Lack of Influence of α -hANP on Ioxaglate-Induced Injury on a Cultured Renal Cell Line, LLC-PK1 Cells

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Dear Sir,

Various atrial natriuretic peptides have recently been reported to be preventive against radiocontrast-induced acute renal failure (ARF) both in man and in animals [1, 2]. We examined the direct toxic effect of ioxaglate, a dimeric radiocontrast agent, in vitro on a cultured renal cell line, LLC-PK1 cells and the effect of α -hANP thereon. The effects of ioxaglate were compared with those of an equiosmolar solution of mannitol.

As urinary excretion of proximal tubular enzymes has proved to be effective in assessing radiocontrast-induced renal injury after major arteriography [3], changes in alkaline phosphatase (ALP), γ -glutamyl transpeptidase (γ -GTP), leucine aminopeptidase (LAP) and N-acetyl- β -D-glucosaminidase (NAG) activity in the medium were used as the markers of cell injury.

LLC-PK1 cells obtained from the American Type Culture Collection (ATCC CRL-1392) were cultured as previously reported [4].

The effects of ioxaglate and mannitol were studied 7 days after seeding when the cells reached a confluent monolayer. Each well was washed twice with 2 ml of Dulbecco's PBS buffer. After removing the buffer, reaction was initiated by 1.0 ml addition of DMEM (Dulbecco's modified Eagle's medium, Nissui Pharmaceutical Tokyo, Japan) supplemented with 0.1% bovine serum albumin, 0.1% glucose, 1 mM CaCl_2 and 20 mM Tris-HEPES (pH 7.4) containing ioxaglate or mannitol. Cells were incubated at 37 °C in an atmosphere of 5% CO_2 -95% air for indicated time intervals. At the end of the incubation period, the medium was removed immediately by suction. The medium samples were then analyzed for enzyme activities. Medium ALP, γ -GTP and LAP were assayed by an autoanalyzer (Hitachi 736-60). Medium NAG was assayed by the m-cresolsulfonphthaleiny-N- β -glucosaminide method (Shionogi NAG Assay Kit, Shionogi, Osaka, Japan).

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Fig. 1. Effect of ioxaglate on medium enzyme activities. Control (O), ioxaglate 10 mM (Δ), 0.1 mM (Δ) and mannitol 10 mM (•). Data are means $\pm$ SD of six determinations. *p < 0.05 vs. control (Dunnett's multiple comparison analysis).

α -hANP stimulation of cGMP was studied in confluent monolayers of LLC-PK1 cells 7 days after seeding. Culture medium was replaced with 1.0 ml DMEM containing 1 mM Mg^{2+} -

isobutylmethylxanthine (IBMX), 0.1% bovine serum albumin, 0.1% glucose, 1 mM CaCl_2 , 20 mM Tris-HEPES (pH 7.4) and ioxaglate or mannitol immediately before the incubation. Vehicle or a pharmacological concentration of α -hANP that would give a maximal response (10^{-6} M) [5] was added to the wells. Cells were incubated at room temperature (20–23 °C). The incubation was stopped by aspirating medium. cGMP was ex-

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protective effect on ioxaglate toxicity, when α -hANP (10^{-6} M) was simultaneously added to the experiment media (fig. 2).

The data indicate that the preventive effect of α -hANP against radiocontrast-induced ARF is not attributable to its direct actions on proximal tubules, but to a modification of the hemodynamic changes induced by radiocontrast agents.

Fig. 2. Effect of α -hANP (10^{-6} M) on medium NAG and γ -GTP. Control (O), α -hANP (10^{-6} M) (Δ), ioxaglate 10 mM (A), and ioxaglate 10 mM + α -hANP (10^{-6} M) ($\bullet$). Data are mean $\pm$ SD of six determinations. * $p < 0.01$ vs. control (Dunnett's multiple comparison analysis).

tracted from cells with 0.5 ml 5% trichloroacetic acid (TCA). The medium and cellular cGMP concentrations were determined by radioimmunoassay with a commercially available [125I] cGMP assay system (Amersham Japan, Tokyo, Japan).

Figure 1 shows that the rates of increase in the medium NAG and γ -GTP were increased 2- to 3-fold by 10 mM ioxaglate ($p < 0.05$). Lower concentration (0.1 mM) of ioxaglate did not affect any of these enzymes. Mannitol 10 mM also significantly increased the medium NAG and LAP. The results indicated that ioxaglate was directly toxic to LLC-PK1 cells and that the toxicity was due to its hyperosmolarity. In the present study, LLC-PK1 cells responded promptly to α -hANP, and c-GMP formation reached a near plateau at 20 min. Ioxaglate did not affect the response of LLC-PK1 cells to α -hANP assessed by c-GMP production (data not shown). Under this absence of inhibitory action on α -hANP, α -hANP did not have a

References

- Kurnik BRC, Kurnik PB, Cutler I, Stom MC, Weisberg LS: Protective effect of atrial natriuretic peptide (ANP) in patients with chronic renal failure (CRF) receiving contrast. *Kidney Int* 1989;35:411.
- Bentley MD, Fiksen-Olsen MJ, Romero JC: Effect of atriopeptin II on contrast medium-induced changes in renal blood flow. *Kidney Int* 1989;36:13–19.
- Hartmann HG, Braedel HE, Jutzler GA: Detection of renal tubular lesions after abdominal aortography and selective renal arteriography by quantitative measurements of brush-border enzymes in the urine. *Nephron* 1985;39:95–101.
- Nakahama H, Fukuhara Y, Orita Y, Yamauchi A, Takama T, Kamada T: Furosemide accelerates gentamicin accumulation in a cultured renal cells (LLC-PK1 cells). *Nephron* 1989; 53: 138–141.
- Wong KR, Xie MH, Shi LB, Liu FY, Huang CJ, Gardner DG, Cogan MG: Urinary cGMP as biological marker of the renal activity of atrial natriuretic factor. *Am J Physiol* 1988;