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Platelet-derived endothelial cell growth factor

Pharmacokinetics, organ distribution and degradation after intravenous administration in rats

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Platelet-derived endothelial cell growth factor (PD-ECGF) stimulates chemotaxis of endothelial cells in vitro and has angiogenic activity in vivo. Recently PD-ECGF was shown to have thymidine phosphorylase activity. In order to study possible therapeutic applications of PD-ECGF we used a rat model to determine its pharmacokinetics and tissue distribution after intravenous injection. [^{125}I]PD-ECGF disappeared from the plasma in a biphasic manner, with estimated distribution and elimination half-lives of 17 min and 7 h, respectively. PD-ECGF was metabolized in the liver, excreted via the bile, and not accumulated in any organ system. The stability and long half-life in the circulation, together with the specificity for endothelial cells, suggest that PD-ECGF may be useful as a therapeutic agent to stimulate re-endothelialization in vivo, or, in view of its thymidine phosphorylase activity, in chemotherapy, by decreasing the pool of available thymidine.

Angiogenesis; Platelet-derived endothelial cell growth factor; Re-endothelialization of blood vessel; Pharmacokinetics; Thymidine phosphorylase; Chemotherapy

1. INTRODUCTION

Platelet-derived endothelial cell growth factor (PD-ECGF) was initially purified from human platelets as a 45 kDa protein [1] and was recently shown to form homodimers [2]. It stimulates chemotaxis of endothelial cells in vitro and has angiogenic activity in vivo [3]. PD-ECGF is also present in human placenta [4]; in addition, it is produced by a limited number of normal and transformed cell lines in culture [5]. Cloning of its complementary DNA revealed that PD-ECGF lacks a hydrophobic leader sequence [3]. Consistent with this observation PD-ECGF is secreted from the producer cells only slowly [5]. More recently, PD-ECGF was found to have sequence homology to *Escherichia coli* thymidine phosphorylase [6] and to have thymidine phosphorylase activity, i.e. catalyzing the reversible phosphorolysis of thymidine. PD-ECGF is therefore a human homologue of *E. coli* thymidine phosphorylase [2]. The mechanism by which PD-ECGF exerts its effects on endothelial cells is unknown, but is likely to be indirect and involve products of its enzymatic activity.

The availability of a cDNA clone made it possible to produce recombinant human PD-ECGF with full biological activity (unpublished results). Therefore, material is now available for studies of the in vivo effects of PD-ECGF when administered to the whole organism. In this report we describe the pharmacokinetics and organ distribution of PD-ECGF in the rat after intravenous injection.

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2. MATERIALS AND METHODS

2.1. *Iodination of recombinant human PD-ECGF*

Recombinant human PD-ECGF (a kind gift from A. Thomason, Amgen Inc., Thousand Oaks, CA) was ^{125}I labeled using the procedure described by Bolton and Hunter [7] with slight modifications. The resulting preparation had a specific radioactivity of 1 μCi/μg. After this procedure PD-ECGF retains full biological activity in a [^{3}H]thymidine incorporation assay in endothelial cells (data not shown).

2.2 *Experimental models and assays*

Animal experiments were conducted using male Wistar-Kyoto rats weighing about 200 g. Animals were anesthetized with an intraperitoneal injection of chloral hydrate (350 mg/kg) and placed upon a heating plate (37°C) to prevent hypothermia. [^{125}I]PD-ECGF (about 0.5 μCi in 0.3 ml phosphate buffered saline, containing 1 mg gelatin per ml) was injected into each animal through a right jugular vein catheter, and blood samples of 0.2–0.3 ml were withdrawn after different time periods via a heparinized left carotid artery catheter. Blood coagulation was prevented by EDTA in the precoated sampling tubes and plasma was prepared by separation of blood cells by centrifugation for 2 min at 10,000 × *g*. The radioactivity in whole blood as well as in plasma and in red blood cells (after removal of the buffy coat) was determined in a γ-counter. The same was done with bile and urine after collection through additional catheters placed in the common bile duct and the urinary bladder. The extent of degradation of the labeled material in the various liquid samples was determined by

precipitation in 10% ice-cold trichloroacetic acid (TCA) on 3MM Whatman paper In addition, the samples were analyzed by SDS-gel electrophoresis (Phast-System, Pharmacia LKB, Uppsala, Sweden) and autoradiography 3 h after the injection of the labeled material, the animals were sacrificed by an intravenous overdose of the anesthetic, various organs were taken out and their wet weight and their content of ^{125}I radioactivity determined The plasma weight was estimated by the use of normative data [8], and its content of ^{125}I radioactivity was calculated by including the samples withdrawn during the experiment. All animal work was performed in accordance with institutional guidelines

As a further test of its stability in rat serum, [^{125}I]PD-ECGF (about 1 μCi in 0.25 ml of the buffer described above) was added to 1 ml of heparinized rat blood and incubated at 37°C. Samples were taken during a 6-h period and analyzed by γ-scintillation and by SDS-gel electrophoresis and autoradiography. Determination of ^{125}I radioactivity in blood, bile, urine and tissue samples was performed by γ-scintillation (PW4800, Philips, The Netherlands) Unless further specified, all data represent the average of triplicates ± S.E.M.

Plasma clearance kinetics of TCA-insoluble ^{125}I radioactivity were estimated using a two-compartment, non-linear regression model (PCNONLIN, Statistical Consultants, Lexington, KY)

3. RESULTS

3.1. *Pharmacokinetics*

The rate of disappearance of PD-ECGF from the blood and its components was studied in a rat model in which [^{125}I]PD-ECGF was injected into the jugular vein, and blood samples were taken after different time periods. [^{125}I]PD-ECGF showed a biphasic clearance from the circulation of the rat; an initial rapid clearance from the plasma (as well as from the whole blood) was followed by a slower phase. The estimated half-life for the initial distribution phase is 17 min, whereas the value for the elimination phase is 7 h (Fig. 1). All radioactivity was precipitable by TCA. More than 90% of the radioactivity in the blood was restricted to the plasma throughout the whole experiment (one time point is shown in Fig. 2). No major degradation product was

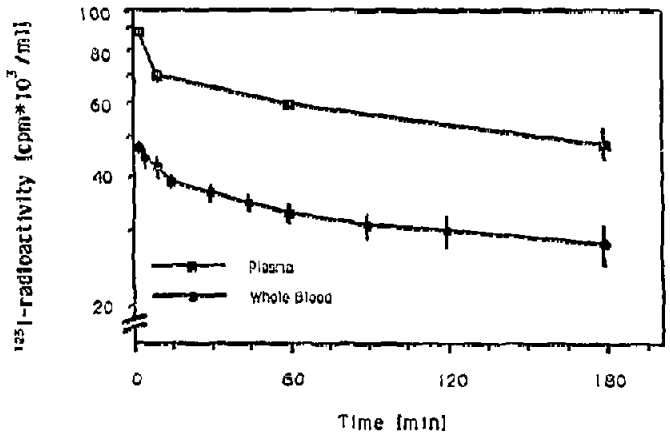

Fig. 1. Disappearance of [^{125}I]PD-ECGF radioactivity from the circulation of rats. [^{125}I]PD-ECGF (0.5 μCi) was administered intravenously and samples (0.2 ml) were taken at different time points. TCA precipitable radioactivity was determined and is shown for the plasma fractions. Results represent mean ± S.E.M. from 3 different experiments.

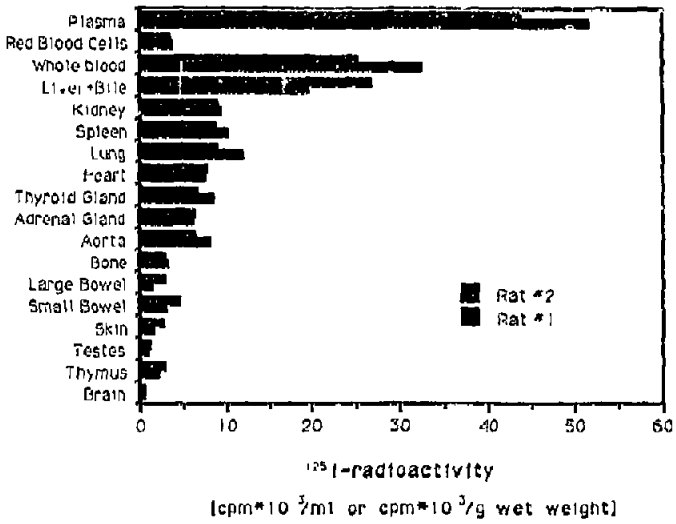

Fig. 2 Organ distribution of ^{125}I radioactivity per volume or wet weight (cpm/ml or cpm/g) after intravenous administration of 0.5 μCi [^{125}I]PD-ECGF into two male rats weighing 194 and 209 g. Tissues were taken 3 h after injection of the labeled material.

detected during the time-course of the experiment in which plasma was analyzed by SDS-gel electrophoresis and autoradiography (data not shown).

The stability of PD-ECGF in vitro was further tested by incubation of [^{125}I]PD-ECGF in rat blood. Over a 6-h period the TCA-precipitable ^{125}I radioactivity remained constant. Analysis of the plasma by SDS-gel electrophoresis and autoradiography demonstrated a 45 kDa band, representing [^{125}I]PD-ECGF. The intensity of this band did not change during the 6-h period, nor did any degradation product occur (data not shown).

3.2. *Organ distribution and degradation*

Three hours after injection of [^{125}I]PD-ECGF animals were sacrificed and the content of ^{125}I radioactivity in various organs and body fluids determined. The highest concentration of radioactivity was still present in the blood and mainly restricted to the plasma (Table I).

Comparing the radioactivity per wet weight or volume there was no accumulation detected in any of the organs analyzed (Fig. 2). Among the different organ systems, the liver plus bile contained the highest concentration of radioactivity. One hour after injection of the labeled protein, high concentrations of radioactivity appeared in the bile, of which less than 10% was precipitable by TCA. Fig. 3 shows an example for the appearance of radioactivity in the bile collected over three consecutive 60-min intervals. Another animal showed a similar pattern, but had a lower bile production and a lower concentration of radioactivity during the observed period of time. Thus, our data indicate that [^{125}I]PD-ECGF is taken up by the liver, degraded and then excreted via the bile. There was no activity detected

Table I

Distribution of ^{125}I radioactivity in various compartments of the body compared to their relative weights

Organ	% of injected radioactivity	% of body weight
Plasma	36.3 ± 4.0	4.0 ± 0
Liver + bile	16.0 ± 2.7	3.6 ± 0
Others (rest)	47.7 ± 2.0	92 4 ± 0
Total recovery	≈100	100

Samples were analyzed 3 h after injection of 0 5 μCi of [^{125}I]PD-ECGF (10^6 cpm) into rats. Results represent mean ± S E.M. from 3 different experiments.

in the urine during the time–course of the experiment (data not shown).

4. DISCUSSION

Using rat as a model we have shown that intravenously administered PD-ECGF disappears from the plasma with a fairly long half-life of about 7 h in the elimination phase. It is not accumulated in any organ system and the organ distribution largely reflects the blood content of the various tissues. One exception is the hepatobiliary system, where PD-ECGF is processed into non-TCA-precipitable molecules and excreted through the bile.

Compared to other peptide growth factors present in platelets, such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), PD-ECGF has a much longer half-life in the plasma. PDGF has a half-life of 2 min, when injected into normal baboons [9] and 5 min in rats [10]. The rapid disappearance from the blood can, to a large extent, be explained by binding to the serum protease inhibitor α_2-macroglobulin [11], which is rapidly cleared from the circulation by binding to hepatocytes [12]. TGF-β has a similar half-life of 2.2 min in rats [13] and is cleared in the same way as PDGF by binding to α_2-macroglobulin [14,15]. In contrast, recombinant latent TGF-β does not associate with α_2-macroglobulin and has an extended plasma half-life of slightly more than 100 min in rats [16], which is somewhat closer to that which we determined for PD-ECGF.

The long plasma half-life of PD-ECGF has important physiological implications. PD-ECGF might stimulate endothelial regeneration of large vessels, and when released from platelets or other producer cells, the biologically active molecule may be carried by the circulation to distant target cells.

The lack of the hydrophobic leader sequence and the comparably long plasma half-life of PD-ECGF, together with the fact that PD-ECGF is not accumulated in any organ system other than the liver, where it is processed and eliminated from the circulation, are compatible with the idea that PD-ECGF functions as a thymidine phosphorylase in vivo. The thymidine

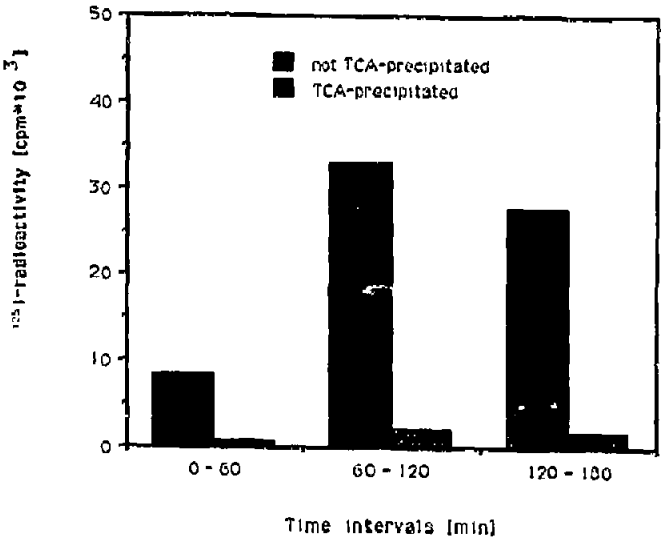

Fig. 3 Total and TCA-precipitable ^{125}I radioactivity in the bile accumulated over three consecutive 60 min time periods after intravenous administration of 0.5 μCi [^{125}I]PD-ECGF into a rat. Bile was collected through a catheter placed in the common bile duct. The amount of bile collected during each of the three 60 min time periods was about 420 μl. TCA precipitation was performed as described in section 2.

phosphorylase activity of PD-ECGF, however, is of potential therapeutic interest itself. Chemotherapy with nucleotide analogues is based on the concept of incorporation of modified nucleotides (instead of thymidine) into the DNA or RNA, resulting in the distortion of fundamental cellular mechanisms such as DNA replication, transcription or translation. The thymidine phosphorylase activity of PD-ECGF could be useful in combination with nucleotide analogues to decrease the pool of available thymidine and to consecutively increase the incorporation rate of the analogue, and thus the efficacy of the chemotherapy.

So far nothing is known about biological effects caused by systemically administered PD-ECGF. The long plasma half-life, however, would enable the factor to act within the circulatory system for several hours after the injection of a single dose. Furthermore, it will be possible to maintain a certain plasma level by application of a few doses per day. This makes PD-ECGF a suitable candidate for in vivo studies, e.g. for stimulation of re-endothelialization after percutaneous transluminal angioplasty of atherosclerotic vessels, where endothelial denudation and insufficient regeneration might be a reason for the rapid development of restenosis (reviewed by [17]). Moreover, it will now be possible to investigate whether PD-ECGF is useful in chemotherapy, based on its thymidine phosphorylase activity.

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