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Influence of Heparin(s) on the Interleukin-1-β-Induced Expression of Collagenase, Stromelysin-1, and Tissue Inhibitor of Metalloproteinase-1 in Human Gingival Fibroblasts

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ABSTRACT. Here, we describe the influence of heparin(s) on the interleukin-1-β (IL-1 β)-induced expression of collagenase (matrix metalloproteinase-1, MMP-1), stromelysin-1 (matrix metalloproteinase-3, MMP-3) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in human gingival fibroblasts (HGF). Amounts of secreted enzymes and inhibitors as well as their mRNA steady-state levels increased significantly following supplementation of HGF culture medium with 2 ng/mL of IL-1 β. Addition of heparin to cell culture medium 1 hour following IL-1 β decreased MMP and TIMP-1 expression in a dose-dependent manner. The inhibitory effect of heparin was significant at a concentration as low as 1 μg/mL. These findings could be reproduced with a low M_r heparin fragment devoid of anticoagulant activity. Heparin and fragments might therefore reduce the excessive proteolytic capacity of the gingival fibroblast during inflammation and could be useful as pharmacological agent(s) in gingivitis and periodontitis. BIOCHEM PHARMACOL **56**;11:1447–1454, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. gingival fibroblasts; interleukin-1; heparin; matrix metalloproteinases; collagenase; stromelysin

The integrity of the extracellular matrix requires a balance between the amount and activity of matrix-degrading proteolytic enzymes and their associated inhibitors [1, 2]. The most prominent of these neutral endopeptidases belong to the MMP family which, to date, comprises at least 15 members [3]. Among them, collagenase (MMP-1§), as initiator of the degradation of interstitial collagens, and stromelysin-1 (MMP-3), first described as a proteoglycanase, constitute pivotal control points for matrix catabolism. The activity of these matrix metalloproteinases is controlled by specific tissue inhibitors of metalloproteinases, i.e. TIMPs, mainly TIMP-1 [1, 2].

Nonspecific inflammation following tissue response to injury is characterized by a local accumulation of neutrophils and mononuclear cells [4]. Release of IL-1 β by mononuclear cells amplifies this inflammatory response [4–8]. IL-1 β is probably the most potent inducer of MMP-1 and MMP-3 expression in fibroblasts, although cells of different tissue origins do not necessarily respond in a similar fashion to their environment and to a given cytokine. Gingival fibroblasts are under constant aggression due to the mechanical forces applied to gingiva, and gingival tissue is susceptible to damage from plaque bacteria and their by-products, resulting in acute inflammation [9]. For instance, the influence of cytokines, particularly IL-1, in the pathogenesis of peridontal disease is well documented [10, 11]. It was demonstrated, using cultured fibroblasts from normal or wounded rabbit oral mucosa, that IL-1 activity expressed by fibroblasts from injured tissue was 3- to 10-fold higher as compared to normal fibroblasts [12]. Loss of coordination between MMPs and their natural inhibitor, i.e. TIMP-1 expression, could thus lead to impaired gingiva remodeling and periodontitis. Mast cells are widely found and randomly distributed in normal connective tissue. Their number has been observed to increase during connective tissue inflammation, and they are known to contain various biologically active substances such as histamine and heparin [13].

Heparin inhibits the proliferation and migration of several cell types [14, 15] and was also recently found to modulate the increased biosyntheses of MMPs following

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§ *Abbreviations:* DMEM, Dulbecco's modified Eagle's medium; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HGF, human gingival fibroblasts; IL-1, interleukin-1; LAH, lactal human hydrolysate; MMP-1, matrix metalloproteinase-1; MMP-3, matrix metalloproteinase-3; and TIMP-1, tissue inhibitor of metalloproteinase-1.

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phorbol ester and IL-1 β treatment of arterial smooth muscle cells and mesangial cells, respectively [16–18]. Growth of HGF could also be partly suppressed by heparin and/or fragments.* These data led us to examine the influence of heparin as well as a heparin fragment devoid of anticoagulant activity on the IL-1-β-induced levels of MMP-1, MMP-3 and TIMP-1 expression in HGF.

MATERIALS AND METHODS

Materials

Cell culture medium, additives and foetal bovine serum were purchased from GIBCO unless otherwise specified. Electrophoresis supplies were obtained from Biorad. The following items were from Sigma Chemical Co.: aminophenylmercuric acetate (APMA), α casein, calf skin collagen type I and porcine skin gelatin. IL-1 β was obtained from Boehringer. MMP-1, MMP-3, and TIMP-1 as well as their corresponding antisera were purchased from Valbiotech. Enzymes and inhibitor were >98% homogeneous by electrophoresis and Western blotting. Heparin and low molecular weight heparin were gifts from M. Petitou (Sanofi Recherche). Heparin was isolated from porcine mucosa (160 USP units/mg; 155 anti-Xa units/mg), and periodate-oxidized heparin fragments (SR 80258A) were obtained after β elimination and borohydride reduction of periodate-oxidized heparin as described [19]. It exhibited $M_r \sim 6{,}000$ (1000–9000 range); OSO_3^- : 3.85 mequiv/g; OSO_3^-/CO_2^- : 2.40/1.0.

Cell Culture

Human gingival fibroblasts were obtained from gingival explants (14–28 years old) with no history of periodontitis. All patients gave their informed consent and denied having recently taken drugs that could affect connective tissue metabolism. Gingival tissue was rinsed three times with DMEM supplemented with 400 μg/mL of penicillin, 400 μg/mL of streptomycin, and 4 μg/mL of fungizone and cut into small pieces. Primary explant cultures were established in 25 cm^2 culture flasks in DMEM containing 20% foetal bovine serum, penicillin (100 μg/mL), streptomycin (100 μg/mL), and fungizone (2 ng/mL). Monolayer cultures were maintained in 5/100 CO_2/air v/v and cell culture medium was changed every 48 hr. After passage, the cells were routinely maintained in 10% foetal bovine serum containing DMEM. HGF were used from passages 3–8. For experiments, cells were seeded at 30,000/cm^2 and grown to subconfluency (100,000/cm^2) in DMEM supplemented with 10% foetal bovine serum, penicillin (100 μg/mL), streptomycin (100 μg/mL), and fungizone (2 ng/mL); they were cultured in serum-free DMEM containing 0.2% LAH obtained from Sigma for 4–48 hr. Fibroblasts were treated or not with IL-1 β (10–100 U/mL) for 1 hr, cell culture medium was withdrawn, and heparin or fragment (0.1–400 μg/mL) in serum-free medium supplemented with LAH was added.

Determination of MMP and TIMP-1 Levels Secreted into Fibroblast Culture Medium

ZYMOGRAPHIC ANALYSES OF COLLAGENASE AND STROMELYSIN. For collagenase, we recently developed a novel zymographic assay in which native type I collagen was incorporated into polyacrylamide gel. A 0.5 mg/mL collagen concentration within gels was found optimal for the detection of procollagenase and its activated form. Briefly, conditioned culture medium was mixed with Laemmli sample buffer containing 0.1% SDS without reducing agent and electrophoresed without boiling, under nondenaturing conditions. Following electrophoresis, SDS was eluted from the gel by 3 successive washings in 2.5% Triton X 100 for 20 min to allow collagen fibrillation and protein to renature. Gels were then immersed for 16–48 hr in 100 mM of Tris, 5 mM of $CaCl_2$, 2 μM of $ZnCl_2$ pH 8.0, and stained with Coomassie blue R.250 to reveal zones of lysis [20]. Casein (1 mg/mL) zymography was used to demonstrate the presence of MMP-3 in HGF-conditioned media [21].

IMMUNOBLOT DETECTION OF COLLAGENASE, STROMELYSIN, AND TIMPS. Aliquots of fibroblast-conditioned media were brought to a final volume of 100 μL with DMEM, centrifuged at 10,000 g to remove cellular debris, and mixed with 10 μL of 1M Tris–Hcl, 150 mM of NaCl, pH 7.5. Samples were applied in triplicate onto nitrocellulose membrane (Biorad), mounted in a slot-blot apparatus. Membranes were air-dried at 22° for 1 hr and treated with 1% blocking solution (Boehringer) for 1 hr at 22° with constant shaking. The filters were then treated with either antistromelysin, anticollagenase, or anti-TIMP monoclonal antibodies, diluted 1/1000 in blocking solution overnight at 22°. Membranes were washed 4 times for 10 min with 50 mM of Tris, 150 mM of NaCl, 0.1% Tween 20 (v/v), pH 7.5 (TBS/Tween), and incubated with peroxydase-labeled second antibodies diluted 1/200 (v/v) in blocking solution. They were then washed extensively with TBS Tween, treated with H_2O_2 diacylhydrazide (Boerhinger) for 1 min, and revealed using a Kodak Biomax MR film. Multiple exposures were examined to ascertain that the results analyzed reflected those produced in the linear range of the film.

Total RNA Extraction and Northern Blot Analyses

For Northern blot analyses, total RNA from each 75 cm^2 flask of fibroblasts was extracted with an Rnazol (1 mL/10^6 cells) kit. The steady-state levels of each mRNA (i.e. the levels resulting from the cumulative effects of both mRNA transcription and mRNA degradation) were determined on Northern blots. Equivalent amounts (1 μg) were electro-

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TABLE 1A. Influence of heparin and heparin fragment (SR 80258A) on the expression of MMP-1, MMP-3, and TIMP-1 by HGF

	MMP-1 Amount (ng/mL)	Variation (as compared to control)	MMP-3 Amount (ng/mL)	Variation (as compared to control)	TIMP-1 Amount (ng/mL)	Variation (as compared to control)
Control	19.9 (±2.1)	1.0	59.6 (±6.2)	1.0	54.7 (±4.9)	1.0
Heparin treatment						
0.1 μg/mL	20.1 (±3.2)	1.01	68.9 (±7.9)	1.15	60.1 (±8.9)	1.10
1 μg/mL	15.2 (±2.3)	0.76	69.4 (±5.8)	1.16	60.8 (±5.2)	1.10
10 μg/mL1	11.9 (±1.6)*	0.59	55.7 (±6.5)	0.93	63.6 (±5.8)	1.16
100 μg/mL	6.7 (±0.6)*	0.33	10.1 (±1.6)*	0.17	57.1 (±3.2)	1.04
Heparin fragment (SR 80258 A) treatment						
0.1 μg/mL	14.6 (±1.5)	0.75	66.6 (±6.4)	1.12	53.7 (±6.1)	0.98
1 μg/mL	12.1 (±1.1)*	0.61	55.5 (±5.3)	0.93	55.5 (±6.3)	1.02
10 μg/mL	8.4 (±0.5)*	0.42	15.6 (±1.5)*	0.26	49.8 (±5.5)	0.91
100 μg/mL	4.2 (±0.5)*	0.21	4.1 (±0.4)*	0.07	49.5 (±4.2)	0.90

Enzymes and inhibitor are quantified in serum-free conditioned media (36 hr of culture) by immunoblot assay. Values are expressed as ng/10^5 fibroblasts and are means of data obtained with 3 different cell lines (experiments in triplicate).

*$P \leq 0.01$ (Student-Fisher t test between control and treatment).

phoresed on 1% agarose, 2.2 M formaldehyde gels that were stained with ethidium bromide before transfer to check RNA integrity and assess levels of the RNA sample loaded. The RNAs were blotted onto nitrocellulose and blots were immersed at 80° under vacuum and hybridized with ^{32}P-labeled nick-translated cDNA inserts for either stromelysin, collagenase, TIMP-1, c-*fos*, or GAPDH. Nick translations were performed with the Amersham rediprime kit. Each probe had a specific activity averaging 10^8 cpm/μg. The characteristics of each probe purchased from Valbiotech are as follows:

MMP-1: Size 3.3 kb: A 415 bp Xbal/Eco RI (135–548) fragment of human interstitial collagenase cDNA spanning the N-terminal protein domain, cloned into the HincII/XBaI site of pGEM2;

MMP-3: Size 3.1 kb: A 475 bp corresponds to the range of 1336 to 1810 of human stromelysin cDNA, cloned into the EcoRI/HindIII sites of pGEM2;

TIMP-1: Size 3.3 kb: A 331 bp San 3 A fragment from p16C8A corresponding to the murine TIMP-1 gene was cloned into a Bam HI site of pGEM1;

GAPDH: Size 4 kb: cDNA coding from murine GAPDH was cloned by dG/dC tailing into the Psti site of pBR 322. The 1050 bp insert was isolated by PstI digestion and cloned into the PstI site of pBlue Script KS;

c-*fos*: a 40 single-stranded synthetise a oligonucleotide

TABLE 1B. Influence of heparin and heparin fragment (SR 80258A) on the IL-1β-induced expression of MMP-1, MMP-3, and TIMP-1 by HGF

	MMP-1		MMP-3		TIMP-1	
	Amount (ng/mL)	Variation (as compared to control)	Amount (ng/mL)	Variation (as compared to control)	Amount (ng/mL)	Variation (as compared to control)
Control	19.9 (±2.1)	1.0	59.6 (±6.2)	1.0	54.7 (±4.9)	1.0
IL-1β (100 U/mL) treatment	48.9 (±4.8)*	2.46	109.8 (±9.4)*	1.84	99.6 (±8.8)*	1.82
IL-1β (100 U/mL1) treatment in presence of heparin						
0.1 μg/mL	53.2 (±6.1)*	2.67	82.6 (±7.3)*	1.38	93.1 (±6.9)*	1.70
1 μg/mL	44.9 (±3.5)*	2.26	71.7 (±5.2)*	1.20	79.5 (±5.8)*	1.45
10 μg/mL	28.7 (±3.6)*	1.44	31.7 (±2.5)*	0.53	64.6 (±5.2)	1.18
100 μg/mL	19.1 (±2.1)	0.96	22.2 (±1.8)*	0.37	59.3 (±6.2)	1.08
IL-1β (100 U/mL) treatment in presence of heparin fragment (SR 80258 A)						
0.1 μg/mL	52.3 (±4.5)*	2.62	58.4 (±6.1)	0.98	89.8 (±9.1)*	1.64
1 μg/mL	40.1 (±4.1)*	2.01	46.6 (±5.2)	0.78	78 (±6.2)*	1.42
10 μg/mL	20 (±3.2)	1.00	31.8 (±1.7)*	0.53	59.3 (±4.5)	1.08
100 μg/mL	18.1 (±1.9)	0.91	27.7 (±3.4)*	0.46	50.8 (±4.8)	0.93

Enzymes and inhibitor are quantified in serum-free conditioned media (36 hr of culture) by immunoblot assay. Values are expressed as ng/10^5 fibroblasts and are means of data obtained with 3 different cell lines (experiments in triplicate).

*$P \leq 0.01$ (Student-Fisher t test between control and treatment).

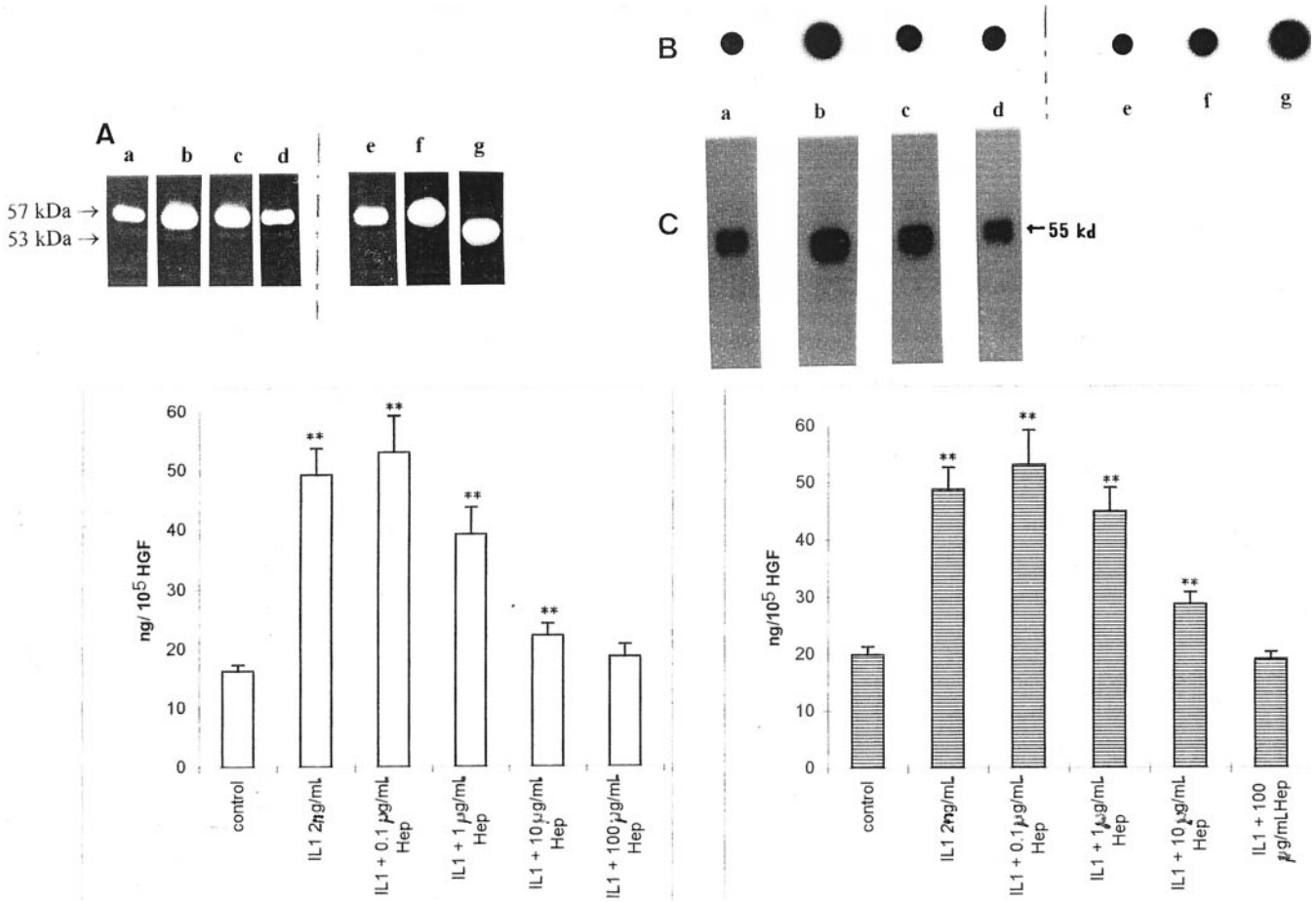

FIG. 1. Effect of heparin on the IL-1β stimulation of collagenase production by HGF. Levels of enzyme, as determined following 36 hr of culture in serum-free conditioned media, were expressed as ng/10^5 fibroblasts. (A) Collagen zymography. Inset: lane a, control; lane b, fibroblasts were treated with 2 ng/mL of IL-1β; lane c, fibroblasts received 2 ng/mL of IL-1β; following 1 hr of culture, the fibroblast culture medium was supplemented with 10 µg/mL of heparin; lane d, same as c, but culture medium contained 100 µg/mL of heparin; lane e, procollagenase (0.1 ng); lane f, procollagenase (0.3 ng); and lane g, procollagenase (0.1 ng) was activated with APMA (1 mM) for 3 hr. Dose-response of the heparin effect. Bars correspond to SEM (from two cell strains; experiments in quadriplicate). **$P \leq 0.01$ (Student-Fisher *t* test between control and treatment). (B) and (C) Immunoblot assay. Inset: lane a, control; lane b, fibroblasts were treated with 2 ng/mL of IL-1β; lane c, fibroblasts received 2 ng/mL of IL-1β; following 1 hr of culture, the fibroblast culture medium was supplemented with 10 µg/mL of heparin; lane d, same as c, but culture medium contained 100 µg/mL of heparin; lane e, procollagenase (0.01 ng); lane f, procollagenase (0.05 ng); and lane g, procollagenase (0.1 ng). Western blots are represented (corresponding to a, b, c, and d) to illustrate the specificity of the antibody used. Dose-response of the heparin effect. Bars correspond to SEM (from two cell strains; experiments in quadriplicate). **$P \leq 0.01$ (Student-Fisher *t* test between control and treatment).

(52% GC) derived from the first exon of c-*fos* was used under high stringency hybridization conditions.

All hybridizations were performed as described. Filters were exposed to Kodak film with a Dupont lightening intensifying screen at −80°. No cross-hybridization could be demonstrated between enzymes under our experimental conditions.

Quantification of Zymogram Lysis Bands and Blots by Image Analysis

The average surface of polyacrylamide gel electrophoresis bands or/and membrane blots was determined semi-automatically following its contour with a calibrated electronic slide as described previously [21]. They were then observed with a C.F.R.126 videocamera, and black and white images were converted into 256 different gray levels using Sophretec image memory, transferred to a BFM microcomputer, and finally analyzed with a software for mathematical morphology. For zymographic analyses, surface (pixels) × gray levels were converted to ng of MMP or TIMP using standard curves incorporated into each experiment.

Statistical Analysis of Data

The statistical significance of the variations observed were analyzed using the Student-Fisher *t*-test.

RESULTS

The levels of MMPs and TIMP-1 were first quantified in the HGF serum-free conditioned media from 6 to 48 hr of

culture. For periods of culture <12 hr, the amounts of secreted MMPs and TIMP-1 fell below the detection limit of our assays; they further accumulated quasi linearly with time of culture up to 48 hr. Following 36 hr of culture in DMEM supplemented with 0.2% LAH, 10^5 HGF secreted 19.9 ± 1.8 ng, 59.6 ± 4.3 ng, and 54.7 ± 2.9 ng of MMP-1, MMP-3, and TIMP-1, respectively (Table 1). Although LAH was previously reported to influence the basal expression of MMP-1 in rheumatoid synovial fibroblasts [7], supplementation of HGF serum-free culture medium with BSA instead of LAH did not modify the levels of enzymes and inhibitor secreted by gingival fibroblasts.

Influence of Heparin(s) on the IL-1-β-Mediated Production of MMP and TIMP-1 HGF

Preliminary experiments intended to evaluate temporal aspects of HGF activation by IL-1 β revealed that the extent of MMP stimulation by cytokine was on average similar at 24, 36, and 48 hr under our culture conditions (not shown). Also, dose-response studies indicated that IL-1 β stimulation of MMP-1 production was optimal for a cytokine concentration equal to 100 UI/mL (2 ng/mL) (Fig. 1). Such a concentration can be considered as saturating, since the Kd value of IL-1 β for its receptor has been reported in the picomolar range [7]. The influence of heparin on the production of MMP-1 was determined using either heparin or its fragment at concentrations ranging from 0.1–400 μg/mL. To avoid possible interaction with IL-1 β or its receptor, heparin(s) was added one hour following supplementation of HGF culture medium with the cytokine. IL-1 β induced a maximal ~3-fold increase in MMP-1 production by HGF; levels of enzyme, as determined by collagen zymography or immunoblot analyses, were equivalent (Fig. 1, A and B), further substantiating the usefulness of collagen zymography in quantifying low amounts of collagenase [20]. Also, whatever the culture conditions, only the zymogen form of MMP-1 could be identified (Fig. 1A).

Figure 1, A and B indicates that heparin was able to significantly decrease the induced IL-1 β expression of MMP-1 in a quasi linear fashion with increasing doses of heparin. At 100 μg/mL of heparin, MMP-1 amounts returned to basal levels of secretion.

The influence of heparin and fragment on the basal and IL-1-β-mediated production of MMP-1, MMP-3, and TIMP-1 was analyzed. Heparin was found to inhibit the basal secretion of both enzymes by HGF while having no effect on TIMP-1 (Table 1A). The inhibitory effect was particularly significant at a high concentration of heparin (100 μg/mL); interestingly, heparin fragments exerted were influenced at a lower concentration. The effect of IL-1 β on MMP-3 expression by HGF was less than that observed for MMP-1, i.e. a 1.84-fold stimulation, but the inhibitory influence was greater at lower concentrations (Table 1B). In particular, 0.1 μg/mL of heparin fragment was capable of totally inhibiting the IL-1-β-induced overexpression of MMP-3.

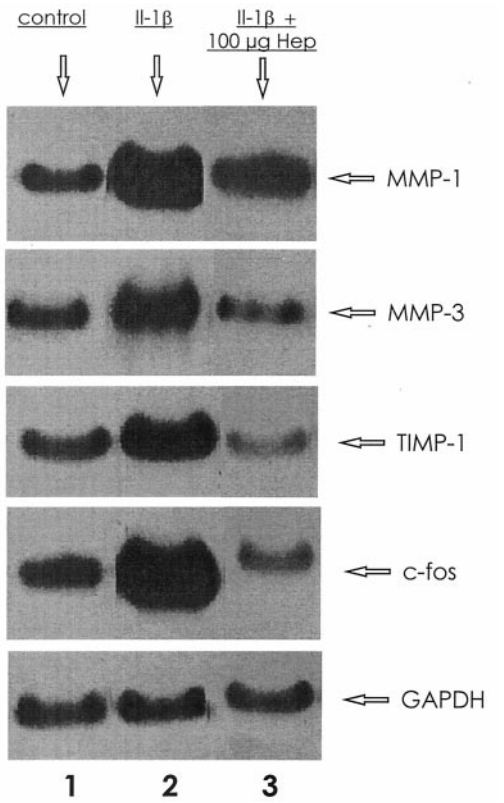

FIG. 2. Constitutive and IL-1β-induced expression of collagenase, stromelysin, TIMP-1 and c-*fos* mRNAs in HGF. Effect of heparin (100 μg/mL). Northern blot analyses of total RNA isolated from HGF treated with DMEM containing 0.2% LAH (lane 1), 0.2% LAH and IL-1β (2 ng/mL) (lane 2), and 0.2% LAH, IL-1β (2 ng/mL) and heparin (100 μg/mL) (lane 3). The Northern blots were hybridized with ^{32}P-labeled cDNA probes. GAPDH was used as control.

In keeping with investigations performed with fibroblasts from other origins [22–24], IL-1 β only induced a modest (1.8-fold) albeit significant increase in TIMP-1 secretion by HGF; as for MMPs, heparin(s) down-regulated the IL-1-β-induced expression of TIMP-1 (Table 1B).

Suppression of IL-1-Induced MMP-1, MMP-3, and TIMP-1 mRNA Expression by Heparin(s)

More generally, mRNA variations parallel the changes in protein synthesis induced by IL-1 [16, 17]. Levels of specific mRNAs were determined by Northern blot analysis following 7 hr of culture to determine whether heparin(s) exerted its influence on IL-1-β-induced secretion of MMP-1, MMP-3, and TIMP-1 at a translational or pretranslational level. c-*fos* expression was analyzed in parallel and mRNA levels were standardized with respect to corresponding GAPDH mRNA levels (Figs. 2 and 3).

Steady-state levels of enzymes and inhibitor mRNAs were highest in the presence of IL-1 β with a 7.8-, 7.1-, and 4-fold induction for MMP-1, MMP-3, and TIMP-1, respec-

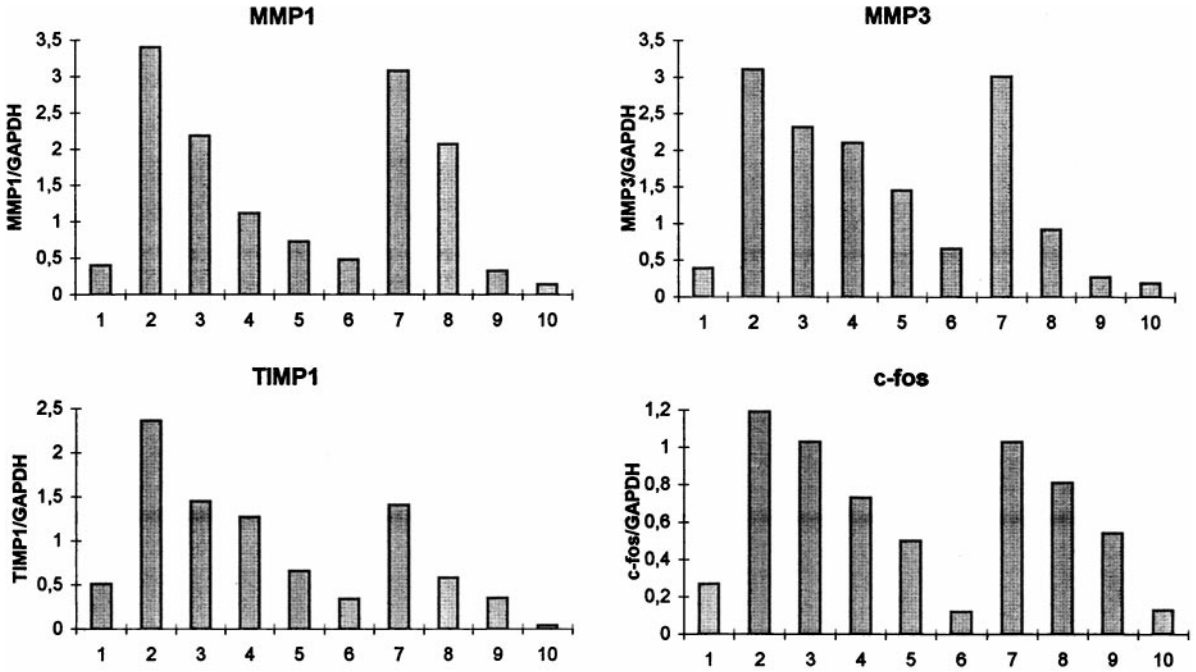

FIG. 3. Down-regulation of the IL-1-β-induced expression of matrix metalloproteinases, TIMP-1, and c-*fos* mRNA steady-state levels by heparin and heparin fragment. Northern blots were quantified by image analysis and results were normalized with GAPDH. Experiments were performed twice with two different HGF strains; the extent of variations was similar and data reported in this figure are means of obtained values: lane 1, control; lane 2, IL-1β 2 ng/mL; lane 3, IL-1β 2 ng/mL + heparin 0.1 μg/mL; lane 4, IL-1β 2 ng/mL + heparin 1 μg/mL; lane 5, IL-1β 2 ng/mL + heparin 10 μg/mL; lane 6, IL-1β 2 ng/mL + heparin 100 μg/mL; lane 7, IL-1β 2 ng/mL + SR 80258A 0.1 μg/mL; lane 8, IL-1β 2 ng/mL + SR 80258A 1 μg/mL; lane 9, IL-1β 2 ng/mL + SR 80258A 10 μg/mL; lane 10, IL-1β 2 ng/mL + SR 80258A 100 μg/mL.

tively; similar variations were observed when mRNA were analyzed for 16 hr following IL-1 supplementation (not shown). Since c-*fos* mRNA expression by IL-1 was described to exhibit a transient character, protooncogene mRNAs were isolated and quantified 3–4 hr after IL-1 β addition to cell culture media. Under such conditions, IL-1 β (2 ng/mL) induced a 4-fold enhancement of c-*fos* mRNA (Figs. 2 and 3).

The IL-1-β-induced steady-state levels of all mRNAs returned to basal levels in the presence of 100 μg/mL of heparin, but a concentration as low as 1 μg/mL could significantly reduce their expression (Fig. 3A). Again, SR 80258A appeared more potent than heparin in inhibiting the IL-1-β-induced stimulation of all mRNAs (Fig. 3B).

DISCUSSION

The regulation of matrix involves a balance between synthesis and organization of its structural constituents and their degradation by metalloendopeptidases belonging to the matrixin family [1, 2]. The activities of these proteinases are, in turn, modulated by specific inhibitors, i.e. tissue inhibitors of metalloproteinases [25, 26]. Fibroblasts, as cells able to synthesize all members of this triad, play a prominent role in maintaining tissue homeostasis both under basal conditions and during repair following injury. Substances which may affect matrix metalloproteinases and inhibitor expression could therefore be considered, depending on the circumstances, as beneficial or detrimental. Previous investigations indicated that heparin could modulate the phenotypic expression of collagen types in fibroblasts and aorta smooth muscle cells [27, 28] and down-regulate the phorbol ester-induced expression of collagenase in arterial cells [16]. This investigation was undertaken to explore the influence of heparin on the expression of collagenase, stromelysin, and TIMP-1 by fibroblasts in culture. We recently evidenced that the expression of neutral proteinases by fibroblasts depended upon their tissular origin and their matrix environment [29, 30]. Gingival fibroblasts were used in the present study mainly in keeping with the critical importance of IL-1 in gingival diseases [2, 5]. It must be mentioned, however, that, on a qualitative basis, most of the heparin effects reported herein also applied to human dermal fibroblasts (not shown).

Gingival fibroblasts secreted, in a constitutive manner, low levels of collagenase: 19 ng/10^5 cells following 36 hr of culture; on average, they produced a 3-fold higher amount of stromelysin, a particularity of this cell type since dermal fibroblasts expressed, on a molar basis, similar quantities of both enzymes.*

IL-1β was found to enhance the transcription of collagenase and stromelysin genes through their 5′TRE sequence, but the extent of induction varied according to the tissular origin of fibroblasts [22–24]. A 2 ng/mL (0.12 pM)

*B. Gogly *et al.*, unpublished results.

concentration of IL-1 β was here found optimal for collagenase stimulation by gingival fibroblasts both at the protein (3-fold increase) and the mRNA level (7-fold increase). Such a cytokine-induced increase in collagenase production is close to values reported by Oshima *et al.*, who used similar gingival fibroblast culture conditions and 1.25 ng/mL of IL-1 [8]. Stromelysin expression, often coordinated with collagenase, was also stimulated by IL-1 β but to a lesser extent. The basal expression of TIMP-1 in gingival fibroblasts was also slightly increased by interleukin-1. TIMP-1, like MMP-1 and MMP-3, contains a TRE regulatory sequence in its promotor [25] and is induced in areas of inflammation where AP-1 expression is high [5, 31]; however, its level of induction by IL-1 β is generally less than those of collagenase [22–24].

The mechanism involved in the inhibitory effect of heparin on several types of gene expression following interleukin treatment of cells is not fully understood. Although IL-1 β is only slowly internalized following binding to its receptor, the direct interaction between heparin and cytokine and its receptors seemed improbable, since in the present investigation heparin supplementation of fibroblast culture medium was delayed by one hour following IL-1 β addition to cells. Generally, substances that either inhibit protein kinase C or elevate levels of cyclic AMP and activate protein kinase A significantly inhibit IL-1-β-mediated effects [24]. Since several transcription factors contain heparin-binding sequences, heparin could regulate gene activity at nuclear sites binding to transcription factors [18]. It could also act upstream by inhibiting either the mitogen-activated protein kinase [34] or with the phosphatidyl inositol 4-phosphate kinase [35], modifying calcium fluxes [36], or by interfering with the sphingomyelin pathway which was implicated in IL-1 β signaling events [37].

Heparin effects on collagenase, stromelysin, and TIMP-1 expression could be reproduced with low M_r fragments devoid of anticoagulant activity. Such low M_r heparin species were also found to inhibit human leucocyte elastase in a noncompetitive hyperbolic manner with Ki $<$ 10-9 M [38], a similar concentration to those required to suppress IL-1-β-induced expressions of collagenase and stromelysin. Elastase was considered to be a marker in periodontal diseases and furthermore can activate prostromelysin, which in turn activates procollagenase [39].

Therefore, heparin fragments, by acting both at the level of collagenase and stromelysin gene transcriptions and at the activation of the secreted zymogens, could be envisaged as useful pharmacological agents for controling matrix destruction as occurs during periodontitis.

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