Plasminogen Activator Inhibitor-1 Messenger RNA Expression Is Induced in Rat Hepatocytes In Vivo by Dexamethasone

By Barbara A. Konkle, Stephen J. Schuster, Michael D. Kelly, Karla Harjes, Daniel E. Hassett, Mindy Bohrer, and Mehdi Tavassoli

Plasminogen activator inhibitor-1 (PAI-1), the major physiologic inhibitor of tissue plasminogen activator (tPA), plays a crucial role in the regulation of fibrinolysis. Both hepatocytes and endothelial cells have been implicated as major sources of plasma PAI-1. To study the relative contribution of these cell types to hepatic PAI-1 production, we have separated hepatocytes and hepatic sinusoidal endothelial cells by fractionation of freshly isolated rat livers using metrizamide density gradients and centrifugal elutriation. In untreated animals, PAI-1 messenger RNA (mRNA) was detected only in the purified endothelial cell fraction, and not in the hepatocyte fraction or in unfractionated liver. However, when the animals were treated with dexamethasone, PAI-1 mRNA expression was transiently induced in the liver. This induction paralleled the appearance of PAI-1 mRNA in purified hepatocytes, while PAI-1 expression in sinusoidal endothelial cells was unchanged. Four hours after dexamethasone treatment, plasma PAI-1 levels were increased approximately twofold over levels measured in animals treated with the diluent alone. These data suggest that PAI-1 production by hepatocytes may contribute to elevated plasma PAI-1 levels in the setting of acute injury and stress.

From the Cardeza Foundation for Hematologic Research, the Department of Medicine, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA; and the Veterans Administration Southeastern Pennsylvania Affiliate Medical Center, Philadelphia, PA; and the Veterans Administration From the Cardeza Foundation for Hematologic Research, the Department of Medicine, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA; and the Veterans Administration Southeastern Pennsylvania Affiliate Medical Center, Philadelphia, PA.

Address reprint requests to Barbara A. Konkle, MD, Cardeza Foundation for Hematologic Research, 1015 Walnut St, Philadelphia, PA 19107.

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From the Cardeza Foundation for Hematologic Research, the Department of Medicine, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA; and the Veterans Administration Southeastern Pennsylvania Affiliate Medical Center, Philadelphia, PA.

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Plasminogen activator inhibitor-1 (PAI-1) is the major physiologic inhibitor of tissue plasminogen activator (tPA). Clinical studies suggest that PAI-1 plays a crucial role in the regulation of fibrinolysis. Elevated plasma PAI-1 levels have been observed in pathologic conditions associated with thrombosis, including myocardial infarction and deep vein thrombosis, but are also seen in a variety of disease states including in critically ill patients, postoperatively, and in some patients with pancreatitis or malignancies.

PAI-1 is produced by a variety of cell types including endothelial cells, cultured hepatocytes, smooth muscle cells, granulosa cells, megakaryocytes, and a number of malignant cell lines. Endothelial cells and hepatocytes are thought to be the major sources of plasma PAI-1, although it is unclear which cell type is the major synthetic site.

Factors that have been shown to modulate PAI-1 expression in liver-derived cultures are largely different from those that appear to modulate PAI-1 expression in endothelium. Tumor necrosis factor (TNF), interleukin-1, and endotoxin all increase PAI-1 production by endothelial cells, but not by primary cultures of human or rat hepatocytes. Dexamethasone and epidermal growth factor increase PAI-1 production in hepatocyte cultures and malignant hepatic cell lines. Insulin increases PAI-1 synthesis in hepatocyte and Hep G2 cultures but has no effect on large vessel endothelial cell PAI-1 production.

Recent studies have suggested that liver-derived PAI-1 may be synthesized primarily in the hepatic sinusoidal endothelium. The technique of centrifugal elutriation allows separation of liver into highly purified hepatocyte, Kupffer cell, and endothelial cell fractions. Using this technique, Kuiper et al. found that the sinusoidal endothelium was the major source of PAI-1 secreted by rat liver cells. By immunoprecipitation, 35S-labeled PAI-1 was detected in conditioned media from short-term cultures of sinusoidal endothelial cells but not in media from hepatocytes or Kupffer cells. Quax et al. reported the presence of PAI-1 messenger RNA (mRNA) in sinusoidal endothelial cells but not in hepatocytes or Kupffer cells in unstimulated rats, as assessed by Northern blotting technique. In vivo, endotoxin treatment markedly induced sinusoidal endothelial PAI-1 mRNA and to a much lesser degree hepatocyte PAI-1 mRNA levels.

In this report, we confirm that PAI-1 mRNA is constitutively expressed in the sinusoidal endothelium in livers from untreated animals. We further report that dexamethasone induces hepatocyte PAI-1 mRNA expression and increases plasma PAI-1 levels in vivo.

MATERIALS AND METHODS

Liver cell fractionation. Whole liver cell suspensions were prepared from adult male Sprague-Dawley rats (150 to 250 g) by in situ perfusion and collagenase digestion using previously described methods. Solutions were warmed to 42°C to maintain an approximate tissue temperature of 37°C during perfusion and the initial perfusion with calcium-free buffer contained 123 mmol/L NaCl, 2.5 mmol/L MgSO4, 4.7 mmol/L KCl, 1.2 mmol/L KH2PO4, 25 mmol/L NaHCO3, 10 mmol/L HEPES, 0.5% bovine serum albumin (BSA), 5 mmol/L dextrose, and 2,000 U/L heparin solution (pH 7.4). This was followed by perfusion with a similar buffer to which 600 mg/L collagenase (type IV, Sigma Chemical Company, St Louis, MO) and 2.5 mmol/L CaCl2 (final concentration) were added. For some experiments, dextrose, heparin, BSA, and NaHCO3 were omitted from the initial calcium-free perfusion.
buffer, MgSO4 reduced to 0.6 mmol/L and 1.0 mmol/L EGTA added.

After collagenase perfusion, the liver was either minced and further digested in vitro at 37°C with agitation or partially decapsu-
lated and mechanically separated into single cell suspensions. After separation of tissue debris by unit gravity sedimentation, the whole liver cell suspension was fractionated either by double-layer metrizamide gradient centrifugation or by centrifugal elutriation as described. For metrizamide gradient separation, the cell suspension was pelleted and resuspended in phosphate-buffered saline (PBS) at a concentration of 4 x 106 cells/mL and layered over a discontinuous gradient of 15% (specific gravity, 1.09 g/mL) and 30% (specific gravity, 1.15 g/mL) metrizamide solutions. After centrifugation at 1,500g for 30 minutes, two cellular fractions were obtained. Nonparenchymal cells appeared above the 15% metrizamide and hepatocytes appeared at the interface of the 15% and 30% solutions. For isolation of endothelial cells, the super-
nates from these centrifugations were pooled and sinusoidal endothelial cells were purified by elutriation using a Beckman JE6-B elutriator rotor (Beckman Instruments Inc, Palo Alto, CA). Approximately 106 cells were loaded into the rotor at 11 ml/min, 2,500 rpm at 20°C and sinusoidal endothelial cells were collected using a flow rate of 22 ml/min. Cells were processed for light and electron microscopy as well as cytochemistry. The purity of sinusoidal endothelial cells prepared by elutriation was greater than 95% with a viability of greater than 99%. This is in agreement with published results. For some experiments cells were fraction-
ated as follows: after separation of tissue debris by unit gravity sedimentation, the whole liver suspension was centrifuged at 50g, the supernatant removed, recentrifuged, and that supernatant applied to a 15% metrizamide cushion for nonparenchymal cell isolation. The initial hepatocyte pellet was washed with PBS containing 0.5% BSA and recentrifuged as above for a total of 5 times to prepare “washed hepatocytes.” These methods resulted in a nonparenchymal cell fraction containing less than 1% hepato-
cytes and a greater than 90% pure hepatocyte fraction.

**RNA isolation and evaluation.** Total cellular RNA was pre-
pared by immediate solubilization of the cell fractions in guanidine HCl (International Biotechnologies, Inc, New Haven, CT) as previously described. Briefly, the solutions were sonicated and centrifuged to remove cellular debris. The RNA was ethanol-
precipitated, phenol-extracted, and then reprecipitated with etha-
nol. The RNA was quantified by absorbance at 260 nm. Except where noted, 10 μg of each sample was electrophoresed under denaturing conditions in a formaldehyde-containing gel and trans-
ferred to a nylon membrane (Hybond-N; Amersham, Arlington Heights, IL) by Northern blotting. The RNA was fixed to the membrane with ultraviolet irradiation. In experiments using rat-specific probes, the blots were prehybridized in 1 mol/L NaCl, 0.1% sodium dodecyl sulfate (SDS), 1.5 mg/mL herring sperm DNA, and 10% dextran for 3 hours at 68°C, and then hybridized with the appropriate radiolabeled probe at 68°C for 12 to 24 hours in the prehybridization solution with the addition of 1.5 mg/mL sonicated herring sperm DNA. Blots were washed to high stringency (0.1X SSC [SSC: 2.25 mol/L NaCl, 0.225 mol/L sodium citrate], 0.1% SDS, 1 mol/L EDTA [pH 8], 10 mol/L sodium phosphate [pH 6.8], at 68°C) and analyzed by autoradiography. Blots were hybridized with human albumin cDNA at 45°C and washed as above except with 2X SSC to 45°C.

**Rat PAI-1, fibrinogen γ-chain (γ-FGN),** from Willebrand factor (vWF) cDNAs, and human albumin and phosphoglycerate kinase (PGK) cDNAs were used as probes. Rat-specific PAI-1 and vWF probes were prepared after human-rat cross-species hybridization did not produce a signal sufficient for evaluation. The rat PAI-1 probe was prepared using the polymerase chain reaction (PCR) to amplify PAI-1 cDNA from reverse transcribed rat total hepatic RNA, using rat-specific primers (published nucleotide sequence nos. 335-364; 667-638). These procedures were performed as previously described. Briefly, approximately 1 μg of total RNA was used as template with 10 U of avian myeloblastosis virus reverse transcriptase (Seikagaku America, Rockville, MD) for 1 hour at 41°C in 50 mmol/L Tris HCl, pH 8.3, 50 mmol/L KCl, 8 mmol/L MgCl2, 10 mmol/L dithiothreitol, dNTPs at 500 μmol/L each in a total volume of 50 μL with 0.06 A260 unit of oligo(dT)16 as primer. Ten microliters of the reverse transcriptase reaction was adjusted to PCR buffer conditions in a total volume of 50 μL with 0.02 A260 units of each PCR primer and 5 U of Taq polymerase (Thermus aquaticus DNA polymerase; Perkin-Elmer Cetus, Nor-
walk, CT). PCR was performed for 30 cycles in an automated thermocycler (Perkin-Elmer Cetus) with cycle times of 1 minute at 94°C and 2 minutes at 72°C. The rat vWF probe was amplified from the same reverse transcribed RNA using primers from human sequence (nucleotides 7116-7155; 7824-7785) and relaxed annealing conditions for the first 5 cycles of the PCR (1 minute at 94°C, 1 minute at 37°C, gradual increase over 2 minutes to 72°C, 2 minutes at 72°C), then standard conditions as above for the next 25 cycles. The amplified cDNAs were electrophoresed through a 1.5% low-melting agarose gel (LMG; Gibco Bethesda Research Labs, Gaithersburg, MD), the desired bands were cut out, diluted in H2O, and a 20 to 40 ng aliquot was labeled with 32P dCTP (Amersham) using random hexamer priming. These reactions yield approximately 800 ng of insert, as determined by purification of a representative band by phenol chloroform extraction from LMG and ethanol precipitation. The DNA was resuspended in H2O and A260 determined. The identity of the rat PAI-1 and vWF probes were confirmed by their ability to detect the appropriate size message in rat RNA, cross-hybridization to the appropriate message species in human umbilical-vein endothelial-cell RNA under low stringency hybridization and washing, and by direct sequencing of the amplified bands using the dideoxy chain-termination method to sequence double-stranded DNA. The sequence of the amplified rat PAI-1 fragment was 100% identical to that previously reported. For the rat vWF fragment, 245 bp of the 708-bp fragment was sequenced and found to have 81% homology with human sequence.

**Dexamethasone treatment.** Tissue culture grade, endotoxin-free dexamethasone (Sigma) was diluted in sterile PBS, 0.22 μm filtered, and 1 mL (0.4 mg/mL) was used for treatment except where indicated. The diluent alone (sterile PBS) was injected into the control animals. Both the PBS diluent and the dexamethasone solution contained less than 0.01 ng/mL of endotoxin as deter-
mined by a limulus amebocyte lystate gel clot assay (Sigma). Male Sprague-Dawley rats (150 to 250 g) were injected intraperitoneally at the times indicated. Dosages were chosen to ensure plasma levels considerably greater than 1 μmol/L, which is the dose that modulates hepatic PAI-1 in culture.

**Hepatocyte culture.** Hepatocytes isolated as described above were cultured overnight in Dulbecco’s Modified Eagle Medium (GIBCO BRL) and penicillin (100 U/mL) streptomycin (100 μg/mL) with either 1% BSA or with 10% fetal bovine serum (FBS) and insulin (0.24 U/mL). The media were removed approximately 3 hours after plating and fresh media applied. The cells were harvested 12 hours later.

**Plasma PAI-1 levels.** Blood for PAI-1 plasma levels was ob-
tained from the inferior vena cava by venipuncture immediately after opening the abdominal cavity. Blood was collected into sodium citrate containing tubes, centrifuged at 7,500g for 10 minutes, and plasma PAI-1 activity determined using Coatest PAI (Kabi Diagnostica, Mölndal, Sweden). The data were analyzed statistically using the Student’s t-test.
RESULTS

Identification of cell fractions. Hepatocytes were identified by the obvious size difference (≥20 μm) in comparison with nonparenchymal liver cells, as well as by characteristic ultrastructure in electron micrographs as shown in Fig 1. Hepatocyte fractions prepared by metrizamide-gradient centrifugation contained greater than 95% hepatocytes. Hepatic sinusoidal endothelial cells prepared by centrifugal elutriation were stained with diaminobenzidine and H2O2 to assess contamination with peroxidase positive Kupffer cells. By electron microscopy, endothelial cells were seen to have a typical vacuolated cytoplasm that differentiates them from Kupffer cells and hepatocytes. Using electron microscopy and peroxidase activity as criteria, these preparations contained greater than 95% peroxidase-negative vacuolated endothelial cells.

RNA analysis of cell fractions from untreated rats. Northern blot analyses of total cellular RNA prepared from the hepatic fractions are shown in Fig 2. PAI-1 mRNA was detected only in the nonparenchymal cell fraction prepared

Fig 1. (A) Photomicrograph (original magnification ×1,000) and (C) electron micrograph (original magnification ×4,500) of purified rat hepatocytes prepared by metrizamide gradient centrifugation; (B) Photomicrograph (original magnification ×1,000) and (D) electron micrograph (original magnification ×10,150) of purified rat sinusoidal endothelial cells prepared by centrifugal elutriation. Differences in cell size and the characteristic vacuolated cytoplasm of endothelial cells are evident.

Fig 2. PAI-1 mRNA expression in liver cell fractions from untreated rats. Northern blot analysis was performed on 10 μg of total cellular RNA from the parenchymal or hepatocyte metrizamide fraction (H), the nonparenchymal metrizamide fraction (NP), and the hepatic sinusoidal endothelial cells (E) further purified from the nonparenchymal fraction by elutriation. (A) The ethidium-stained ribosomal (rRNA) in the gel before transfer. For (B), the Northern blot was probed with rat PAI-1 cDNA, showing the single, approximately 3-kb rat PAI-1 mRNA species. For (C) and (D), the blots were probed with rat vWF cDNA and human albumin cDNA, respectively.
PAL1 expression is induced in hepatocytes by metrizamide gradient and in the endothelial cells further purified by elutriation. Endothelial cell-specific vWF mRNA was detected similarly, confirming the identity of these cellular fractions as endothelial cells. Albumin mRNA, on the other hand, was detected predominantly in the hepatocyte fractions. Similar results were obtained in four independent experiments from different rat livers.

**Effect of dexamethasone on hepatic PAI-1 mRNA levels.** Rats were treated with dexamethasone (0.4 mg) or the diluent PBS alone and killed at various points in time. PAI-1 mRNA was undetectable by Northern blot analysis of total liver RNA from untreated or PBS treated rats. With dexamethasone treatment, there was an induction of PAI-1 mRNA in unfractionated liver that peaked at 4 to 6 hours, and was again undetectable after 8 hours (Fig 3A). As shown in Fig 3A, analysis of the hepatocyte fraction at 4 hours showed induction in this cell fraction. Because the PAI-1 mRNA level in the nonparenchymal fraction was not noticeably increased over that seen in other untreated control animals, and because nonparenchymal liver cells contain approximately 50 times less total cellular RNA than equal numbers of hepatocytes, the PAI-1 signal seen in 5 μg of RNA from purified hepatocytes could not represent endothelial cell contamination.

To confirm that dexamethasone induced hepatocyte PAI-1 expression, rats were treated simultaneously with dexamethasone or the diluent alone. Four or 6 hours later, the animals were killed, and total liver RNA was isolated and evaluated for PAI-1 mRNA expression as shown in Fig 3B. PAI-1 mRNA was induced in the livers of dexamethasone-treated but not PBS-treated animals. In animals other than those used for the data shown in Figs 2 and 3, the dexamethasone induction of hepatic PAI-1 in vivo was confirmed in total liver RNA from nine rats, metrizamide separated hepatocyte RNA from two rats, and was not seen in eight PBS-treated and eight untreated controls.

Repeated treatment of the rats with dexamethasone did not further increase the hepatic PAI-1 mRNA levels (Fig 3C). Despite a repeat injection, PAI-1 mRNA induction remained transient and was undetectable 8 hours after the first injection and 4 hours after the second injection of dexamethasone. In one experiment, treatment with higher dose dexamethasone (10 mg vs 0.4 mg) did not appear to further increase the PAI-1 mRNA levels at 6 hours (data not shown). Overnight culture of hepatocytes from untreated animals induced PAI-1 expression (Fig 3C), confirming previously reported data by Heaton et al. This induction was seen in the absence or presence of insulin and FBS added to the media.

**Effect of dexamethasone on rat plasma PAI-1 levels.** PAI-1 activity was determined in plasma from animals treated with dexamethasone or the PBS diluent (Fig 4). Four hours...
after treatment with dexamethasone (0.4 mg), plasma PAI-1 activity was approximately twofold that measured in PBS-treated animals. Similar values were obtained 6 hours after treatment. The difference in PAI-1 levels between dexamethasone- and PBS-treated animals was statistically significant after both 4 and 6 hours of treatment ($P = .02$; $P = .004$, respectively).

**DISCUSSION**

Vascular endothelium has been shown to be a major synthetic site of PAI-1 in vivo. Consistent with this, our data show that freshly isolated sinusoidal endothelial cells express PAI-1 mRNA. Although we cannot rule out some induction of PAI-1 synthesis in these cells by endotoxin contamination during isolation, the finding of PAI-1 expression in sinusoidal endothelium has now been confirmed in three independent investigations. Kuiper et al. found PAI-1 to be the major protein secreted by freshly-isolated rat sinusoidal-endothelial cells. Studies have suggested that damage to the sinusoidal endothelium during storage of donor livers is a major factor contributing to liver failure after transplantation. A deficiency of PAI-1 production, possibly related to the endothelial injury, could contribute to the postperfusion hyperfibrinolysis seen after liver transplantation.

It is known that the hepatocyte has the capacity to synthesize PAI-1. Malignant hepatic cell lines produce PAI-1 and have been used in numerous studies to elucidate the regulation of PAI-1 expression. Furthermore, cultures...
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D: Plasma levels of a specific inhibitor of tissue-type plasminogen activator (tPA/mL of plasma under the experimental conditions used. Each sone (0.4 ma). PAL1 activity is shown as arbitrary units (AU) of activator (and urokinase) in normal and pathological conditions.

The difference in PAL1 levels between the dexamethasone- and PBS-treated animals was statistically significant at both 4 and 6 hours after treatment (P = .02; P = .004, respectively).

Fig 4. Plasma PAL1 activity in dexamethasone-treated rats. PAL1 activity was determined in platelet-free plasma samples from rats 4 or 6 hours after treatment with (a) the PBS diluent or (b) dexamethasone (0.4 mg). PAL1 activity is shown as arbitrary units (AU) of inhibitor. One AU is defined as the amount that inhibits 1 IU of tPA/mL of plasma under the experimental conditions used. Each value shown represents the mean ± SD of determinations in four rats.

The difference in PAL1 activity between the dexamethasone- and PBS-treated animals was statistically significant at both 4 and 6 hours after treatment (P = .02; P = .004, respectively).

The findings reported here, that the freshly isolated hepatocyte does not express PAI-1 mRNA as assessed by Northern blotting, confirm published data by Quax et al.18 Because the hepatocytes account for most of the RNA in the liver, low-level expression, not detectable by Northern blotting, could result in significant PAI-1 production. However, data reported by Laishes et al.36 in 1976 on the fibrinolytic activity of rat liver cells suggest that unstimulated hepatocytes do not synthesize a significant amount of PAI-1. By using fibrin-agar overlays, they showed lytic activity from individual hepatocytes. Treatment of the cells with dexamethasone inhibited the lysis, presumably by inducing PAI-1 expression in these cells. The malignant rat hepatoma tissue culture (HTC) cells produced only trace PAI-1 expression in these settings. Further studies of in vivo modulation of sinusoidal endothelial and hepatocyte PAI-1 production will elucidate their respective roles in the modulation of plasma PAI-1.

Our data, and that of others, suggest that cell culture alone induces the hepatocyte to produce PAI-1. Kuiper et al.17 did not detect isolated hepatocyte PAI-1 synthesis, but their cells were in culture for only 4 hours. Because PAI-1 may be involved in modulation of cell proliferation,37,38 it may be induced in the setting of cell growth and matrix deposition.

Hepatic PAI-1 production may play a role in acute stress and inflammation. The study by Quax et al.18 suggested that PAI-1 expression is induced in hepatocytes in endotoxin-treated animals. However, the amount of induction was small compared with the marked induction in the sinusoidal endothelium. de Boer et al.39 recently reported that TNF increases PAI-1 expression in HepG2 cultures, although others have reported no effect on PAI-1 expression in primary cultures of human or rat hepatocytes.30,31 Our study shows that dexamethasone treatment of rats can induce PAI-1 mRNA expression in hepatocytes in vivo, and increase plasma PAI-1 activity. In our studies, the PAI-1 mRNA signal in 5 µg of purified hepatocyte RNA from stimulated animal was similar to that seen in 5 µg of sinusoidal endothelial cell RNA from stimulated or unstimulated animals. However, hepatocytes, which are 60% of the cells in liver,40 contain about 50 times more RNA than the other liver cells.40 Thus, hepatocytes represent the major source of PAI-1 production in this setting.

Plasma PAI-1 is known to be increased in a number of clinical situations including, postoperatively, in acutely ill patients, and in some patients with malignancies or pancreatitis.1,14 Hepatocyte PAI-1 production could be induced in these settings. Further studies of in vivo modulation of sinusoidal endothelial and hepatocyte PAI-1 production will elucidate their respective roles in the modulation of plasma PAI-1.

REFERENCES

6. Sawdey M, Podor TJ, Loskutoff DJ: Regulation of type 1
8. Kooistra et al., Integration of transferrin receptors in endothelial cells of the rat liver by centrifugal elutriation. Gastroenterology 75:30, 1978
14. Alessi et al., Juhan-Vague et al., Kooistra et al., Deeleck et al., Collen et al.: Insulin stimulates the synthesis of plasminogen activator inhibitor 1 in the human hepatocellular carcinoma cells Hep G2. Thromb Haemost 60:491, 1988
17. Kuiper et al., Kamps et al., van Berkelaar et al., JAC: Identification of the inhibitor of the plasminogen activator as the major protein secreted by endothelial rat liver cells. FEBS Lett 245:229, 1989
18. Quax et al., van den Hoogen et al., Verheijen et al., Padro et al., Zehetb, Gelehrter et al., Van Berkelaar et al., Kuiper et al., Emeis et al.: Endotoxin induction of plasminogen activator and plasminogen activator inhibitor type 1 mRNA in rat tissues in vivo. J Biol Chem 265:15563, 1990
19. Soka et al., Pavazza et al.: Liver endothelium and not hepatocytes or Kupffer cells have transferrin receptors. Blood 63:270, 1984
25. Michel et al., Markham et al., Orkin et al.: Isolation and DNA sequence of a full-length cDNA clone for human X chromosome-encoded phosphoglycerate kinase. Proc Natl Acad Sci USA 80:472, 1983
27. Ginsburg et al., Konkle et al., Gill et al., Montgomery et al., Bockenstedt et al., Johnson et al., Yang et al.: Molecular basis of human von Willebrand disease: Analysis of platelet von Willebrand factor mRNA. Proc Natl Acad Sci USA 86:3723, 1989
32. McKeown et al., Edwards et al., Phillips et al., Iu et al., Harvey et al., Petrunka et al., Strasberg et al.: Sinusoidal lining cell damage: The critical injury in cold preservation of liver allografts in the rat. Transplantation 46:178, 1988
33. Caldwell-Kenkel et al., Currin et al., Tanaka et al., Thurner et al., Lemasters et al.: Reperfusion injury to endothelial cells following cold ischemic storage of rat livers. Hepatology 10:292, 1989
34. Harper et al., Luddington et al., Jennings et al., Rearden et al., Seaman et al., Carroll et al., Klink et al., Smith et al., Rolles et al., Calne et al.: Coagulation changes following hepatic revascularization during liver transplantation. Transplantation 48:603, 1989
35. Sprengers et al., Prinzen et al., Kooistra et al., van Hinsbergh et al.: Inhibition of plasminogen activators by conditioned medium of human hepatocytes and hepatoma cell line HepG2. J Lab Clin Med 105:751, 1985
38. Cajot et al., Bamat et al., Bergonzelli et al., Kruithof et al., Medcalf et al., Testuz et al., Sordet et al.: Plasminogen-activator inhibitor type 1 is a potent natural inhibitor of extracellular matrix degradation by fibrosarcoma and colon carcinoma cells. Proc Natl Acad Sci USA 87:6393, 1990
39. de Boer et al., Abbinck et al., Brouwer et al., Meijer et al., Roem et al., Voorn et al., Lammers et al., van Mourik et al., Hack et al.: PAI-1 synthesis in the human hepatoma cell line HepG2 is increased by cytokines—Evidence that the liver contributes to acute phase behavior of PAI-1. Thromb Haemost 65:181, 1991
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