Kidney International, Vol. 45 (1994), pp. 137–142

Heparin inhibits endothelin-1 production in cultured rat mesangial cells

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Heparin inhibits endothelin-1 production in cultured rat mesangial cells. The present study was designed to examine whether heparin inhibits basal or stimulated endothelin-1 production by arginine vasopressin (AVP) and platelet-derived growth factor (PDGF) in cultured rat mesangial cells. In addition, the reversibility of the heparin effect on mesangial cell endothelin-1 production was examined. AVP and PDGF stimulated endothelin-1 secretion in a concentration-dependent manner in these cells. Heparin (10 to 100 U/ml) exhibited concentration-related inhibition of AVP- and PDGF-stimulated endothelin-1 secretion. Heparin also had weak but significant inhibitory effects on basal endothelin-1 secretion in these cells. The protein kinase (PKC)-activating phorbor ester, phorbor myristate acetate (PMA), stimulated endothelin-1 secretion and heparin inhibited PMA-stimulated endothelin-1 secretion. In addition, the inhibitory effect of heparin was completely abolished in PKC-depleted mesangial cells. Mesangial cells which were exposed to a high concentration (100 U/ml) of heparin for 24 hours were capable of producing endothelin-1 after a short lag period of removal of heparin from the culture medium. These mesangial cells also showed recovery of responses to AVP and PDGF by secreting a significantly greater amount of endothelin-1 than the non-stimulated level. These results indicate that heparin potently inhibits mesangial cell endothelin-1 production, especially when stimulated by AVP or PDGF. This inhibitory effect of heparin is probably PKC dependent, and reversible.

Glomerular mesangial cells are directly related to glomerular function through their contractility and proliferation. These cells have receptors specific to various vasoactive peptides and growth factors such as arginine vasopressin (AVP) [1, 2], endothelin [3, 4] and platelet-derived growth factor (PDGF) [5, 6]. Recent evidence indicates that AVP and PDGF bind to their specific receptors, and induce the contraction and proliferation of mesangial cells [1, 7–9].

Endothelin-1 exerts a potent vasoconstrictive effect on a variety of blood vessels including renal artery [10–12]. This peptide is also shown to bind to its specific receptors and induce the contraction and proliferation of glomerular mesangial cells [3, 13]. Endothelin has been reported to play a role in variety of diseases that affect the kidney, including severe hypertension [14–16], acute renal failure [17–19] and cyclosporine nephrotoxicity [20, 21]. In fact, we have previously shown that plasma endothelin-1 levels are significantly increased in hypertensive patients with impaired renal function and in severe hypertensive rats with renal damage [14, 15]. Furthermore, recent studies demonstrated constitutive expression of endothelin-1 transcripts and peptide secretion in cultured rat mesangial cells [22, 23]. This endothelin-1 production by mesangial cells is found to be stimulated by AVP and PDGF [2, 24, 25]. These observations have led to the possibility that endothelin-1 may play a role as a modulator of renal function. On the other hand, recently we [26] have shown that heparin treatment reduces vascular endothelial cell production of endothelin-1 and lowers blood pressure in spontaneously hypertensive rats (SHR). Therefore, if heparin inhibits mesangial cell production of endothelin-1, heparin treatment may modulate renal function in certain pathological states, including hypertension and glomerular diseases.

Accordingly, the present study was designed to test the two hypotheses: (1) that heparin inhibits basal endothelin-1 secretion in cultured rat mesangial cells; and (2) that heparin inhibits AVP- and PDGF-stimulated endothelin-1 secretion in these cells. In addition, we examined whether this inhibitory effect of heparin on mesangial cells was reversible.

Methods

Materials

AVP, PDGF, angiotensin II (Ang II), phorbor myristate acetate (PMA) and heparin were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). RPMI 1640, trypsin, Versene® and fetal calf serum (FCS) were purchased from GIBCO Laboratories (Grand Island, New York, USA). Flasks were purchased from Becton Dickinson and Co. (Oxnard, California, USA). Endothelin-1, endothelin-2, endothelin-3, and big endothelin-1 (porcine, 1-39) were purchased from Peptide Institute, Inc. (Osaka, Japan). Endothelin-1 antiserum was purchased from Peninsula Laboratories, Inc. (Belmont, California, USA). ^{125}I-endothelin-1 was purchased from Amersham Japan, Inc. (Tokyo, Japan).

Mesangial cell culture

Glomeruli from Sprague-Dawley rats weighing 50 to 100 g were isolated by sieving with stainless steel and nylon meshes

Received for publication April 1, 1993
and in revised form August 3, 1993
Accepted for publication August 8, 1993

under sterile conditions as previously reported [23]. The isolated glomeruli were then cultured in RPMI 1640 medium containing 20% FCS and antibiotics. The identity of the mesangial cells was confirmed by the following criteria: (1) morphology; (2) typical microfilaments seen by transmission electron microscopy; (3) survival in a medium containing D-valine substituted for L-valine, indicating the existence of D-amino acid oxidase; (4) resistance to puromycin aminonucleoside (10 μg/ml) but susceptibility to mitomycin C (10 μg/ml); (5) presence of receptors specific to Ang II and contraction in response to Ang II; and (6) absence of immunofluorescence with factor VIII antibody. The cultures were maintained at 37°C with atmospheric air and 5% CO_2, and subculture was carried out after treatment with Versene® followed by trypsin. Cells after three to seven passages were used for this experiment.

Pharmacological treatment

The culture medium was removed and the cell monolayers were washed twice with serum-free RPMI-1640. Various concentrations of AVP (10^{-6} M, 10^{-7} M and 10^{-8} M) or PDGF (50 ng/ml, 5 ng/ml and 0.5 ng/ml) were added to the individual wells, and the cells were incubated at 37°C for 24 and 48 hours. These concentrations of AVP and PDGF are shown to stimulate mesangial cell production of endothelin-1 in cultured rat or human mesangial cells [24, 25]. In separate experiments, various concentrations of heparin (100 U/ml, 10 U/ml and 1 U/ml) were added in the absence or presence of 10^{-7} M AVP or 5 ng/ml PDGF, and the cells were incubated at 37°C for 24 hours.

In final experiments, we tested whether mesangial cells which were exposed to a high concentration of heparin (100 U/ml) for 24 hours were capable of producing endothelin-1 after a short lag period of removal of heparin. The heparin-containing medium was removed and replaced with medium plus 0.5% FCS or medium plus 0.5% FCS and 10^{-7} M AVP or 5 ng/ml PDGF. Accumulated endothelin-1 levels at 0 to 24 hours, at 24 to 48 hours, and at 48 to 72 hours after the removal of heparin were measured.

All experiments were performed with 2 ml of RPMI-1640 under quiescent (0.5% FCS) conditions. After the incubation, the medium was aspirated and centrifuged at 3000 × g for 10 minutes, and the supernatant was collected and stored at −80°C until radioimmunoassay.

Measurement of endothelin-1 concentration

Immunoreactive (ir)-endothelin-1 was extracted as previously described [27]. Briefly, 1.5 ml of each sample was diluted with 4 ml of 4% acetic acid. After centrifugation, the solution was pumped at the rate of 1 ml/min through a Sep-Pak C_{18} cartridge (Millipore Corp., Milford, Massachusetts, USA). After evaporation of the elute with 86% ethanol in 4% acetic acid by centrifugal evaporator (Model RD-31, Yamato Scientific Co., Tokyo, Japan), the dry residue was dissolved in the assay buffer described below. The recovery rate was found by adding three different quantities of cold endothelin-1 (10, 50 and 100 pg/ml) to serum-free RPMI 1640. Recovery was 69 ± 2%.

The ir-endothelin-1 concentration was assayed using endothelin-1 antiserum and ^{125}I-endothelin-1 as a tracer. This antibody reacts 100% with endothelin-1 and cross reacts 7% with endothelin-2, 7% with endothelin-3 and 35% with big endothelin-1(porcine, 1-39). The antiserum did not cross react with somatostatin, β-endorphin, human secretin, Ang II, AVP, or PDGF.

Table 1. Effects of arginine vasopressin and platelet-derived growth factor on endothelin-1 secretion in cultured rat mesangial cells

	Endothelin-1 level, *pg/ml*	
	24 hr	48 hr
Baseline	5.2 ± 0.7	10.8 ± 1.2
AVP		
10^{-8} M	10.3 ± 1.0[a]	20.3 ± 1.5[a]
10^{-7} M	15.7 ± 1.2[ab]	29.7 ± 1.8[ab]
10^{-6} M	23.6 ± 1.9[abc]	36.2 ± 2.5[abc]
PDGF		
0.5 ng/ml	14.6 ± 2.0[a]	27.5 ± 3.2[a]
5 ng/ml	23.5 ± 3.1[ad]	42.6 ± 4.5[ad]
50 ng/ml	38.1 ± 5.7[ade]	51.2 ± 6.8[ad]

Values are mean ± SD, assays made of 4 cell cultures incubated for times indicated. Each assay was done in duplicate. AVP, arginine vasopressin; PDGF, platelet-derived growth factor.
[a] Significant difference compared with baseline level, $P < 0.05$
[b] Significant difference compares with 10^{-8} M AVP, $P < 0.05$
[c] Significant difference compared with 10^{-7} M AVP, $P < 0.05$
[d] Significant difference compared with 0.5 ng PDGF, $P < 0.05$
[e] Significant difference compared with 5 ng PDGF, $P < 0.05$

Radioimmunoassay was performed in an assay buffer of 0.01 M sodium phosphate, pH 7.4, containing 0.05 M NaCl, 0.1% bovine serum albumin, 0.1% Nonidet P-40 and 0.01% NaN_3, as previously described [28]. In brief, rehydrated antiserum (100 μl) was added to 100 μl of the sample or 100 μl of standard endothelin-1 dissolved in the assay buffer and the mixture was incubated for 24 hours at 4°C. Approximately 15,000 cpm of ^{125}I-endothelin-1 was added to each reaction and incubated for an additional 24 hours. After this incubation, 100 μl of diluted normal rabbit serum and 100 μl of diluted goat anti-rabbit immunoglobin G were added and the mixture was again incubated for 24 hours. After the third incubation, the precipitate was collected by centrifugation at 1700 g for 30 minutes. The supernatant was removed by aspiration and the pellet was counted for ^{125}I with a gamma counter. The level of detectable ability of this assay was 0.2 pg/ml with a range of 0.2 to 200 pg/ml. The interassay variation was 13% and the intra-assay variation was 7%.

AVP, PDGF and heparin did not interfere with the radioimmunoassay.

Calculations and statistical analysis

The statistical significance of differences in the results were evaluated by analysis of variance, and P values were calculated by Scheffe's method [29]. Values are expressed as mean ± SD.

Results

Effects of AVP and PDGF on mesangial cell endothelin-1 secretion

AVP and PDGF increased ir-endothelin-1 secretion in a time-dependent fashion. These stimulatory effect were clearly concentration-dependent (Table 1).

Effects of heparin on basal and stimulated mesangial cell endothelin-1 secretion

Figure 1 shows effect of heparin on basal mesangial cell ir-endothelin-1 secretion. Heparin in small dose (1 U/ml) did not

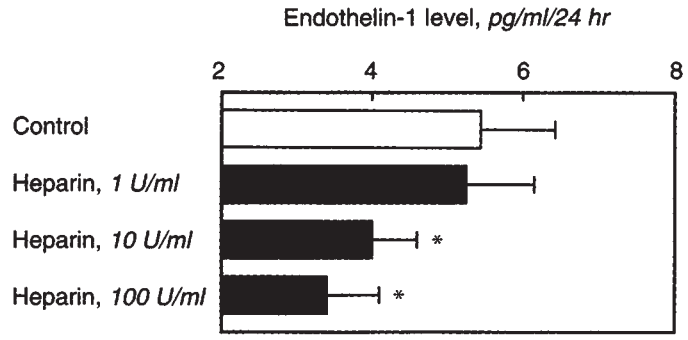

Fig. 1. *Effect of heparin on basal ir-endothelin-1 secretion from cultured mesangial cells.* Cells were exposed to different concentrations of heparin for 24 hours. * Significant difference compared with control level ($P < 0.05$). Each point is the mean of six measurements.

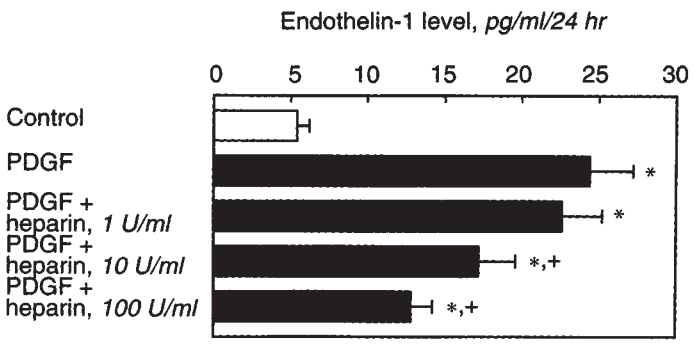

Fig. 3. *Effect of heparin on ir-endothelin-1 secretion from cultured mesangial cells treated with PDGF.* Cells were exposed to different concentrations of heparin in addition to 5 ng/ml PDGF for 24 hours. * Significant difference compared with control level ($P < 0.05$). + Significant difference compared with values when PDGF only was added ($P < 0.05$). Each point is the mean of six measurements.

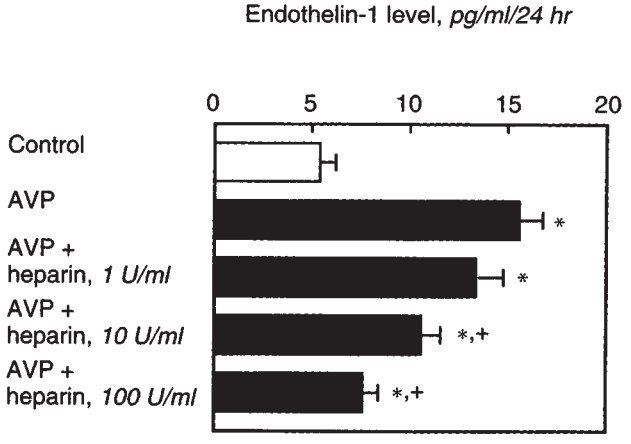

Fig. 2. *Effect of heparin on ir-endothelin-1 secretion from cultured mesangial cells treated with AVP.* Cells were exposed to different concentrations of heparin in addition to 10^{-7} M AVP for 24 hours. * Significant difference compared with control level ($P < 0.05$). + Significant difference compared with values when AVP only was added ($P < 0.05$). Each point is the mean of six measurements.

affect basal ir-endothelin-1 secretion (5.2 ± 1.0 pg/ml) compared to control (5.4 ± 0.9 pg/ml). However, in higher doses (10 and 100 U/ml) heparin showed weak but significant inhibitory effects (10 U/ml, 4.0 ± 0.6 pg/ml; 100 U/ml, 3.4 ± 0.7 pg/ml).

Figure 2 shows effect of heparin on AVP-stimulated ir-endothelin-1 secretion from cultured mesangial cells. Heparin in small dose (1 U/ml) did not affect ir-endothelin-1 secretion (13.0 ± 1.4 pg/ml), whereas 10 and 100 U/ml heparin exhibited concentration-related inhibition (10 U/ml, 10.5 ± 1.2 pg/ml; 100 U/ml, 7.6 ± 0.9 pg/ml) of AVP-stimulated ir-endothelin-1 secretion (15.5 ± 1.7 pg/ml). However, even in a highest dose (100 U/ml) heparin could not inhibit the secretion to the control level (7.6 ± 0.9 pg/ml vs. 5.4 ± 0.8 pg/ml).

Figure 3 shows effect of heparin on PDGF-stimulated ir-endothelin-1 secretion from cultured mesangial cells. Heparin in small dose (1 U/ml) did not affect ir-endothelin-1 secretion (21.6 ± 2.5 pg/ml), whereas 10 and 100 U/ml heparin exhibited concentration-related inhibition (10 U/ml, 17.3 ± 2.3 pg/ml; 100 U/ml, 12.7 ± 1.5 pg/ml) of PDGF-stimulated ir-endothelin-1 secretion (24.3 ± 3.0 pg/ml). However, even in a highest dose heparin could not inhibit the secretion to the control level (12.7 ± 1.5 pg/ml vs. 5.3 ± 0.9 pg/ml).

To test the hypothesis that heparin inhibits mesangial cell production of endothelin-1 through the inhibition of PKC, the effect of the PKC-activating phorbor ester, PMA, on ir-endothelin-1 secretion and the effect of heparin on PMA-stimulated ir-endothelin-1 secretion were examined. PMA stimulated ir-endothelin-1 secretion in a concentration-dependent fashion (control, 5.5 ± 0.9 pg/ml; PMA 10^{-10} M, 7.8 ± 1.0 pg/ml; PMA 10^{-9} M, 10.5 ± 1.1 pg/ml; PMA 10^{-8} M, 14.7 ± 1.9 pg/ml; Fig. 4). Heparin clearly inhibited this PMA-stimulated secretion in a concentration-dependent fashion (heparin 1 U/ml + PMA 10^{-8} M, 12.9 ± 1.3 pg/ml; heparin 10 U/ml + PMA 10^{-8} M, 9.4 ± 0.7 pg/ml; heparin 100 U/ml + PMA 10^{-8} M, 5.8 ± 0.8 pg/ml; Fig. 4). Actually, in a highest dose (100 U/ml) heparin completely inhibited PMA-stimulated ir-endothelin-1 secretion.

To confirm the importance of PKC-dependent mechanisms in the inhibition of endothelin-1 by heparin, the effect of heparin on ir-endothelin-1 secretion was examined in PKC-depleted mesangial cells. PKC-depletion was made by preincubation with a high dose of PMA (10^{-7} M) for 24 hours. The inhibitory effect of heparin on ir-endothelin-1 secretion was completely abolished in PKC-depleted cells (Table 2).

Reversibility of the inhibitory effect of heparin on endothelin-1 secretion

Next, we tested whether the inhibitory effects of heparin on mesangial cell ir-endothelin-1 secretion was reversible. At 24 hours after exposure to high concentration (100 U/ml) of heparin, the heparin-containing medium was removed and replaced with medium plus 0.5% FCS or medium plus 0.5% FCS and 10^{-7} M AVP or 5 ng/ml PDGF. Accumulated endothelin-1 levels at 0 to 24 hours, at 24 to 48 hours, and at 48 to 72 hours after the removal of heparin were 3.8 ± 0.5 pg/ml, 4.8 ± 0.6 pg/ml, and 5.8 ± 0.9 pg/ml, respectively (Fig. 5). Mesangial cells which were previously exposed to heparin were capable of producing ir-endothelin-1 after a short lag period of removal of heparin from the culture medium. These mesangial cells were also capable of responding to AVP and PDGF and of secreting significantly greater amounts of endothelin-1 than non-stimulated levels (AVP 0 to 24 hr, 7.0 ± 1.2 pg/ml; AVP 24 to 48 hr,

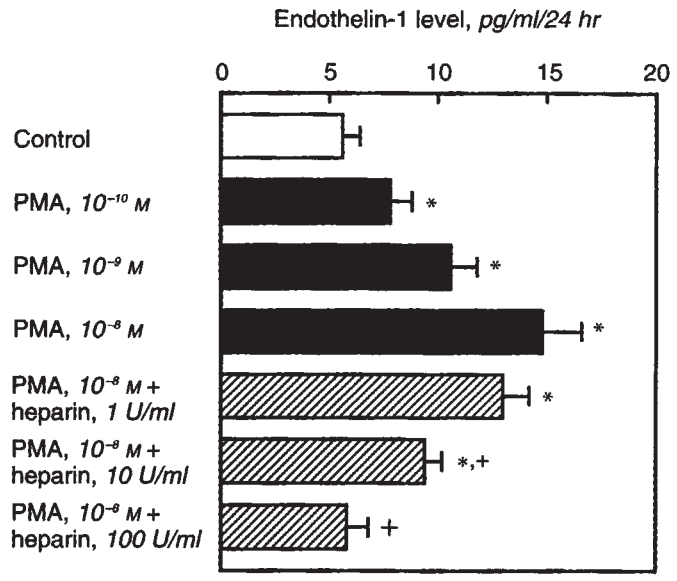

Fig. 4. *Effect of the PKC-activating phorbor ester, phorbor myristate acetate (PMA), on ir-endothelin-1 secretion and effect of heparin on PMA-stimulated ir-endothelin-1 secretion from cultured mesangial cells.* Cells were exposed to different concentrations of PMA for 24 hours. In the same experiment, cells were exposed to different concentrations of heparin in addition to 10^{-8} M PMA for 24 hours. * Significant difference compared with control level ($P < 0.05$). + Significant difference compared with values when 10^{-8} M PMA only was added ($P < 0.05$). Each point is the mean of six measurements.

Table 2. Effect of heparin on endothelin-1 secretion in cultured rat mesangial cells with or without PKC depletion

	Heparin *100 U/ml*		
	(−)	(+)	*P*
PKC depletion			
(−)	5.5 ± 0.9	3.5 ± 0.6	< 0.05
(+)	3.4 ± 0.7	3.2 ± 0.8	NS

Values are mean ± SD, and assays were made of 4 cell cultures incubated for 24 hours. Each assay was done in duplicate. To deplete PKC, the cells were preincubated with 10^{-7} M PMA for 24 hours. Abbreviations are: PKC, protein kinase C; NS, not significant; PMA phorbor myristate acetate.

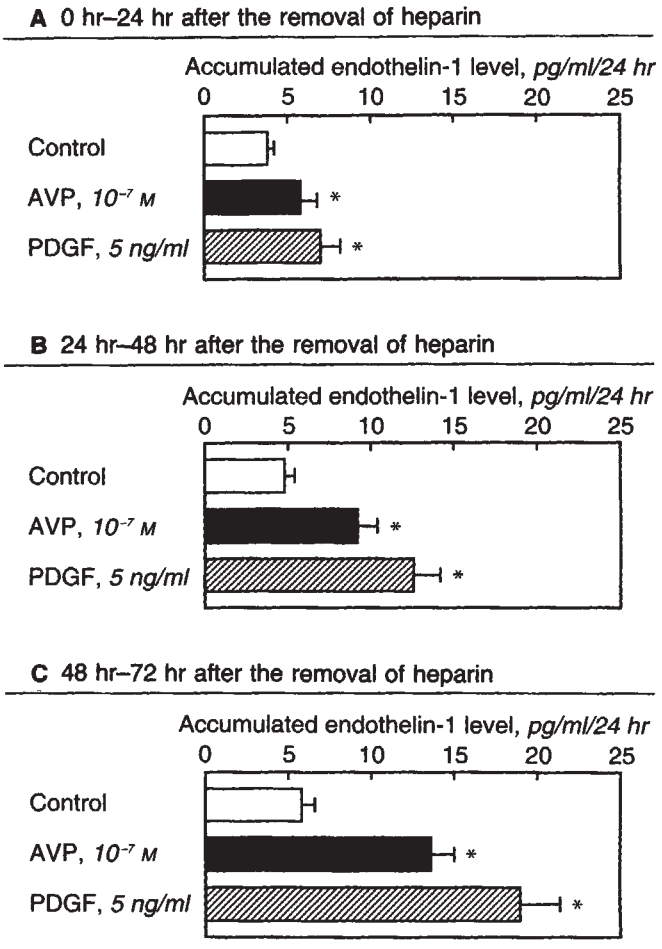

Fig. 5. *Reversibility of the heparin effect on mesangial cell endothelin-1 production.* Heparin-containing medium was replaced with medium alone or medium plus 10^{-7} M AVP or 5 ng/ml PDGF. Accumulated endothelin-1 levels at 0 to 24 hours, at 24 to 48 hours, and at 48 to 72 hours after the removal of heparin were shown. * Significant difference compared with control level (with 0.5% FCS only) ($P < 0.05$). Each point is the mean of six measurements.

9.2 ± 1.2 pg/ml; AVP 48 to 72 hr, 13.6 ± 1.2 pg/ml) (PDGF 0 to 24 hr, 7.0 ± 1.2 pg/ml; PDGF 24 to 48 hr, 12.6 ± 1.6 pg/ml, PDGF 48 to 72 hr, 19.0 ± 2.4 pg/ml; Fig. 5). Furthermore, the above-mentioned heparin-containing medium did not contain detached cells. The inhibitory effects of heparin on mesangial cell endothelin-1 production thus appear to be reversible and not simply a function of killing mesangial cells.

Discussion

In the present study, we have confirmed the previous reports that AVP as well as PDGF stimulates a vasoconstrictive and growth-promoting peptide, endothelin-1, in cultured rat mesangial cells [2, 24, 25] and we have presented data for the first time that heparin inhibits AVP- and PDGF-stimulated endothelin-1 secretion in these cells. We also have shown that heparin was weak but significant inhibitory effects on basal endothelin-1 secretion in cultured rat mesangial cells. The present results are in agreement with our recent report [26] that heparin inhibits endothelin-1 secretion in cultured rat aortic endothelial cells.

Next, we showed that the PKC-activating phorbor ester, PMA, stimulated endothelin-1 secretion and heparin clearly inhibited PMA-stimulated endothelin-1 secretion. Actually, a high dose (100 U/ml) of heparin completely suppressed PMA-stimulated endothelin-1 secretion. However, this suppressive effect of heparin was abolished in PKC-depleted mesangial cells. Therefore, our findings suggest that heparin suppresses mesangial cell production of endothelin-1 probably through a mechanism of inhibition of PKC. Although PKC-depletion prevented the heparin-induced fall in endothelin-1 production, the baseline levels with PKC-depletion were similar to the heparin-induced fall. One possible explanation is that the basal endothelin-1 production is also PKC-dependent and heparin inhibits the basal endothelin-1 production as well as AVP- and PDGF-stimulated production most likely through a common mechanism of inhibition of PKC. We speculate that even in

basal conditions mesangial cell production of endothelin-1 may be stimulated by endogenously produced substances, such as PDGF, through the activation of PKC. Actually, previous evidence indicates that mesangial cells themselves produce a PDGF-like molecule [9] and PDGF induces its own messenger RNA in mesangial cells [30].

Heparin has many pharmacologic effects other than anticoagulation. Previous studies indicate that heparin treatment reduces blood pressure and improves renal function in several experimental hypertensive models [31–34]. In addition, Castellot et al [35] have demonstrated that heparin inhibits mesangial cell proliferation. In the present study, we showed that heparin inhibits basal and AVP- or PDGF-stimulated mesangial cell production of endothelin-1. Taken together, these findings indicate that endothelin-1 has vasoconstrictive effect on renal vasculature and some mitogenic activities for glomerular mesangial cells. Thus heparin treatment by its inhibitory effects on production of endothelin-1 in vascular endothelial and mesangial cells, as well as by its anticoagulation and antimitogenic action, may help to attenuate hypertension and glomerular dysfunction.

Finally, we showed that mesangial cells which were exposed to a high concentration of heparin for 24 hours were capable of secreting endothelin-1. These mesangial cells also were capable of responding to AVP and PDGF and of secreting greater amount of endothelin-1 than non-stimulated level. These results suggest that the inhibitory effect of heparin on mesangial cell production of endothelin-1 is reversible and not simply a function of killing mesangial cells. Actually, heparin-containing medium in our experiment did not contain detached cells.

Overall, our data indicate that heparin inhibits mesangial cell endothelin-1 secretion probably through the inhibition of PKC and this effect is reversible. Therefore, heparin treatment may modulate glomerular hemodynamics in part through the inhibition of mesangial endothelin-1 production, especially when endogenous endothelin-1 production is enhanced by AVP or PDGF. If so, this action may be added to the anticoagulation and antimitogenic action [35] as yet another effect of heparin of benefit in certain pathological states. However, it remains to be clarified whether heparin has a role in modulating endothelin-1 production in glomerular mesangial cells *in vivo*, since high concentrations of heparin are required to inhibit the AVP or PDGF effect on endothelin-1 production. In addition, further investigation will be necessary to elucidate the exact cellular mechanism by which heparin inhibits mesangial cell endothelin-1 production.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (572-690-231-646) and by a Grant of the Osaka Heart Club.

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References

1. Ausiello DA, Kreisberg JI, Roy C, Karnovsky MJ: Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin. *J Clin Invest* 65:754–760, 1980
2. Kohno M, Horio T, Ikeda M, Yokokawa K, Fukui T, Yasunari K, Murakawa K, Kurihara N, Takeda T: Natriuretic peptides inhibit mesangial cell production of endothelin induced by arginine vasopressin. *Am J Physiol* 33:F678–F683, 1993
3. Simonson MS, Wann S, Mene P, Dubyak GR, Kester M, Nakazato Y, Sedor JR, Dunn MJ: Endothelin stimulates phospholipase C. Na^+/H^+ exchange, c-*fos* expression and mitogenesis in rat mesangial cells. *J Clin Invest* 84:708–712, 1989
4. Sugiura M, Snajdar RM, Schwartzberg M, Badr KF, Magami T: Identification of two types of specific endothelin receptors in rat mesangial cell. *Biochem Biophys Res Commun* 161:1396–1401, 1989
5. Mene P, Simonson MS, Dunn MJ: Physiology of the mesangial cells. *Physiol Rev* 69:1347–1424, 1989
6. Sterzal BB, Lovett DH: Interactions of inflammatory and glomerular cells in the response to glomerular injury, in *Contemporary Issues of Nephrology* (vol. 18), edited by Brenner BM, Stein JH, Wilson CB, New York, Churchill Livingstone, 1988, pp. 137–173
7. Ganz MB, Perfetto MC, Boron WF: Effects of mitogens and other agents on rat mesangial cell proliferation, pH, and Ca^{2+}. *Am J Physiol* 28:F269–F278, 1990
8. Mene P, Abboud HE, Dubiak GR, Scarpa A, Dunn MJ: Effects on PDGF on inositol phosphates, Ca^{2+}, and contraction of mesangial cells. *Am J Physiol* 253:F458–F463, 1987
9. Abboud HE: Platelet-derived growth factor and mesangial cells. *Kidney Int* 41:581–583, 1992
10. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kabayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (London)* 332:411–415, 1988
11. Murakawa K, Kohno M, Yokokawa K, Yasunari K, Horio T, Kurihara N, Takeda T: Endothelin-induced renal vasoconstriction and increase in cytosolic calcium in renal vascular smooth muscle cells. *Clin Exp Hypertens* A12:1037–1048, 1990
12. Yokokawa K, Kohno M, Murakawa K, Yasunari T, Horio T, Takeda T: Acute effects of endothelin on renal hemodynamics and blood pressure in anesthetized rats. *Am J Hypertens* 2:715–717, 1989
13. Floege J, Topley N, Resch K: Regulation of mesangial cell proliferation. *Am J Kidney Dis* 17:673–676, 1991
14. Kohno M, Yasunari K, Murakawa K, Yokokawa K, Horio T, Fukui T, Takeda T: Plasma immunoreactive endothelin in essential hypertension. *Am J Med* 88:614–618, 1990
15. Kohno M, Murakawa K, Horio T, Yokokawa K, Yasunari K, Fukui T, Takeda T: Plasma immunoreactive endothelin-1 in experimental malignant hypertension. *Hypertension* 18:93–100, 1991
16. Yokokawa K, Tahara H, Kohno M, Murakawa K, Yasunari K, Nakagawa K, Hamada T, Otani S, Yanagisawa M, Takeda T: Hypertension associated with endothelin-secreting malignant hemangioendothelioma. *Ann Intern Med* 114:213–215, 1991
17. Firth JD, Ratcliffe, Raine AEG, Ledingham JGG: Endothelin: An important factor in acute renal failure. *Lancet* 2:1179–1181, 1988
18. Tomita K, Ujiie K, Nakanichi T, Tmura S, Matsuda O, Ando M, Shichiri M, Hirata Y, Marumo F: Plasma endothelin levels in patients with acute renal failure. *N Engl J Med* 321:1127, 1989
19. Kon V, Yoshioka T, Fogo A, Ichikawa I: Glomerular actions of endothelin in vivo. *J Clin Invest* 83:1762–1767, 1989
20. Kon V, Sugiura M, Inagami T, Harvie BR, Ichikawa I, Hoover RL: Role of endothelin in cyclosporine-induced glomerular dysfunction. *Kidney Int* 37:1487–1491, 1990
21. Takeda M, Breyer MD, Noland TD, Homma T, Hoover R, Inagami T, Kon V: Endothelin-1 receptor antagonist: Effects of endothelin- and cyclosporine-treated mesangial cells. *Kidney Int* 42:1713–1719, 1992
22. Sakamoto J, Sakai S, Hirata Y, Imai T, Ando K, Ida T, Sakurai T, Yanagisawa M, Masaki T, Marumo F: Production of endothelin-1 by rat cultured mesangial cells. *Biochem Biophys Res Commun* 169:462–468, 1990
23. Kohno M, Horio T, Ikeda M, Yokokawa K, Fukui T, Yasunari K, Kurihara N, Takeda T, Johchi M: Angiotensin II

stimulates endothelin-1 secretion in cultured rat mesangial cells. *Kidney Int* 42:860–866, 1991
24. BAKRIS G, FAIRBANKS R, TRAISH AM: Arginine vasopressin stimulates human mesangial cell production of endothelin. *J Clin Invest* 87:1158–1164, 1991
25. SAKAMOTO H, SASAKI S, NAKAMURA Y, FUSHIMI K, MARUMO F: Regulation of endothelin-1 production in cultured rat mesangial cells. *Kidney Int* 41:350–355, 1992
26. YOKOKAWA K, MANDAL AK, KOHNO M, HORIO T, MURAKAWA K, YASUNARI K, TAKEDA T: Heparin supresses endothelin-1 action and production in spontaneously hypertensive rats. *Am J Physiol* 263:R1035–R1041, 1992
27. KOHNO M, YASUNARI K, YOKOKAWA K, MURAKAWA K, HORIO T, TAKEDA T: Inhibition by atrial and brain natriuretic peptides of endothelin-1 secretion after stimulation with angiotensin II and thrombin of cultured human endothelial cells. *J Clin Invest* 87: 1999–2004, 1991
28. KOHNO M, YASUNARI K, MURAKAWA K, YOKOKAWA K, HORIO T, FUKUI T, TAKEDA T: Release of immunoreactive endothelin from porcine aortic strips. *Hypertension* 15:718–723, 1990
29. WALLENSTEIN S, ZUCKER CL: Some statistical methods usefulin circulation research. *Circ Res* 47:1–9, 1980
30. ABBOUD HE, POPTIC E, DICORLETO P: Production of platelet-derived growth factor-like protein by rat mesangial cells in culture. *J Clin Invest* 80:675–683, 1987
31. MANDAL AK, OLEINICK SR, JAMES TM, WISE W, LONG H, NORDQUIST JA, BELL RD, YUNICE AA, PARKER D: Glomerular thrombosis in spontaneously hypertensive rat II. Effects of Heparin. *Microvasc Res* 16:373–390, 1978
32. SUSIC D, MANDAL AK, KENTERA D: Heparin lowers the blood pressure in hypertensive rats. *Hypertension* 4:681–685, 1982
33. WILSON SK, SOLEZ K, BOITNOTT J, HEPTINSTALL RN: The effect of heparin treatment on hypertension and vascular lesions in stroke-prone spontaneously hypertensive rats. *Am J Pathol* 102: 62–71, 1981
34. PURKERSON ML, TELIEFSEN DM, KLAHR S: N-desulfated/acetylated heparin ameliorates the progression of renal disease in rats with subtotal renal ablation. *J Clin Invest* 81:69–74, 1988
35. CASTELLOT JJ, HOOVER RL, HAPPER P, KARNOVSKY MJ: Heparin and glomerular epithelial cell-secreted heparin like species inhibit mesangial cell proliferation. *Am J Pathol* 120:427–435, 1985