the regression analysis permits the estimation of hepatic and renal clearance without interference of concomitant synthesis by the same organ. Secondly, two-way analysis of variance of the results indicated that the observed changes in t-PA-level, both antigen and activity, by passage through the liver become greater with increasing levels of the enzyme under the influence of DDAVP, but are independent of the individual. In conclusion, in the liver a fixed fraction of the inflowing t-PA is removed from the blood and the fraction not removed is represented by the slope (a) of the regression curve.

For t-PA antigen an apparent half-life of 4.1 minutes and for t-PA activity 3.1 min was calculated (Table 5). These figures differ only slightly. If this difference is confirmed and strengthened by future studies, it would suggest that part of the antigen is cleared at a slower rate, which is not surprising. It has recently been appreciated that the t-PA activity is influenced by an inhibitor of t-PA present in normal plasma (31). This fast-acting PA-inhibitor is synthesized by endothelial cells and hepatocytes in

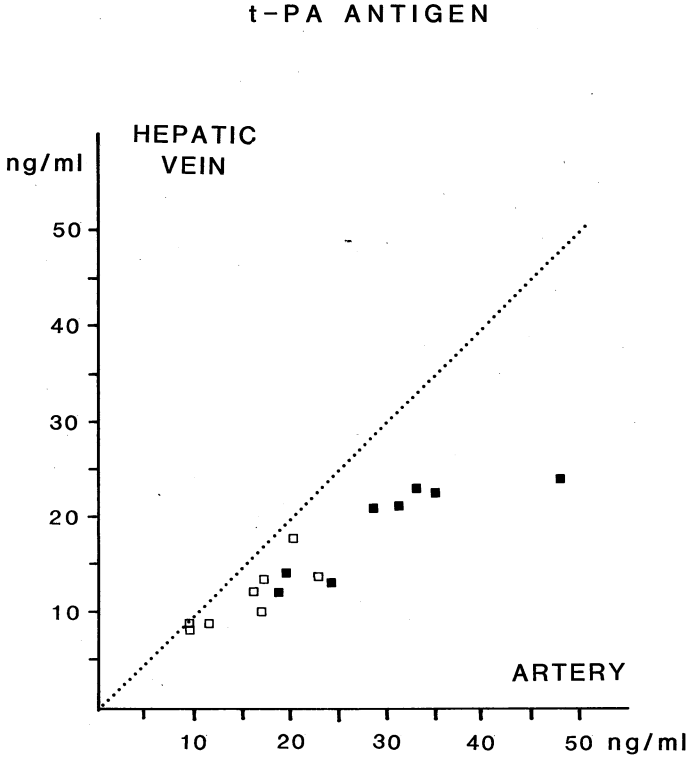

Fig. 4 Orthogonal regression analysis of t-PA antigen level in hepatic artery and vein in ng/ml. Open symbols = before DDAVP; closed symbols = after DDAVP

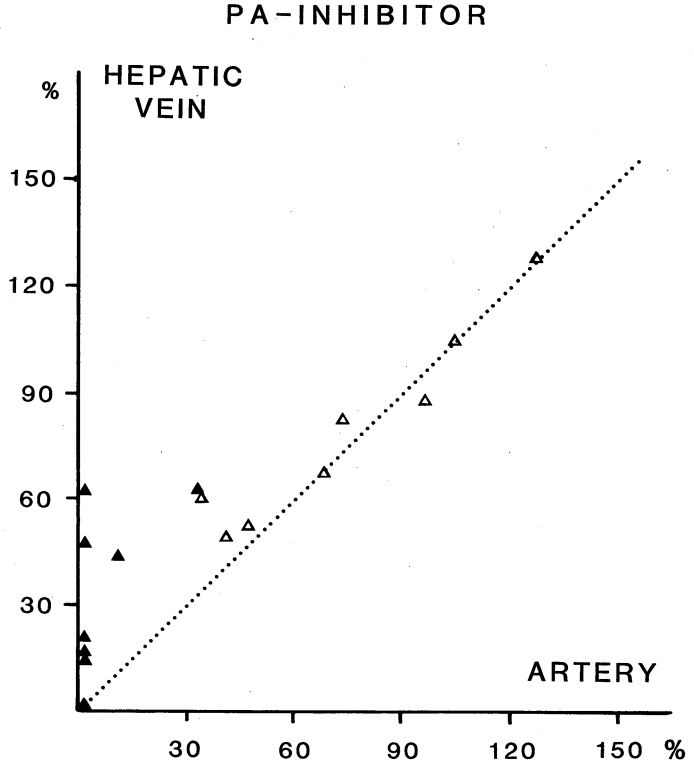

Fig. 5 Orthogonal regression analysis of PA-inhibitor in hepatic artery and vein, expressed as percentage of normal. Open symbols = before DDAVP; closed symbols = after DDAVP

Table 5 Tentative calculation of $t_{1/2}$ of t-PA from the slopes (a) in Table 5 according to formula's (5) and (6) in Materials and Methods, assuming an average normal distribution volume and hepatic plasma flow

	before DDAVP	after DDAVP	all
t-PA activity	3.8	2.9	3.1
t-PA antigen	5.3	4.1	4.1
free t-PA inhibitor	10.4	–	–

culture (32–35), but the main site of production in the body is not yet known. The presence of free PA-inhibitor in normal blood (19, 29, 36, 37) and in the blood of our patients before DDAVP had been injected, suggests that the greater part, if not all, of t-PA circulates in a complex with this inhibitor. Only after stimulation of the release of t-PA from its stores, for instance by injection of DDAVP, does free t-PA appear in the blood, and neutralize any circulating PA-inhibitor (31). The decrease in fibrinolytic activity of the blood upon passage through the liver, in particular after DDAVP-injection, is consistent with the view that the liver removes most of the freely circulating t-PA. This is in agreement with the elimination of t-PA from a perfusate by isolated rat livers (38) and with the observed accumulation of radiolabeled t-PA in the liver in mice (4), rabbits (3, 5) and in man (27). The t½ of disappearance of about 3 minutes calculated by us, is the same as has been found with the other approach in rabbits and mice (3–6). It is only slightly shorter than the half-life measured in dogs, baboons and in man (6, 27–30) after injection of a therapeutic dosage of t-PA. An even shorter disappearance time of endogenous t-PA in man, 2 to 3 minutes, but still in agreement with the present findings, can be read from the decline of the t-PA level after cessation of the cardiopulmonary bypass procedure in open heart surgery (39). That the hepatic extraction of t-PA proceeds with approximately the same speed as the removal of t-PA from the entire ciruclation, supports the hypothesis that, also in man, the liver is the main site of elimination of t-PA from the blood.

To the best of our knowledge, only one article has hitherto been published on the fibrinolytic activity in hepatic venous and arterial blood in man (40). The authors did not find a significant arterio-venous difference, which at first sight is in contradiction with the rapid clearance of blood from plasminogen activator by the liver. However, as is obvious from the comparison of the two methods of analysis used by us, the liver appears to only slightly decrease the concentration of normal levels of t-PA-activity as measured in euglobulin fractions of plasma, and an arteriovenous difference might well be missed. As a matter of fact, little or no t-PA circulates in a free form in baseline conditions. The t-PA activity measured in euglobulin fractions reflects the partial dissociation of a complex (with PAI) during the preparation of the euglobulin fractions, These complexes occur in both in-flowing and out-flowing blood, testified by the difference between the changes in t-PA-antigen level and t-PA activity. High levels of (free) t-PA-activity are reduced dramatically by the liver (Table 3, Fig. 3).

The partial reversibility of the complex of t-PA with its inhibitor (21) may be relevant in two other respects. In the first place, any contribution to the circulating activator-inhibitor complex, including small amounts of t-PA produced by the liver and instantaneously bound to an inhibitor, would raise the fibrinolytic activity as it is measured in euglobulins. In the second place, the reappearance of free PA-inhibitor in hepatic vein blood concomitantly with reduction of the high fibrinolytic activity after DDAVP (Table 3) could be explained by the liberation of inhibitor from this reversible complex. The alternative, the release of free PA-inhibitor from the liver itself, cannot be excluded. Our results suggest that the complex of t-PA and its inhibitor is eliminated at a lower rate than free t-PA, since t-PA-antigen is removed less fast than t-PA-activity.

Calculation of the clearance of PA-inhibitor following the same principle as used for t-PA, results in an apparent half-life of 10.4 minutes. This figure is in the same range as the value obtained by transfusion experiments (41).

The t-PA-antigen level decreased by approximately 30% upon passage of the blood through the liver both before and after DDAVP. Mixture of t-PA-rich arterial blood with portal venous blood (see Fig. 1), if devoid of t-PA, would give a similar decrease. Little information is available about the t-PA content or fibrinolytic activity of portal vein blood, which accounts for about two thirds of the blood flow to the liver. The spleen is the major organ in the splanchnic circulation known to have a variety of tasks in modifying the quality of the blood, but there is no evidence that removal of the spleen changes the fibrinolytic activity in non-cirrhotic patients, neither in baseline conditions nor after stimulation (42, 43). Strangely enough, Kwaan et al. (42) could not detect any fibrinolytic activity in splenic vein blood. Leaving aside the question whether the spleen or the liver removes free t-PA, the splanchnic area should, for our purpose, be regarded as one compartment (see Fig. 1). An influcence of DDAVP on the total blood flow through the liver has not been found in a previous study (24). Now evidence is presented that DDAVP does not influence the rate of disappearance, neither of fibrinolytic factors, nor of active renin (data not shown). Despite the apparent clearance of the blood from t-PA-antigen, the linear regression curve suggests that the liver also contributes to the t-PA-antigen level of the blood. In view of the low t-PA activity in hepatic vein blood, any t-PA produced by the liver will be bound to an inhibitor.

We failed to show a rise in the plasma-urokinase activity level in response to DDAVP. This confirms previous findings by ourselves (7).

The concentration of factor VIII-antigen does not change appreciably upon the passage of the blood through the liver, neither before nor after DDAVP. Any hepatic extraction or contribution of the factor VIIIR:Ag would remain hidden from observation because of its relatively long half-life in the circulation. Likewise, due to their relatively long survival time many other proteins did not exhibit a measurable A-V-difference, even when they are synthesized by the liver, e.g. antithrombin III, α_2-antiplasmin, plasminogen, fibrinogen and coeruloplasmin.

The increase in t-PA-antigen and in t-PA activity of the blood during passage through the kidney under basal conditions (see Table 2), suggests some contribution by the kidney to the t-PA-level of the blood. This had been anticipated in view of the extensive vascularization of the organ and of similar findings by others (10–12). However, regression analysis (Table 4) does not support this view, the intercepts with t-PA antigen and activity being not significantly different from zero (Table 4) and we tend to conclude that the kidneys do not alter significantly the concentration of t-PA-antigen and t-PA-activity of the blood. Production of u-PA by the kidney could not be demonstrated by mere comparison of arterial and venous activities. Regression analysis did not reveal production of u-PA in the kidney either. Our observations lend no support for the hypothesis that the kidneys eliminate inhibitors from the blood.

The novel approach of estimating the elimination rate of circulating endogenous fibrinolytic activators from the blood during its passage through an organ, by regression analysis of concentrations of in-flowing and out-flowing blood, opens a way to study the same parameters for other substances. The only prerequisite is that the ratio C_V/C_A can be measured accurately enough over the organ. In general this is the case if $0.1 \leq C_V/C_A$

$\leqslant$ 0.9 or (via formula's 6 and 7) 2.1 $\leqslant$ t½ (min) $\leqslant$ 21. In accordance, substances that are removed almost completely from the blood in a single passage through the liver ($C_A/C_A \sim 0$), like indocyanine green (44), have an apparent half-life of a little more than 2 minutes when measured in this way (24, 45). For obvious reasons the catheterization cannot be performed in critically ill patients. Therefore, the procedure is not suitable for the study of a number of substances which are present merely in disease states and which are cleared from the blood by the liver, as for example activated clotting factors (46) and fibrin- and fibrinogen degradation products (47).

The relevance of our results for clinical medicine is, first of all, a confirmation of the short half-life of endogenous t-PA in the human circulation and secondly that clearance of the blood from t-PA occurs mainly in the liver. Apparently, this holds true also for the exogenous enzyme. Impairment of liver function would allow any free t-PA to circulate longer than normal. Under basal conditions, where there is an excess of PA-inhibitor, the impairment has few consequences. An inappropriate elevation of t-PA levels would, however, become conspicious either after stimulation of the release, as has been found by several investigators (48–51), or after exogenous administration, or, theoretically, after lowering of the inhibitor level. Such a high level of t-PA might considerably shorten the survival of any fibrin formed intravascularly and cause bleeding from fresh wounds (52).

As for the kidney, the body could probably dispense with the contribution made by this organ to the blood fibrinolytic activity. In patients with terminal renal insufficiency and in anephric patients, we have found a normal or even high baseline fibrinolytic activity (6, 22). The capability of some anephric patients to respond to DDAVP with a rise in fibrinolytic activity (53, 6, 22) indicates that for stimulation of t-PA-release with vasopressin derivatives the kidney is not required either.

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