the predicted v-*erb*-B product and the EGF receptor (*11*), as well as possible links between other oncogene products and growth factor–hormone receptors that share tyrosine kinase activity, have provided further support for this concept.

This study has shown that v-*sis* translational products synthesized by and associated with SSV-transformed cells specifically bind PDGF receptors, stimulate tyrosine phosphorylation of the PDGF receptor, and induce DNA synthesis in quiescent fibroblasts. In each case, we were able to establish the v-*sis*–coded nature of these activities by specific inhibition with antibodies to different regions of the v-*sis* gene product. These findings demonstrate that the SSV-transforming protein is functionally equivalent to PDGF.

PDGF provides a proliferative stimulus only to those cells that have specific receptors for the growth factor. We observed a strict correlation between those cell types with PDGF receptors and those susceptible to transformation by SSV. Although there are undoubtedly other differences between fibroblasts and endothelial or epithelial cells in addition to their PDGF receptor status, our findings strongly suggest that the v-*sis* gene product must interact with the PDGF receptor to manifest its transforming activity. If so, the PDGF-like activities of the v-*sis* translational product are those functions responsible for its transforming ability.

After synthesis on membrane-bound ribosomes, the $p28^{sis}$ monomer has been shown to form rapidly a $p56^{sis}$ homodimer in the endoplasmic reticulum. This homodimer then travels through the Golgi apparatus toward the cell periphery, where it is further processed at both termini (*6*). Recent evidence indicates that the low-density lipoprotein receptor travels along a similar intracellular pathway (*12*). Moreover, sequence analysis of the EGF receptor predicts a protein whose receptor domain is translocated across the endoplasmic reticulum with its kinase domain occupying a cytoplasmic orientation (*13*). If the PDGF receptor were processed in an analogous manner, its ligand binding domain might proceed along the same intracellular route as newly synthesized *sis*/PDGF-2–like dimers. Our study establishes that several forms of the *sis*/PDGF-2 product, including the $p56^{sis}$ homodimer, have potent mitogenic activity. Thus, our results are consistent with the possibility that the critical transforming interaction of the *sis* product with its receptor target occurs during their transport along the same intracellular route toward the cell periphery.

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Leukotrienes as Mediators in Tissue Trauma

Abstract. *A significant increase in the production of cysteinyl leukotrienes was observed after mechanical or thermal trauma in the anesthesized rat. The amount of biliary* N*-acetyl-leukotriene* E_4*, which represents a suitable indicator for blood plasma leukotrienes, was used as a measure of leukotriene generation. Cysteinyl leukotrienes were rapidly eliminated from blood plasma into bile where* N*-acetyl-leukotriene* E_4 *was the major metabolite. Leukotrienes were at a much lower concentration in blood plasma than in bile and differed in the pattern of metabolites. The detected amounts of leukotrienes were sufficient to induce known phenomena associated with trauma, such as tissue edema and circulatory and respiratory dysfunction. Increased leukotriene generation appears to play an important role in the pathophysiology of tissue trauma.*

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In the mammalian organism, severe tissue trauma is followed by a local inflammatory reaction and a systemic shock-like reaction. Knowledge of the biochemical events underlying these reactions is limited despite the frequent occurrence of trauma-induced lesions.

There are many similarities between the symptoms associated with trauma and the effects of injected cysteinyl leukotrienes. Leukotriene C_4 (LTC_4) and LTD_4 induce tonus changes in small vessels, tissue edema caused by extravasation of plasma, myocardial depression, bronchoconstriction, and lethal shock under conditions of impaired cardiovascular compensation (*1–5*). The dihydroxylated leukotriene LTB_4, in contrast, seems to exert its main effects on local leukocyte immigration and activation (*3–5*). Additional indications of a role for leukotrienes in the pathophysiology of tissue injury come from pharmacological studies demonstrating beneficial effects of inhibitors of leukotriene biosynthesis or action in the treatment of trauma (*6*).

Cysteinyl leukotrienes are formed by conjugation of the 5-lipoxygenase product LTA_4 with glutathione, yielding LTC_4. This peptide leukotriene is con-

verted to LTD_4 and LTE_4 by consecutive release of the γ-glutamyl and the glycinyl moieties (*3–5*). The *N*-acetylation product of LTE_4, LTE_4NAc, has been identified recently in rat bile and feces by several chromatographic methods and by mass spectrometry (*7, 8*).

Experiments were performed on female Wistar rats (180 to 250 g) that had been anesthesized with pentobarbital. Determination of cysteinyl leukotriene concentrations in biological fluids was achieved by the sequential use of reversed-phase high-performance liquid chromatography (RP-HPLC) and a radioimmunoassay (RIA) (*7, 9*).

Whenever cysteinyl leukotrienes appear in blood plasma, they are rapidly eliminated from the circulation (*5, 7–9*). In the rat, the initial plasma half-life of injected $[^3H]LTC_4$ was 30 seconds. $[^3H]LTC_4$ and its metabolites were removed mainly by the liver and recovered in bile (Fig. 1).

As shown in Fig. 2, rat plasma and bile differed in composition and concentration of endogenous cysteinyl leukotrienes. The concentration of these leukotrienes in bile was up to 100 times as great as that in plasma. In bile LTE_4NAc was the major detectable metabolite of LTC_4; the amount of LTD_4 and LTE_4 together was less than 5 percent of the LTE_4NAc. Plasma contained LTE_4 as the predominant metabolite, amounting to 1.7 ± 1 nmol/liter (mean ± standard deviation for 11 animals), whereas LTE_4NAc and LTD_4 constituted but a minor and varying fraction. This LTE_4 concentration was determined in plasma obtained immediately after the trauma induced by abdominal incision and exposure of the aorta for puncture. Apparent control samples were taken 30 minutes after abdominal incision and contained less than 0.5 nmol of LTE_4 per liter of plasma. LTE_4NAc and LTE_4 were also the major metabolites of injected $[^3H]LTC_4$ in bile and blood plasma, respectively, as determined by RP-HPLC.

Measurement of endogenous leukotrienes in blood plasma and bile sampled immediately after opening of the abdominal cavity for puncture of the abdominal aorta and for bile duct cannulation (Fig. 2 and Table 1, "bile duct surgery") represent Lts posttraumatic values since the sampling method is itself traumatic. The biliary LTE_4NAc concentration continuously decreased after bile duct cannulation, reaching a constant basal level within about 4 hours. Therefore, a less harmful access to the biliary compartment was required for studying the effects of trauma on the concentration of

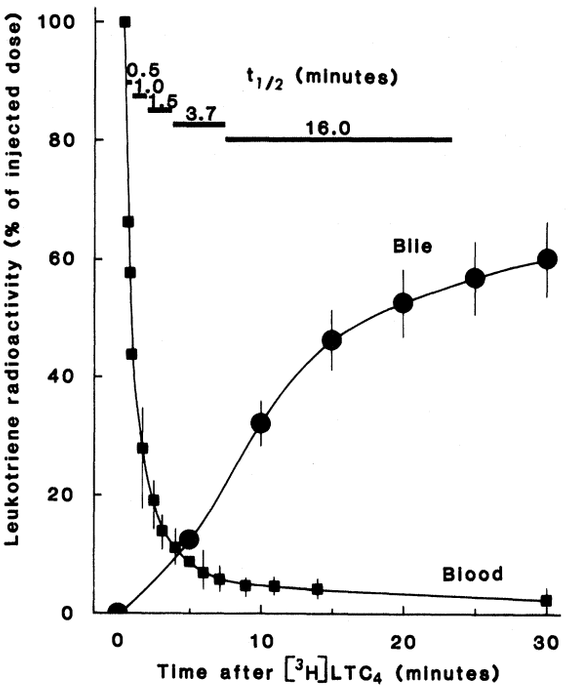

Fig. 1. Time course of $[^3H]LTC_4$ elimination from blood into bile of rats. $[^3H]LTC_4$ (8.5 μCi per kilogram of body weight) was injected intravenously into anesthesized rats. Bile and small amounts of blood from the abdominal aorta were sampled continuously. Data are expressed as percent of injected ^3H circulating in blood (means from three rats; vertical bars indicate the range) or accumulated in bile (means from 21 rats ± standard error of the mean). Horizontal bars with numbers indicate half-life times (minutes) of ^3H radioactivity in blood. According to chromatographic analyses (*7, 9*), radioactivity in blood and bile is associated with $[^3H]LTC_4$ and its metabolites.

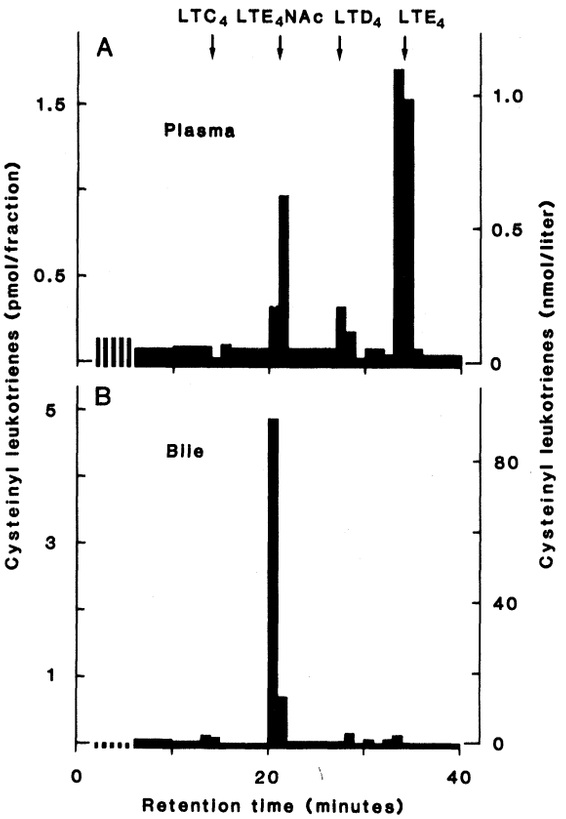

Fig. 2. Cysteinyl leukotrienes in plasma and bile after surgical trauma required for sample collection. Leukotrienes were detected by the combined use of RP-HPLC and RIA. (A) Blood was collected after abdominal incision from the aorta of anesthesized rats, given to an ice-cold solution containing (at final concentrations) heparin (3000 U/liter), EDTA (270 μ*M*), indomethacin (1.7 μ*M*), 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (HTMP, 60 μ*M*) and stored under argon. Blood plasma was deproteinized by addition of methanol at a final concentration of 80 percent in the presence of 1 m*M* HTMP. After a minimum period of 3 hours at −20°C and subsequent centrifugation, aliquots of the supernatants were evaporated to dryness and suspended in 30 percent methanol before application to HPLC. Under the conditions used, tracer $[^3H]LTC_4$ was not metabolized during the preparation of blood for HPLC. The recovery of $[^3H]LTC_4$ added to plasma averaged 86 percent after deproteinization and HPLC. (B) Bile was collected 15 to 45 minutes after bile duct cannulation. Samples were processed and analyzed as described (*7*). The HPLC solvent was composed of a mixture of methanol, water, and acetic acid (65:35:0.1 by volume), and 1 m*M* EDTA, *p*H 5.6; the flow rate was 1 ml/min. Arrows indicate retention times of $[^3H]$leukotriene standards. Concentrations of cysteinyl leukotrienes in the HPLC-fractions were determined by RIA and expressed according to their individual cross-reactivities in the assay, which amounted to 100, 44, 6, and 29 percent on a molar basis for LTC_4, LTD_4, LTE_4, and LTE_4NAc, respectively. At retention times shorter than 6 minutes (broken lines), determination of leukotrienes by RIA was impossible because of the high leukotriene binding in these fractions.

Table 1. Elevation of biliary LTE_4NAc concentration induced by trauma. Control bile was obtained after a minimum of 20 minutes following tapping of the subcutaneous loop of the bilioduodenal anastomosis tubing that had been implanted 1 week before. For abdominal surgery, burn injury, or bone fracture, bile was collected from the anastomosis tubing 20 to 80 minutes after the traumatic manipulation (Fig. 3). For bile duct surgery, bile was collected 15 to 45 minutes after abdominal incision and bile duct cannulation; in this extensive surgical intervention, in contrast to the other traumatic manipulations, surgical tissue injury was induced for the first time. LTE_4NAc was determined as in Figs. 2 and 3. Results are given as mean ± standard deviation for n animals. Values after trauma differed from the control value by a statistically significant amount at the $P < 0.05$ level (Wilcoxon test with Bonferroni correction).

Treatment	n	LTE_4NAc in bile nmol/liter
Control	14	8 (±6)
Abdominal surgery	5	62 (±23)
Burn injury	5	86 (±49)
Bone fracture	5	121 (±54)
Bile duct surgery	4	334 (±194)

endogenous cysteinyl leukotrienes. This was achieved by implanting bilioduodenal anastomosis tubing into the rats 1 week before the experiment. For bile sampling, a subcutaneous loop of the anastomosis tubing was carefully tapped. A transient elevation of biliary leukotrienes was detected during the initial 20 minutes after the bilioduodenal anastomosis was opened. After this time the concentration of cysteinyl leukotrienes in bile was low unless the animals were subjected to further experimental manipulation (Fig. 3 and Table 1).

Burn injury of the back, fracturing of the bones of the hind limbs, or opening of the peritoneal cavity followed by rummaging of the bowels served as models of trauma in the anesthesized rat. Figure 3 shows the time course of generation and biliary appearance of LTE_4NAc after these interventions. The increase in the biliary concentration of cysteinyl leukotrienes was rapid although transient, with a maximum value 1 hour after trauma and a return toward control levels within about 3 hours.

We showed earlier that biliary LTE_4NAc generation was increased in rats under endotoxin shock (*7, 9*). Preliminary data from our laboratory indicate that endogenous cysteinyl leukotrienes can also be detected in human bile during the tissue injury associated with acute pancreatitis. Furthermore, synthesis of leukotrienes has been shown after brain ischemia and reperfusion in gerbils (*10*). In addition to enhanced production of these leukotrienes (Figs. 2 and 3 and Table 1), an increased formation of prostaglandins and thromboxane has been shown after severe trauma (*11*). These cyclooxygenase-derived metabolites of arachidonate mediate, potentiate, or antagonize some of the biological effects of leukotrienes (*2, 3, 12*).

Our results provide support for a mediator role of leukotrienes in trauma. This mediator role is probably not confined to the local area of tissue directly affected by the traumatic lesion. Concentrations of cysteinyl leukotrienes in the nanomolar range in blood plasma and subsequently in bile are sufficient to explain some of the systemic effects of severe tissue injury (*1–5*). Our findings offer new perspectives for the understanding of the pathophysiological consequences of trauma and for appropriate therapeutic intervention.

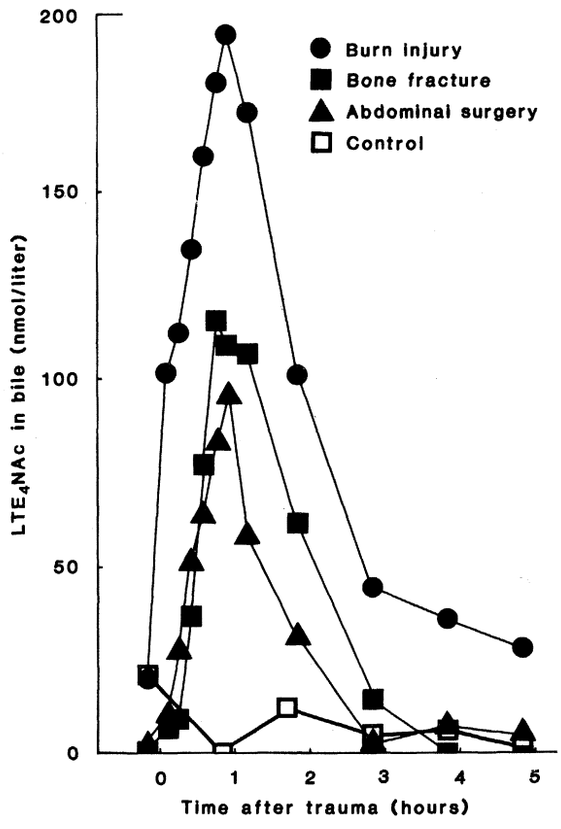

Fig. 3. Time course of generation and biliary appearance of cysteinyl leukotrienes after trauma. Endogenous leukotriene formation was followed by measurement of the LTE_4NAc concentration in bile collected from a subcutaneous loop of a bilioduodenal anastomosis tubing. Each LTE_4NAc value was determined by RP-HPLC and subsequent RIA (*7*) as in Fig. 2. Control: rats without trauma, except for opening of the subcutaneous part of the bilioduodenal anastomosis tubing 20 minutes before the first measurements shown. Burn injury: second- to third-degree burn injury was imposed on the back with an area corresponding to about 18 percent of the body surface of rats by pressing a red-hot metal plate for 1 second on the shaved skin. Bone fracture: bones of the hind legs were fractured twice using a pair of pliers. Abdominal surgery: the peritoneal cavity was opened by a longitudinal cut from the upper abdomen to the pelvis and bowels were rummaged for 5 minutes. During and after these traumatic interventions the rats were under deep pentobarbital anesthesia.

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