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In vivo metabolism of human tissue-type plasminogen activator

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The turnover of human tissue-type plasminogen activator (t-PA), purified from cell culture fluids and radiolabelled with ^{125}I, was studied in 2 healthy males. After injection, the plasma radioactivity initially disappeared with a half-life of about 3 min, but after 30 min reached a plateau level which persisted for several hours. The radioactive material had then already been converted to forms soluble in 10 % trichloroacetic acid (TCA). Surface counting rates indicated a rapid uptake in the liver during the first 10 min after injection, followed by a rapid release from the liver, beginning already at 25 min, which coincided with the appearance of the TCA-soluble radioactive material. After 18 h, about 80 % of the injected dose had already been excreted in the urine. We conclude that, in humans, injected t-PA is rapidly cleared from the circulation, mainly by the liver, and within 30 min metabolized to low molecular weight forms which ultimately (within 1 d) appear in the urine.

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The fibrinolytic enzyme systems in human plasma play an important role in protection against thromboembolic incidents (1). By employing newly-developed specific assay methods for tissue-type plasminogen activator (t-PA) on a clinical material of young patients with idiopathic thromboembolic diseases, we were recently able to show that a reduced activity in the extrinsic fibrinolytic system is characteristic of these patients (2). Therefore, the possible use of t-PA as a therapeutic agent in thrombotic conditions seems to be physiologically attractive (3, 4). However, the turnover characteristics of t-PA in humans have not been studied so far. In this article we report our recent investigations on this subject.

Material and methods

Human tissue-type plasminogen activator (single-chain form) was prepared from melanoma cell cultures (5) and labelled with ^{125}I by the iodogen method (6). Free ^{125}I was removed by chromatography on anti-t-PA IgG covalently coupled to Sepharose 4B (5). Two different radiolabelled preparations were used. They were characterized by a clot lysis activity assay (7) and by an enzyme-linked immunosorbent assay (2), and were found to retain their full activity within experimental error. The incorporation of ^{125}I amounted to 0.7 and 2.2 atoms of iodine per t-PA molecule, respectively. For the turnover studies, 9 µg and 4 µg of the two preparations,

respectively, in volumes of 0.2 ml, were injected intravenously, yielding an injected dose of 1.2–1.5 MBq.

The labelled material was free of pyrogens when tested in rabbits.

Two healthy males aged 27 and 38 volunteered for the study (two of the authors). Thyroid uptake of iodine was blocked by a daily dose of 150 mg KI orally, starting the day before injection. During the first 2 h of the turnover experiments, the subjects were kept resting in a seated position.

Plasma samples were obtained by drawing 9 volumes of blood, taken by venepuncture, into 1 volume of 0.13 mol/l sodium citrate in siliconized Vacutainer tubes (Becton Dickinson, Stockholm, Sweden). After centrifugation at 2000 *g* for 10 min, 1 ml plasma was pipetted off and counted for radioactivity in a Multigamma counter (LKB, Stockholm, Sweden). Then the plasma was precipitated by 10% TCA, and again centrifuged. The clear supernatant was pipetted off and its radioactivity was compared to that of the pellet.

Surface radioactivity counting was performed by a scintillation counter (Thrombograph 11, Novo, Hadsund, Denmark). Countings were performed over the heart, liver, right kidney and spleen consecutively for 10 sec each, every 2 min. For optimal results, the exact positions of these organs were visualized by X-rays prior to injection of label.

Results

Turnover of human tissue-type plasminogen activator in plasma

Figure 1 shows the plasma radioactivity disappearance curves for the ^{125}I-labelled t-PA in 2 healthy males. For the first 10 min, the disappearance is approximately exponential, with half-lives of 3 and 4 min, respectively. After 20 min, when about 10% of the injected dose remains in the circulation, the radioactivity starts to rise again slightly. As seen from the figure, this rise is accounted for by the appearance of TCA-soluble radioactive material in the plasma.

Surface counting

In Figure 2, the results from the surface countings are shown relative to the heart count, which is defined as 100% at each time point. As seen, there is a rapid increase in activity over the liver during the first 10 min after injection of label, then a plateau at 700% and 1000% of the heart activity, respectively, is reached, and at 25 min a rapid drop in the liver activity begins. At 60 min, the liver activity has already leveled off to roughly equal the heart activity. There are also small increases in activity over the kidney and spleen during the first 10 min. After about 20 min, significant activity also appears over the bladder, indicating that a build-up of label in the urine has started.

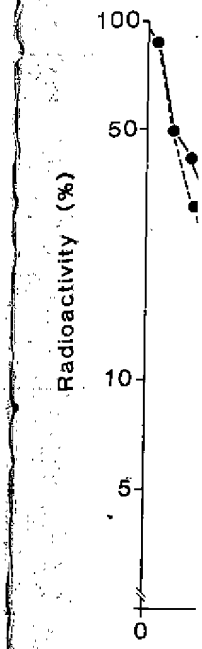
Total body radioactivity

The clearance of the injected radioactivity from the body was studied by measuring volumes and radioactivities of the voided urine portions during the first 18 h after injection of label. As seen from Figure 3, 80% of the injected dose is excreted from the body already after the first 18 h.

Discussion

We have injected 9 μg and 4 μg, respectively, of two ^{125}I-labelled t-PA preparations into 2 healthy males. This leads to an initial plasma concentration of about 4 ng/ml and 1 ng/ml, respectively, of the injected protein. This should be compared to the normal resting value of 4 ng/ml which we recently established utilizing a new ELISA for t-PA (2). Therefore, the injected amounts of t-PA fall in the physiological concentration range of this protein.

We have demonstrated that injected ^{125}I-labelled t-PA is cleared from the the circulation with a half-life of 3–4 min, and is probably mainly taken up by the liver. The degradation in the liver takes about 15 min, and subsequently the TCA-soluble end products are expelled from the liver,



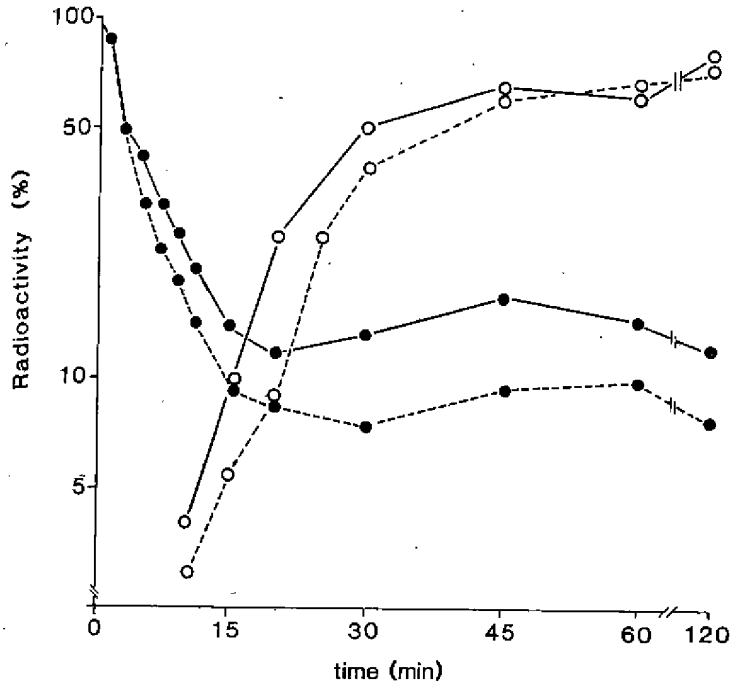


Figure 1. Turnover of ^{125}I-labelled t-PA in 2 healthy male subjects. Plasma radioactivity (●) is expressed as a fraction (%) of the injected dose. The fraction of the plasma radioactivity which is soluble in 10 % trichloroacetic acid is also given (○).

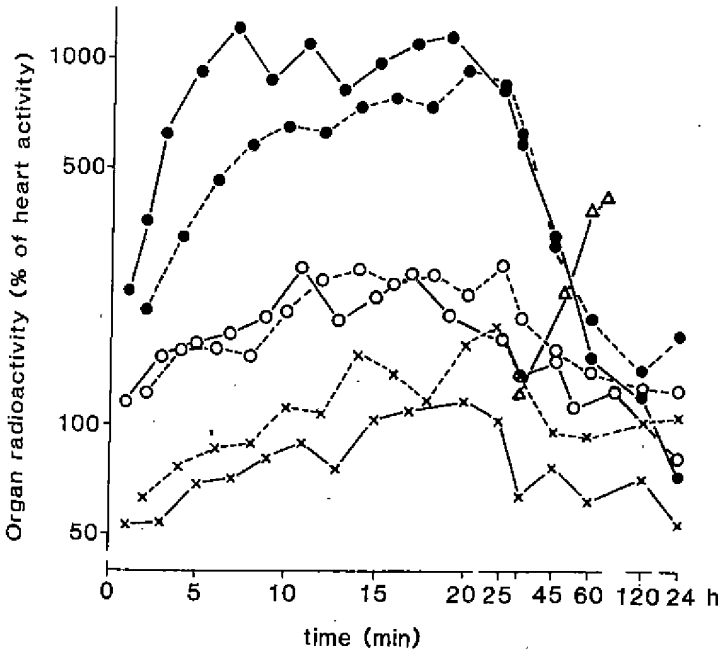


Figure 2. Organ distribution of injected ^{125}I-labelled t-PA. Surface counting was performed over heart, liver (●), spleen (○), right kidney (×) and the bladder (△). All values are expressed relative to the heart activity, which is defined as 100 % at each time point.

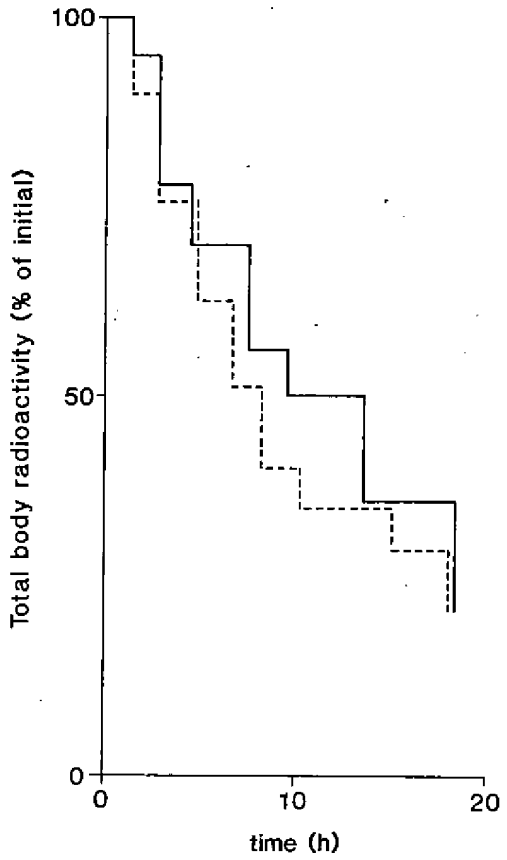


Figure 3. Remaining total body radioactivity after injection of ^{125}I-labelled t-PA. The difference between injected dose and cumulative urinary excretion of label, I-ΣU_t, is plotted against time after injection.

leading to the unusual increase in the plasma radioactivity curve seen between 30 and 60 min (Figure 1) instead of the expected continuous exponential decline. Simultaneously, the label begins to appear in the urine (Figure 2). Within 18 h, 80% of the injected dose has been excreted in the urine (Figure 3).

The turnover of human t-PA has been studied previously in rabbits (8). The results were very similar to our findings in humans, both with respect to the organ distribution and the plasma radioactivity half-life of 2–3 min, compared to 3–4 min in humans. This is remarkable, since usually the turnover rates of a plasma protein in different species are inversely proportional to the bodyweight (8), and furthermore, previous studies have implicated t-PA half-lives in humans of 10–15 min (2, 9). However, using a new specific activity assay for t-PA, a value of about 5 min was estimated (10).

Our finding of the very short in vivo half-life of injected purified t-PA in humans may therefore explain the limited success so far in the attempts to use t-PA as a thrombolytic agent in humans, or as a probe for the scintigraphic detection of deep vein thrombosis (11).

Acknowledgements

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