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Upregulation of Osteopontin in Ischemia-Induced Renal Failure in Rats: A Role for ET-1?

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In this study, the involvement of osteopontin in a rat model of ischemia-induced acute renal failure (ARF) was evaluated. In unilaterally nephrectomized Sprague Dawley rats where the left artery was occluded for 30 min., plasma creatinine levels increased significantly within two hours following reperfusion indicating the onset of renal failure. Northern analysis of kidney cortical RNA from these rats showed a time-dependent increase in osteopontin mRNA expression that was significantly higher than sham-operated rats. Since endothelin-1 (ET-1) is implicated as a mediator of acute renal failure, we evaluated its effects on osteopontin expression in a rat mesangial cell-line. Data from *in vitro* studies indicated that endothelin-1 (ET-1) caused a modest but reproducible increase in osteopontin mRNA in these cells. While the signal for osteopontin upregulation in the rat model is not known, ET-1, which is known to be increased during ischemia, may contribute at least in part to this process.

Acute renal failure (ARF) is a common and serious clinical condition that occurs in man following transient circulatory impairment. Experimental ARF is characterized by changes in renal hemodynamics, tubular obstruction, and back leak of filtrate and vascular reactivity (1,2). A number of animal models simulating the effects of experimental ARF have been developed in order to gain better insight into the mechanisms leading to renal failure (3,4). The ARF model used in the present study is a well characterized model (5,6) and is being used routinely in our laboratory for evaluating possible therapeutic agents and also for understanding the mechanisms involved in this disease process.

Osteopontin [OPN] is a negatively charged, glycosylated phosphoprotein expressed in many tissues including kidney. OPN, which is a secreted calcium binding protein, has been implicated in a number of cellular functions and features that are either directly or evolutionarily related to inflammation and tissue repair (7). In normal rat kidney, osteopontin protein and mRNA is observed in the descending limbs of the loop of Henle and in papillary surface epithelium away from the papillary tip (8). Enhanced expression of the protein has been described in various disease states including some models of renal injury (9,10), artherosclerotic plaques and during restenosis following angioplasty (11) Endothelin-1 (ET-1), a potent vasoconstrictor peptide hormone, is another factor that has been implicated to play a mediator role in acute renal failure (12-14). A number of observations which support this function include, elevated concentrations of ET in patients on hemodialysis (15), and more significantly, the beneficial action of endothelin antibody and ET antagonist in ischemia-induced ARF (16-18).

In the present study the kinetic expression of osteopontin mRNA was investigated in a rat model of ARF. The contribution of ET-1 in stimulating the expression of osteopontin in a rat mesangial cell line was also investigated.

MATERIALS AND METHODS

Rat mesangial cells. The rat mesangial cell line were provided by Dr. C. Albrightson from this institution. The cells were routinely maintained in RPMI-1640 medium supplemented with 15% FBS, insulin and antibiotics such as penicillin and streptomycin.

Animal preparation. All procedures were approved by the Institutional Animal Care and Use Committee (SmithKline Beecham Pharmaceuticals) and were in accordance with National Institutes Health Guidelines for the care and use of animals.

Unilaterally nephrectomized male Sprague-Dawley rats (Wilmington, MA) were used. The right kidney was removed from young rats (175-200 g) 10-14 days prior to the ischemic challenge. To induce ARF, the left artery was occluded for 30 min. under pentobarbital anesthesia (40 mg/kg i.p.). Body temperature was maintained at 37°C. Sham operated rats were used as control. In this model, plasma creatinine levels are monitored and used as an index of impairment of renal function. Blood was collected either from chronically implanted aortic line or by cardiac puncture. The kidney was removed either immediately preceding reperfusion (0h) or 2, 5 and 24 h following reperfusion; the cortex was dissected and frozen in liquid nitrogen.

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TABLE 1

Length of reperfusion (h)	Plasma creatinine (mg%)
0	0.48 ± .05
2	1.0 ± 0.1
5	1.7 ± 0.2
24	4.2 ± 0.3

Note. Plasma creatinine levels increase in a time-dependent manner following reperfusion.

Creatinine analysis. Plasma creatinine was measure by a Synchron AS-8 Clinical System Analyzer. Values are expressed as mean ± SEM. Statistical analysis was performed using the ANOVA test.

Northern blot analysis. Total RNA was isolated from kidney cortex of sham, 0, 2, 5 and 24 h post ischemic rats or the rat mesangial cells according to a modified procedure of Chomczynski and Sacchi (19). RNA was fractionated by electrophoresis on 1% agarose-formaldehyde gels, transferred to UV Duralon nylon membrane and cross-linked to the membrane using a UV Stratalinker-180. The blots were prehybridized at 37°C in 10 ml of 35% formamide, 50 mM $Na_2HPO_4.H_2O$, 0.75 M NaCl, 5 mM EDTA, pH 7.4, 0.2% SDS, 8% dextran sulphate and 250 mg/ml salmon sperm DNA. Next $^{[32]}$P-labeled osteopontin cDNA was added and the blot hybridized at 42°C for 18 h. The blots were washed twice with 6 X SSC and then exposed to Kodak X-Omat AR film with intensifying screens, at −70°C. Ethidium bromide-stained 18 and 28 S ribosomal RNA served as internal control to correct for differences in RNA loading.

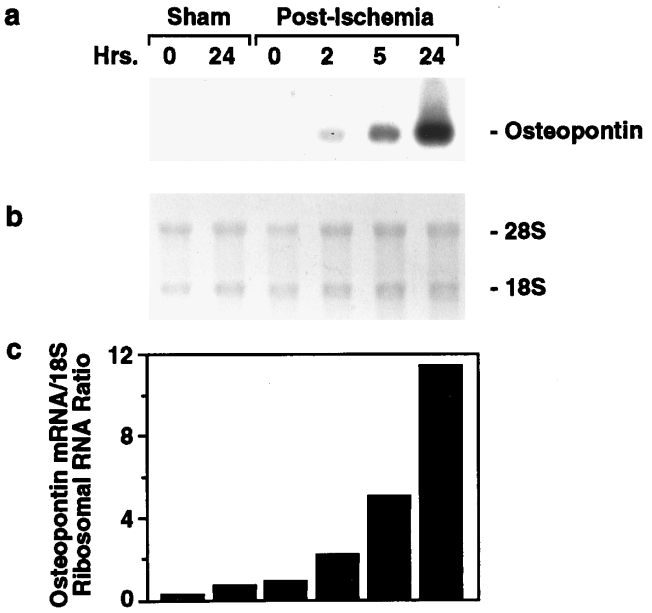

FIG. 1. Northern analysis of mRNA isolated from rat kidney cortex following a 30 min. occlusion of the renal artery. Steady state levels of mRNA's was determined by Northern blotting as described under Materials and Methods. Results were quantitated by densitometry. **A)** osteopontin transcript lanes; **B)** ethidium bromide stained gel of the same blot showing 18 and 28 S ribosomal RNA; **C)** graphic representation of densitometric scanning (osteopontin/18 S ratio).

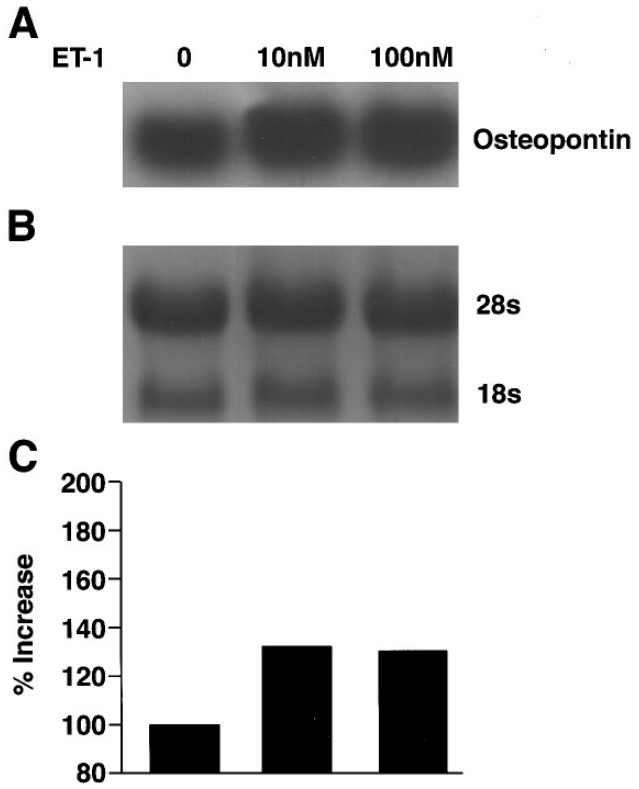

FIG. 2. Endothelin-1 stimulates osteopontin mRNA expression. Rat mesangial cells were stimulated with ET-1 (10 and 100 nM) for 16 hr and total RNA isolated at the end of the incubation. Osteopontin mRNA expression was determined by Northern blot analysis as described under 'Material and Methods'. **A)** osteopontin transcript lanes; **B)** 18 and 28 S ribosomal RNA; **C)** osteopontin/18S ratio.

RESULTS AND DISCUSSION

Following the 30 min. renal artery occlusion, plasma creatinine levels increased in a time-dependent manner as shown in Table 1. The maximum increase in creatinine levels which was 7-8 fold higher than controls, and seen 24-hr post reperfusion, indicates severe impairment of renal function. In this model, the plasma creatinine levels return to normal within one week.

Since the involvement of osteopontin has been suggested in some models of renal injury, it was of interest to evaluate whether there was any change in the levels of osteopontin in this model of ARF. As shown in Figure 1a, there was a time-dependent increase in the mRNA levels of osteopontin. This increase correlated well with the increase in plasma creatinine levels. Similar time-dependent increase in ET-1 has been reported by Shibouta et al (15) in the same model of ARF in rat. The quantitation of the mRNA levels determined by calculating the ratio of osteopontin mRNA and the 18S ribosomal RNA (Fig 1b) is shown in Figure 1c.

Experiments were also performed using rat mesangial cells to test whether ET-1 can stimulate the expres-

sion of osteopontin. Mesangial cells have been shown to display high density and high affinity ET_A receptors (20). Furthermore, ET has been shown to cause proliferation of these cells. Pretreatment of these cells with ET-1 resulted in 50-60 % increase in osteopontin mRNA levels (Figure 2).

Collectively, these data suggest that ET-1 may be an important, if not the only, contributory factor responsible for the upregulation of osteopontin in our model of ARF. Further experiments are required to prove whether the increased levels of osteopontin is beneficial or detrimental in this process.

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