Pharmacokinetics and Tissue Distribution of Recombinant Human Transforming Growth Factor Beta$_1$ After Topical and Intravenous Administration in Male Rats

Thomas F. Zioncheck,[1,3] Sharon A. Chen,[1] Louise Richardson,[1] Marina Mora-Worms,[2] Catherine Lucas,[2] Derf Lewis,[1] James D. Green[1] and Joyce Mordenti[1]

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Recombinant human transforming growth factor beta (rhTGF-β_1) enhances the healing process after topical application to various animal wound models. A detailed pharmacokinetic and tissue distribution study was performed to support the clinical development of rhTGF-β_1 for wound healing indications. Rats received radioiodinated or unlabeled rhTGF-β_1 as an intravenous (iv) bolus or as a topical formulation applied to a full thickness wound. Plasma concentrations of TGF-β_1 were estimated from TCA-precipitable radioactivity or were measured by ELISA. Following iv administration, the initial half-life was rapid (<11 min), regardless of whether radiolabeled or unlabeled rhTGF-β_1 was used. The terminal half-life was long (163 min) when the test material was radioiodinated and administered as a trace dose and relatively short (≤61 min) when given at high doses and assayed by ELISA. Analysis of plasma radioactivity by SDS-PAGE revealed a time-dependent clearance of the 25-kDa parent molecule without a significant appearance of lower molecular weight radiolabeled metabolites. The majority of the radioactivity was associated with highly perfused organs, known iodide elimination pathways, and the thyroid at 1 and 8 hr after iv injection. After topical administration of a high dose (0.8 mg/kg), no immunoreactive TGF-β_1 was detectable in plasma samples taken over a 48-hr period. However, trace amounts (≤0.05 ng/mL) of acid-precipitable radioactivity were detected in plasma after topical application of [^{125}I]rhTGF-β_1 (1 μg/kg, 126 μCi/kg). A significant portion (35%) of the [^{125}I]rhTGF-β_1 persisted intact (25 kDa) at the wound site 24 hr after application. In conclusion, rhTGF-β_1 was rapidly cleared after iv bolus administration and distributed primarily to the liver, lungs, kidney, and spleen. Little systemic exposure was observed after applying a single topical dose of rhTGF-β_1 to a wound, and the intact molecule persisted at the wound site.

KEY WORDS: recombinant human transforming growth factor beta$_1$; wound-healing; pharmacokinetics; plasma-based enzyme-linked immunosorbent assay (ELISA); tissue distribution.

INTRODUCTION

Transforming growth factor beta$_1$ (TGF-β_1) is a ubiquitous multifunctional polypeptide growth factor (1–3). It was originally described as a peptide produced by retrovirally transformed cells that could induce normal rat kidney fibroblasts to form colonies in soft agar (4). Subsequently, the molecule was purified to homogeneity from normal human platelets (5), placenta (6), and bovine kidney (7). The human TGF-β_1 complimentary DNA was cloned and is currently being expressed in a mammalian cell culture system (8). The cloning, expression, and large-scale purification of recombinant human transforming growth factor-β_1 (rhTGF-β_1) have allowed numerous *in vitro* and *in vivo* studies that have enhanced our knowledge of the structure, function, and potential clinical utility of the molecule.

The *in vitro* biological effects of TGF-β_1 may be classified as proliferative, antiproliferative, or unrelated to proliferation. For example, TGF-β_1 has been shown to be a potent mitogen for some cells of mesenchymal origin such as fibroblasts (9) and osteoblasts (10,11), yet displays a reversible growth inhibitory activity on epithelial, endothelial, neuronal, hematopoietic, and lymphoid cell types (12). In addition to proliferative effects, TGF-β_1 also enhances the formation of extracellular matrix, a process of major importance in embryogenesis and tissue repair. This is accomplished in part by upregulating the synthesis of collagen and fibronectin while concomitantly inhibiting the proteolytic degradation of newly formed matrix (2,13).

Based on these results, numerous *in vivo* studies have evaluated the potential clinical utility of TGF-β_1 as a wound-healing agent. For example, a single application of TGF-β_1 to a soft tissue wound induces the formation of granulation tissue and increases the rate of healing, and the strength of healed wounds in various animal models (14–16). In this report, we describe the disposition of [^{125}I]rhTGF-β_1 and unlabeled rhTGF-β_1 in rats after administering an iv bolus dose using both trichloroacetic acid (TCA)-precipitable radioactivity and an enzyme-linked immunoassay (ELISA) to monitor plasma concentrations. The molecular form of the circulating radioactivity was characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. Radioactivity in various tissues was assessed 1 and 8 hr after injection. Because the proposed therapeutic use for rhTGF-β_1 is for topical application to promote wound-healing, we applied [^{125}I]rhTGF-β_1 formulated in a 3% methylcellulose gel to a full-thickness rat wound model to study the absorption kinetics of [^{125}I]-rhTGF-β_1. These dermal studies were designed to mimic the clinical use of the compound. Preclinical evaluation of the extent of systemic absorption, distribution, and metabolism of this molecule is particularly important considering its diverse biological actions and potency.

MATERIALS AND METHODS

Iodination of rhTGF-β_1. Recombinant human TGF-β_1 was expressed in Chinese hamster ovary cells and purified as described previously (8). RhTGF-β_1 was iodinated using the sequential chloramine-T procedure reported by Frolick *et al.* (17). The resultant preparation had a specific activity of 126 μCi/μg and maintained full biological activity as assessed in a mink lung epithelial cell (Mv1Lu) bioassay (18).

Intravenous administration of rhTGF-β_1. For iv admin-

[1] Department of Safety Evaluation, Genentech, Inc., South San Francisco, California 94080-4990.
[2] Department of Medicinal and Analytical Chemistry, Genentech, Inc., South San Francisco, California 94080-4990.
[3] To whom correspondence should be addressed.

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istration, all rats received a bolus injection via the femoral vein cannula followed by 0.5 mL of warm saline to ensure complete delivery of the test material. The first and second dose groups (six rats/group) received 0.1 or 1.0 mg/kg of rhTGF-β_1, respectively. The third group (n = 6) received 0.5 mL of a 170-ng/mL solution of [^{125}I]rhTGF-β_1, providing a total dose of 10.9 μCi/rat and an average dose of 50 μCi/kg (0.4 μg/kg). All animals that received the radioactive dose also received three doses of 50 mg sodium iodide (0.5 mL of a 10% solution) intraperitoneally, approximately 48, 24, and 0.5 hr before [^{125}I]rhTGF-β_1 dosing, in an attempt to minimize organ-specific uptake of ^{125}I.

Topical Administration of rhTGF-β_1 in a Rat Wound Model. Male Sprague Dawley rats were obtained from Charles River Breeding Laboratories (Portage, MI). Two days before dosing, cannulae were implanted into the jugular and or the femoral veins of the rats while anesthetized (ketamine, 80 mg/kg; and xylazine, 4 mg/kg). On the day of dosing, rats in the topical dose group were anesthetized, and two 8-mm-diameter (0.5-cm^2) wounds were made on the left and right dorsal skin region using a biopsy punch. Wounds were full thickness, penetrating the dermis and subcutaneous tissue to the *panniculus carnosis* muscle-layer. Two groups of rats were dosed topically (six rats per group). The total topical dose volume was 0.2 mL (two wounds/animal, 0.1 mL/wound site), resulting in a total dose of 0.2 mg unlabeled rhTGF-β_1 and an average dose of 0.8 mg/kg. Rats treated with [^{125}I]rhTGF-β_1 received an average dose of 126 μCi/kg (1.0 μg/kg). The wounds were covered with Tegaderm (3M Medical-Surgical Division, St. Paul, MN) and then wrapped with adhesive tape.

Collection of Blood Samples. Blood samples (~0.3 mL) were removed from the jugular vein catheter and mixed with EDTA (2.5 m*M* final concentration). After iv administration, three rats per dose group were sampled at 1, 3, 5, 7, 10, 15, 20, 25, 30, 45, and 60 min. The other half of the group was sampled at 1 and 60 min and 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 hr. Similarly, after topical dosing half the animals were sampled at 1, 10, 20, and 45 min and 1.5, 2, 6, 8, and 24 hr, and the other half at 5, 15, 30, and 60 min and 1.5, 4, 6, 30, and 48 hr. Blood samples were then subjected to centrifugation for 5 min at 10,000*g* to pellet blood cells. The plasma was removed immediately and was stored at −70°C.

Organ Harvest. Animals were euthanized by CO_2 asphyxiation after the last blood samples were collected. The adrenal glands, bladder, duodenum, heart, liver, lungs, spleen, and thyroid were then harvested from each animal. The organs were blotted dry and weighed and the amount of radioactivity associated with each tissue was quantified in a gamma counter. The total blood volume was estimated as 8% of the total body weight (19).

Homogenization and Fractionation of Wound Site Tissue. After euthanasia, bandages were removed and the wounds were wiped with dry gauze pads to remove any superficial methylcellulose gel still present at the wound site. Wounds were excised by surgically removing 1 cm^2 of tissue encompassing the wound, including the membraneous muscle tissue layer just beneath the site. All tissues were weighed, and the radioactivity present was determined using a gamma counter. Excised wounds were washed with 5 vol of 20 m*M* sodium acetate, pH 5, containing 1 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride (PMSF) and 10 μg/mL leupeptin (buffer A). The washed tissue was frozen at −20°C prior to homogenization in buffer A.

ELISA. The concentration of TGF-β_1 in the plasma was measured by a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) specific for human TGF-β_1. An affinity-purified murine anti-human TGF-β_1 monoclonal antibody (12H5) was used for coating, and a horseradish peroxidase-conjugated affinity-purified murine anti-human TGF-β_1 monoclonal antibody (4A11) was used for detection (20). Plasma samples were diluted in a buffer containing 25 m*M* Hepes, 4 m*M* EDTA, 0.5% bovine serum albumin, 0.05% polysorbate 20, and 0.01% thimerosal, pH 6.5, to produce TGF-β_1 concentrations within the range of the assay (0.3 to 20 ng/mL). The interassay precision (%CV) was less than 15%.

TCA Precipitation Assays. The total radioactivity present in 0.1 mL of each plasma sample was quantitated using a gamma counter (5000 series, Packard Instruments, Downers Grove, IL). Samples were incubated for 1 hr on ice after the addition of an equal volume of chilled 20% TCA. The supernatant was removed after centrifugation for 5 min at 12,000*g*, and the radioactivity in the precipitated plasma proteins was quantified in the gamma counter.

Immunoprecipitation and Electrophoresis. Plasma from one rat in the topical dose group was immunoprecipitated by adding a 1:1000 dilution of anti-rhTGF-β_1 antiserum (21) for 2 hr at room temperature, followed by an overnight incubation at 4°C with protein G-Sepharose. The immune complexes were washed five times with RIPA buffer (1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 100 m*M* NaCl, 1 m*M* EDTA, 20 m*M* Tris–HCl, pH 7.4), boiled in tricine gel sample buffer, and centrifuged at 12,000*g*. Samples were applied to 16% tricine gels and radioactive bands were detected by autoradiography.

Pharmacokinetic Analysis. Mono- or biexponential equations were fitted to the immunoreactive and TCA-precipitable plasma concentration–time data after iv administration using weighted [weight = 1/(predicted concentration)^2] nonlinear least-squares regression analysis (NONLIN84, Version 1987, Statistical Consultants, Lexington, KY) on combined data from all animals dosed with radioactivity and individual animals dosed with unlabeled protein. The coefficients and exponents of the exponential equations were used to calculate the area under the serum concentration–time curve (AUC), clearance (CL), half-lives ($t_{1/2}$), and initial volume of distribution (V_1) (22), as well as the volume of distribution at steady state (V_{ss}), permanence time in the plasma (T_1), and mean exit time from the plasma (Ω_1) (23), assuming that the system was linear and state determined.

An exponential equation with first-order initial (rising) (k_1) and terminal (declining) (k_2) phases was fitted to the TCA-precipitable plasma concentration–time data after topical administration by performing a nonlinear least-squares regression analysis (NONLIN84) on combined unweighted data from all animals. Half-lives ($t_{1/2}k_1$ and t_1k_2) were calculated by dividing ln(2) by the corresponding k. The observed maximum concentration (C_{max}) and the time of the observed C_{max} (T_{max}) were obtained directly from the data. In addition, the AUC following topical administration, theoretical

C_{max}, and theoretical T_{max} were calculated from the estimated parameters.

RESULTS

Pharmacokinetics of rhTGF-β_1 After iv Administration. After iv administration of 0.1 mg/kg, the immunoreactive rhTGF-β_1 plasma concentration–time data were described by a monoexponential equation (Fig. 1A). Combined data from all the animals administered 1.0 mg/kg rhTGF-β_1 iv were described by a biexponential equation (Fig. 1A). It is possible that the 0.1 mg/kg dose would have resulted in a biphasic disposition if rhTGF-β_1 concentrations were measured by a more sensitive ELISA. Table I summarizes the results describing the disposition of rhTGF-β_1 after iv administration. For reporting purposes, CL, V_1, and V_{ss} were normalized for animal weight (W) and listed as CL/W, V_1/W, and V_{ss}/W, respectively. The available data suggest that the pharmacokinetics of immunoreactive rhTGF-β_1 were linear after iv administration of 0.1 and 1.0 mg/kg. Only 2% of the area under the curve was associated with the terminal half-life after 1.0 mg/kg, and the monoexponential equation used to describe the 0- to 60-min data adequately predicted the time course of rhTGF-β_1 plasma concentrations from the six rats dosed iv with 1.0 mg/kg (data not shown).

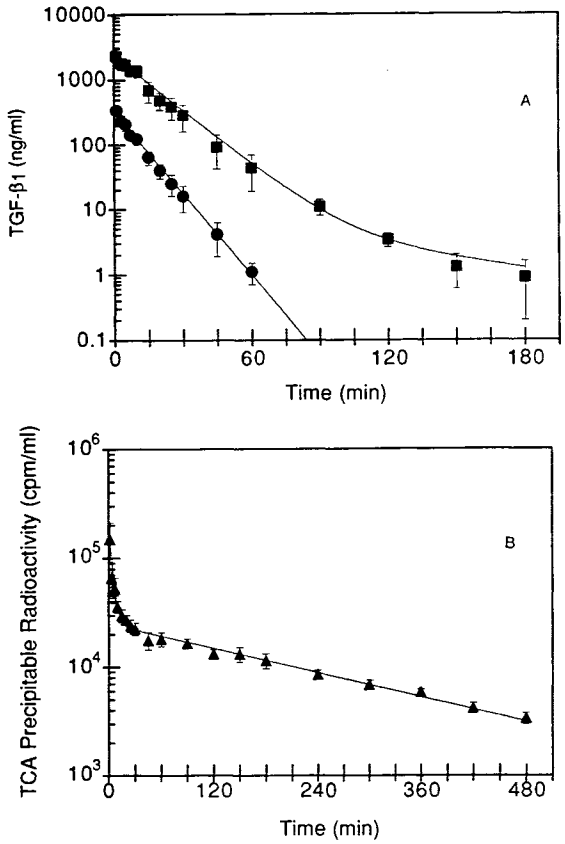

Fig. 1. The mean ± SD plasma concentration–time data for TGF-β_1 in male rats after iv bolus administration. The fitted pharmacokinetic equation is superimposed on the data. (A) The rats were dosed with 0.1 mg/kg (filled circles) and 1.0 mg/kg (filled squares), and the data were quantified by an ELISA. (B) The rats were dosed with 50 μCi/kg [^{125}I]rhTGF-β_1 and the concentrations represent the TCA-precipitable radioactivity in the samples.

Characterization of Radioactivity in Plasma After iv Administration of [^{125}I]rhTGF-β_1. Greater than 95% of the radioactivity was TCA precipitable when [^{125}I]rhTGF-β_1 was incubated in plasma for 24 hr at 37°C *in vitro*. However, 10 min after iv administration, only 81.6 ± 2.2% of the circulating radioactivity was TCA precipitable. This value decreased to 29.7 ± 1.1% after 60 min (data not shown). The relatively rapid metabolism of [^{125}I]rhTGF-β_1 *in vivo* compared to that observed *in vitro* is presumed to be due to organ-specific processing (24). Thus, TCA-precipitable radioactivity rather than total radioactivity was used as a measure of [^{125}I]rhTGF-β_1 concentrations in plasma.

The TCA-precipitable radioactivity–time data for the six rats after iv administration were described by a biexponential equation that is superimposed on the mean data in Fig. 1B. In contrast to the concentration–time data quantified in the ELISA, most of the area under the TCA-precipitable plasma radioactivity–time curve was associated with the terminal half-life (Table I). Plasma samples were separated by electrophoresis and visualized by autoradiography in an effort to characterize plasma radioactivity. A time-dependent decrease in the intensity of the 25-kDa [^{125}I]rhTGF-β_1 band was observed (Fig. 2). The 25-kDa radioactive band was faint in the 60-min sample and undetectable in the 120- to 480-min samples (data not shown). Notably, no lower molecular weight degradation products were observed in the plasma samples, with the exception of a faint band (~12.5 kDa) corresponding to rhTGF-β_1 monomer (also present in the tracer preparation).

Pharmacokinetics of rhTGF-β_1 After Topical Administration. Plasma samples collected over a 48-hr period after topical treatment with a very high dose of rhTGF-β_1 (0.8 mg/kg) did not have detectable levels of rhTGF-β_1 when assayed by ELISA. These results suggest that rhTGF-β_1 (or immunoreactive rhTGF-β_1 metabolites) did not accumulate or persist in plasma after an 0.8 mg/kg dose was applied to an open wound. The possibility of systemic absorption of low levels of rhTGF-β_1 was further addressed using [^{125}I]rhTGF-β_1. The dehalogenation or processing of [^{125}I]rhTGF-β_1 after application to the wound site complicated the data interpretation, therefore all plasma samples were TCA precipitated to determine radioactivity–time data for the six rats after topical administration. Absorption kinetics were described by an exponential equation with first-order absorption and elimination that is superimposed on the data in Fig. 3. The observed C_{max} value (3700–10,130 cpm/mL) were similar to the calculated C_{max} values (5161 cpm/mL), and the observed T_{max} value (120–480 min) was similar to the calculated T_{max} value (339 min) (Table II).

Characterization of Radioactivity in Plasma After Topical Administration of [^{125}I]rhTGF-β_1. The TCA-precipitable radioactivity in plasma after topical application of [^{125}I]rhTGF-β_1 was small (C_{max} range, 3700–10,130 cpm/mL) compared to total radioactivity (C_{max} range, 26,940–74,960 cpm/mL). Therefore, plasma samples from one rat in this group were immunoprecipitated with polyclonal antibodies directed against rhTGF-β_1 in an attempt to concentrate the radiolabeled peptides prior to electrophoresis. Tricine gels were used for this analysis to enhance the resolu-

Table I. Pharmacokinetic Parameters for rhTGF-β_1 in Male Rats After Intravenous Bolus Administration (Mean ± SD, Except When Only One Value Was Available)

Dose	0.1 mg/kg[a]	1.0 mg/kg[b]	~50 μCi/kg
Number of rats	6	6	6
Weight (kg)	0.239 ± 0.003	0.241 ± 0.010	0.219 ± 0.014
Observed C_{max} at 1 min	310 ± 49 ng/mL	2316 ± 770 (ng/mL)	155,573 cpm/mL
V_1/W (mL/kg)	308	424	384
V_{SS}/W (mL/kg)[c]	308	458	2417
CL/W (ml/min/kg)	29.1	26.9	11.3
$t_{1/2}\alpha_1$ (min)	7.3 (100% AUC)	10.7 (98% AUC)	2.5 (9% AUC)
$t_{1/2}\alpha_2$ (min)	n.a.[d]	61 (2% AUC)	163 (91% AUC)
T_1 (min)[c]	10.6	15.7	34.0
Ω_1 (min)	10.6	17.0	213

[a] Pharmacokinetic analysis of combined rat data from 0 to 60 min (n = 6 rats).
[b] Pharmacokinetic analysis of combined rat data from 0 to 180 min (n = 6 rats).
[c] $V_{SS}/W = V_1/W$ and $T_1 = \Omega_1$ when data are described by a monoexponential equation.
[d] Not available when data are described by a monoexponential equation.

tion of potentially low molecular weight (greater than 2-kDa) radiolabeled metabolites. A very faint 14- to 18-kDa band was observed after immunoprecipitation, and it appeared most predominately in the 90- and 120-min time samples (data not shown). Approximately 65% of the TCA-precipitable radioactivity in the plasma samples was recovered in the washed precipitate. These data suggest that little or no intact rhTGF-β_1 systemically circulate after topical administration to a wound model.

Organ Distribution of Radioactivity After Administration of [^{125}I]rhTGF-β_1. The percentage dose per organ and tissue-to-blood ratio was calculated for each organ collected. Following iv administration, the test material distributed to highly perfused organs (Fig. 4). For example, at 1 hr, only the blood (7%) and three of the organs sampled (liver, 14%; lung, 5%; and kidney, 4%) had greater than 1% of the dose; at 8 hr, no organs except blood (2%) had greater than 1% of the dose. All the tissues sampled at 1 hr and only the bladder, lung, and thyroid at 8 hr had a tissue-to-blood ratio significantly greater than 1. A tissue-to-blood ratio greater than 1 for a given organ suggests a specific accumulation of the radiolabel in that organ. The tissue distribution of radioactivity was also analyzed 24 and 48 hr after topical administration to a wound despite the relatively high percentage (>67%) of TCA-soluble radioactivity in the circulating blood pool. Blood contained approximately 1% of the radioactive dose at 24 hr and approximately 0.2% at 48 hr (data not shown). None of the organs sampled contained more than 0.2% of the radioactive dose over the course of 48 hr.

Select organs were homogenized 1 hr after iv bolus administration and subjected to TCA precipitation and immunoprecipitation in an effort to characterize the accumulated radioactivity observed in these organs. The radioactivity in liver, kidney, and spleen was 66 ± 10, 61 ± 8, and 55 ± 18% TCA-precipitable, respectively, and migrated predominantly at 25 kDa after electrophoresis and autoradiography. Thus, we conclude that the majority of radioactivity present in these organs at the 1-hr sampling time represented intact [^{125}I]rhTGF-β_1. In contrast, the majority of the radioactivity was TCA soluble 8 hr after iv dosing and 24 or 48 hr after topical dosing.

Characterization of [^{125}I]rhTGF-β_1 at the Wound Site. The molecular form of the [^{125}I]rhTGF-β_1 present at the wound-site 24 and 48 hr after topical administration to a rat wound model was quantitated and characterized. There was less radioactivity associated with a 1-cm^2 section of tissue around the wounds at 48 hr (~10% of the total radioactivity applied; n = 2) than at 24 hr (~35% of the total radioactivity applied; n = 3), suggesting that greater absorption of the dose had occurred with time (Table III). The wounds were excised, homogenized, and subjected to electrophoresis under reducing and nonreducing conditions. Radioactive bands were visualized by autoradiography (Fig. 5). The majority of the radiolabeled protein retained at the wound site migrated at 25 kDa, however, a small amount of a high molecular weight aggregate that did not enter the resolving gel was observed. Furthermore, the [^{125}I]rhTGF-β_1 present in the wound site homogenate was recovered in the pellet fraction after extraction and centrifugation in the presence of both nondetergent- and detergent-containing buffers. This may reflect aggregation or association with extracellular matrix proteins or other cellular components that were also insoluble under the extraction conditions.

DISCUSSION

Other researchers have used TGF-β_1 purified from platelets and a rat model to study the disposition of [^{125}I]TGF-β_1 after iv bolus administration (24,25). Trace doses were administered and plasma concentrations were estimated from total radioactivity. Data analysis in these reports was limited to initial half-lives (2.2 ± 0.1 and 2.7 ± 0.4 min, respectively). In the present report, we used a recombinant form of the molecule produced in a mammalian cell expression system and a novel plasma-based ELISA, specific for human TGF-β_1, to define plasma concentrations. This assay allowed for a detailed pharmacokinetic analysis of two relatively high iv bolus doses (1.0 and 0.1 mg/kg) of unmodified rhTGF-β_1.

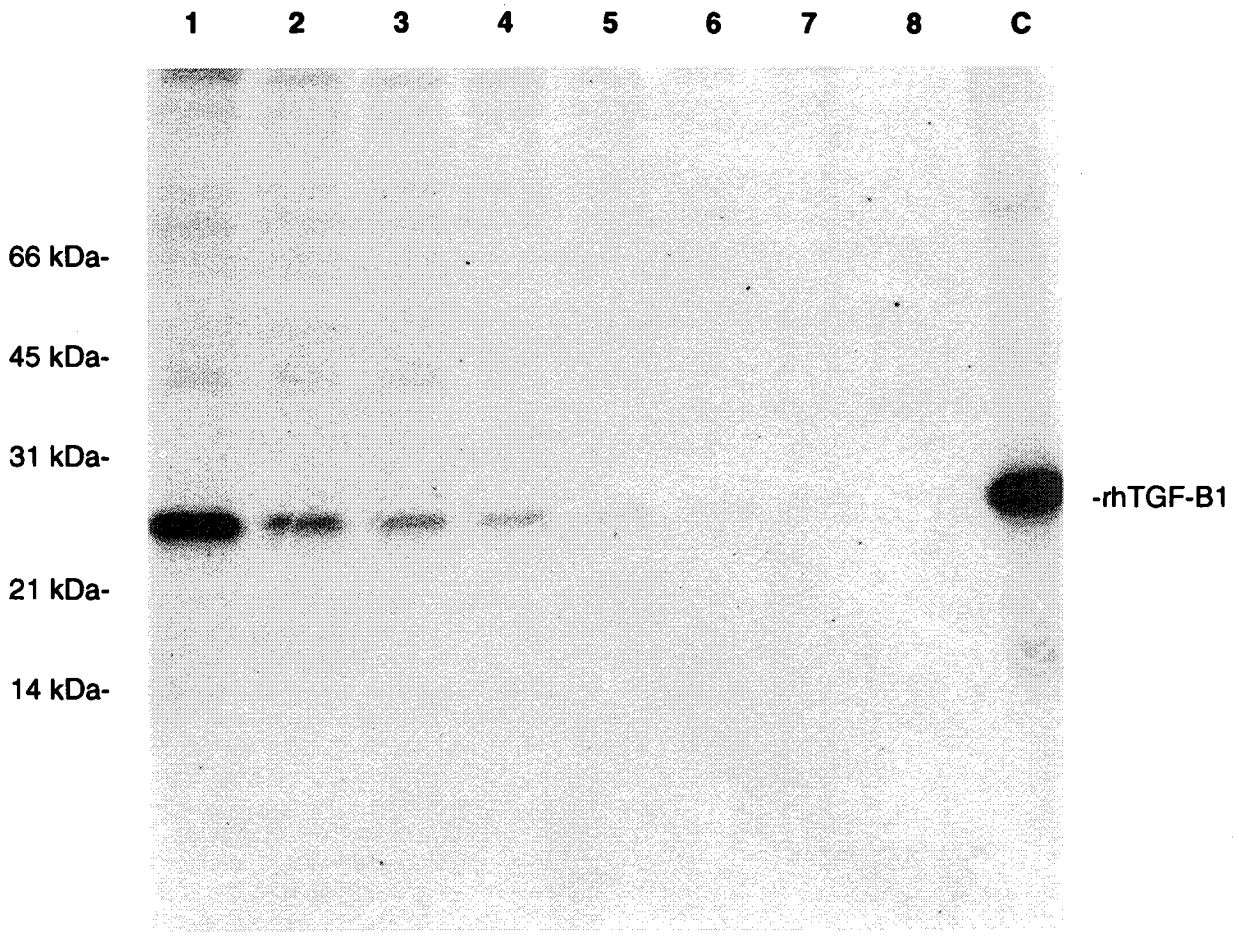

Fig. 2. SDS-PAGE and autoradiography of the plasma samples collected at various times after one of the rats received an iv bolus dose of [^{125}I]rhTGF-β_1. Five microliters of plasma was loaded onto lanes 1–8 (1, 3, 5, 7, 15, 30, 45, and 60 min, respectively) of a 10–20% SDS-PAGE gradient gel and were compared to the tracer preparation in lane 9 (C). Radioactive bands were visualized by autoradiography after a 2-week exposure at −70°C.

For comparison to previously published reports we also studied the kinetics of a trace iv bolus dose of radioiodinated rhTGF-β_1. The sampling schedule outlined in this report provided sufficient plasma concentration-versus-time data to describe the pharmacokinetics of [^{125}I]rhTGF-β_1 over an extended time period. The plasma disappearance of TCA-precipitable radioactivity after an iv dose of [^{125}I]rhTGF-β_1 was biphasic. The initial half-life derived for [^{125}I]rhTGF-β_1 was 2.5 min, nearly identical to that reported for radioiodinated TGF-β_1 purified from natural sources. A time-dependent disappearance of a 25-kDa radioiodinated band was observed in the samples 1–60 min after dosing (as determined by SDS-PAGE and autoradiography). No detectable 25-kDa band was observed beyond 60 min. The molecular form of the radioactivity giving rise to the long terminal

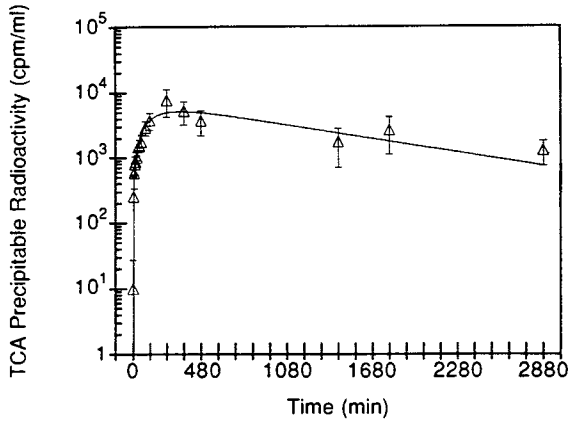

Fig. 3. Mean TCA-precipitable plasma concentrations (cpm/mL) in male rats after a topical dose of [^{125}I]rhTGF-β_1 was applied to a full-thickness wound. The pharmacokinetic equation is superimposed on the data. Symbols are the mean ± SD data from the six rats.

Table II. Pharmacokinetic Parameters for [^{125}I]rhTGF-β_1 in Male Rats After Topical Administration to a Full-Thickness Wound

Dose (μCi/kg)	~126
Number of rats	6
Weight, mean ± SD (kg)	0.233 ± 0.017
Observed C_{max}, range (cpm/mL)	3700–10130
Calculated C_{max} (cpm/mL)	5161
Observed T_{max}, range (min)	120–480
Calculated T_{max} (min)	339
$t_{1/2}/k_1$ (min)	858
$t_{1/2}k_2$ (min)	95

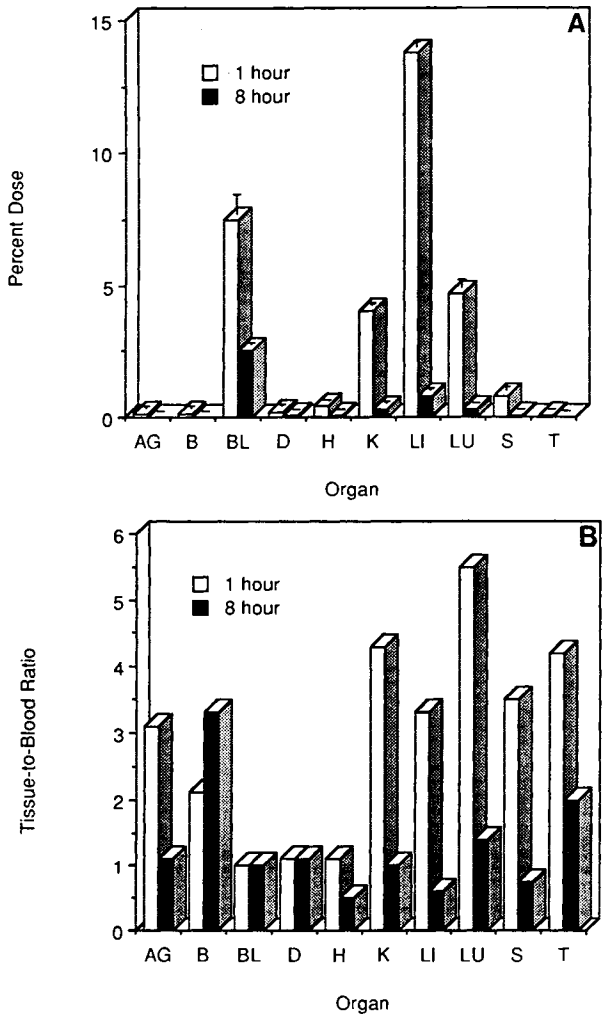

Fig. 4. Tissue distribution of radioactivity after iv administration of [^{125}I]rhTGF-β_1. (A) Percentage of dose in organs. (B) Mean tissue-to-blood ratios. Male rats (n = 3/time point) were euthanized 1 or 8 hr after a single bolus dose of [^{125}I]rhTGF-β_1. The following tissues were harvested and weighed, and tissue-associated radioactivity was determined using a gamma counter: adrenal gland (AG), bladder (B), blood (BL), duodenum (D), heart (H), kidney (K), liver (LI), lung (LU), spleen (S), and thyroid (T). Data are mean ± SD.

phase and relatively large AUC is not presently known but may represent small radiolabeled degradation products. In contrast, almost all of the AUC from the ELISA data was associated with the initial half-life. The ELISA data revealed that the clearance of antigen from the plasma is approximately 2.5 times faster than that of the TCA-precipitable material and may be a better representation of the disposition of intact rhTGF-β_1 based on the absence of detectable [^{125}I]rhTGF-β_1 beyond 60 min. Furthermore, *in vitro* spike experiments have shown that TGF-β_1 antigen levels detected in the ELISA correlate well with values obtained in a TGF-β_1 bioassay.

TGF-β_1 isolated from natural sources associates with both native and methylamine activated forms of α_2-macroglobulin, but it preferentially binds to the conformationally activated "fast form" (26). LaMarre *et al.* have suggested that activated α_2-macroglobulin can mediate the *in vivo* distribution and plasma clearance of TGF-β_1 via an α_2-macroglobulin receptor-dependent mechanism in mice (27). We have examined the binding of rhTGF-β_1 to human α_2-macroglobulin after *in vitro* incubation by native gel electrophoresis and chemical cross-linking. RhTGF-β_1 also binds both native and activated forms, with an apparent preference for the methylamine activated fast form (data not shown). The potential role of α_2-macroglobulin in the clearance of rhTGF-β_1 is the subject of further work.

Table III. Recovery of Radioactivity in the Bandages and Wound Site at the Termination of the Experiment

	Radioactivity recovered			
	Bandage (μCi)	Wound (μCi)	Total (μCi)	Total (% dose)
	24 h after topical administration (n = 3)			
Mean	5.19	5.32	10.51	35.47
SD	2.87	2.71	5.08	17.13
	48 hr after topical administration (n = 2)			
Mean[a]	1.56, 0.65	2.99, 1.00	4.55, 1.65	15.35, 5.57
SD	ND[b]	ND	ND	ND

[a] One of the rats in this group died 90 min after dosing and the wound site was not harvested; therefore, the mean was not calculated and the two individual values are reported.
[b] Not determined (n = 2).

Recombinant growth factors such as TGF-β_1 may be an important new class of pharmaceutical agents used to heal wounds. Based on the potency and diverse actions of TGF-β_1, it was of interest to monitor the systemic levels of rhTGF-β_1 after topical application. Although circulating TCA-precipitable radioactivity was detected in the plasma after topical dosing, the concentration was very low (estimated C_{max}, ≤0.05 ng/mL), and immunoprecipitation was required to concentrate the radioactivity prior to electrophoresis and autoradiography. The results of this experiment revealed a very faint (14- to 18-kDa) band in the 90- and 120-min samples, suggesting that the radioactivity circulating in plasma after topical application of [^{125}I]rhTGF-β_1 was not intact rhTGF-β_1 (25 kDa). It is assumed that this represents an immunoreactive [^{125}I]rhTGF-β_1 degradation product. Importantly, a significant portion of the AUC was associated with the terminal half-life; times when no immunoprecipitable parent molecule (25 kDa) was detectable.

From the analysis of the molecular form of the radiolabeled protein by electrophoresis and autoradiography, it appears that rhTGF-β_1 is not absorbed intact. Thus, pharmacokinetic equations for the TCA precipitation data (after topical or iv administration) describe the time course of different molecular forms of [^{125}I]rhTGF-β_1 and/or radiolabeled protein fragments originating from [^{125}I]rhTGF-β_1. For this reason, it was not possible to determine if the absorption of TCA-precipitable radioactivity following topical administration was rapid or if the kinetics were absorption rate limited. Bioavailability data were not reported due to the differences in the molecular entities that composed the TCA-precipitable radioactivity curves for each route of administration.

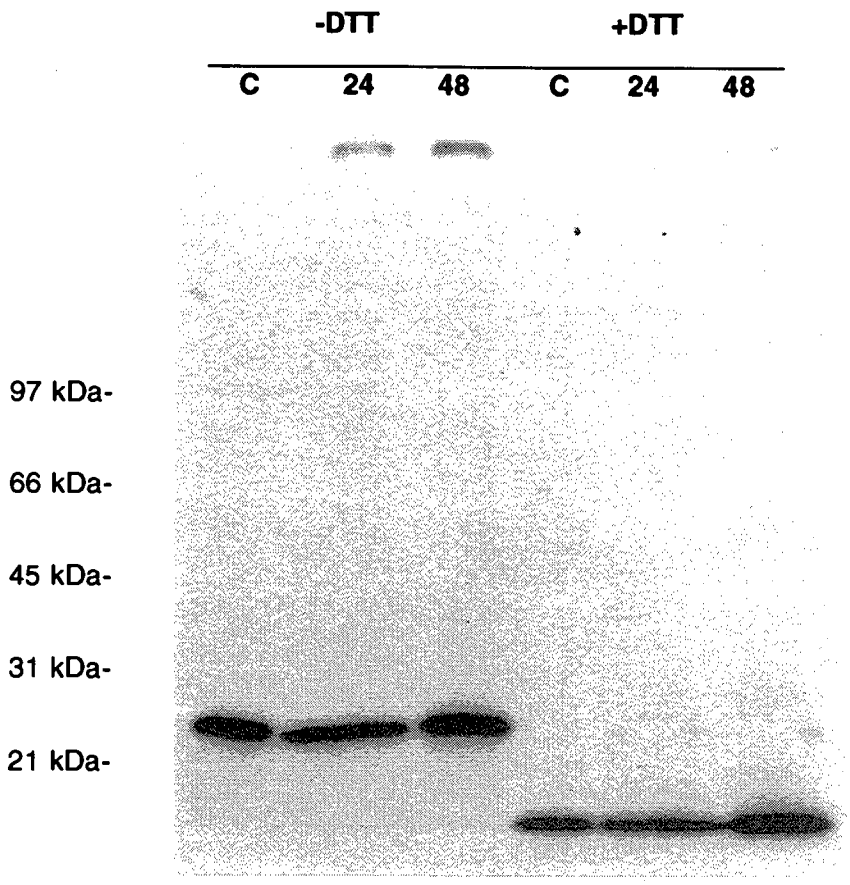

Fig. 5. Characterization of [^{125}I]rhTGF-β_1 at the wound site by SDS-PAGE and autoradiography. Samples were homogenized as described under Materials and Methods and applied to a 4–20% gradient SDS-PAGE mini-PROTEAN II gel (Bio-Rad, Richmond, CA) prior to autoradiography using Kodak X-Omat film.

Wakefield and colleagues reported a similar tissue distribution of radioactivity 30 min after an iv injection of [^{125}I]TGF-β_1, purified from human platelets (25). The distribution to liver is consistent with this organ playing a role in processing TGF-β_1 (24). The repeated systemic administration of high doses of rhTGF-β_1 to rats is known to produce lesions in multiple target organs including liver and kidney. However, high-dose dermal application resulted in local effects at the wound site without systemic toxicity (28). Systemic toxicity would not be expected based on our results demonstrating little or low level absorption of rhTGF-β_1 antigen after topical application to a wound. Numerous studies have demonstrated that a single topical application of TGF-β_1 to soft tissue wounds accelerates the process of tissue repair (14–16). The fact that 35% of the test material persisted intact and tightly associated with the wound site tissue 24 hr after application may explain why comparable accelerated healing has been observed after a single or multiple topical dose(s) (16).

In conclusion, rhTGF-β_1 is rapidly cleared from the systemic circulation after iv administration and distributes primarily to the liver, lung, kidney, and spleen. Little or no immunoreactive or radioactively labeled rhTGF-β_1 enters the systemic circulation when TGF-β_1 is applied topically to a wound model. However, 35 and 10% of the dose was recovered intact from the wound site 24 and 48 hrs, respectively, after a single application. These conclusions are important in developing dosing strategies for clinical studies and minimize the concern of systemic toxicity following topical application to soft tissue wounds.

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