Effects of ascorbic acid, aminoguanididine, Sorbinil and Zopolrestat on sorbitol and betaine contents in cultured rat renal cells

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Abstract. Aldose reductase inhibitors (ARI) have been developed to reduce the conversion of high glucose levels to sorbitol, an important renal osmolyte that may rise to damaging levels in many tissues during diabetic hyperglycemia. Ascorbic acid (AA) and aminoguanidine (AMG) have also been reported to reduce sorbitol levels in diabetes: AMG in rat kidney, and AA in guinea pig lens and human erythrocytes. We tested the effects of AMG, AA, and Pfizer’s Sorbinil and Zopolrestat for 48 h on primary rat renal cell cultures, established from renal inner medullas of male Wistar rats 8–12 weeks old. Osmolyte contents in scraped cells were analysed by HPLC: 100 µM Sorbinil and 20 µM Zopolrestat decreased sorbitol levels ($P<0.05$ and $P<0.001$, respectively), and increased the content of another osmolyte, betaine ($P<0.01$ and $P<0.01$, respectively). The quantity of ATP in cells was unchanged, suggesting no short-term problems. In contrast, 10 mM AMG and 10 mM AA had no effect on sorbitol contents (in contrast to some previous studies). We then tested aldose reductase (AR) activity in crude homogenates of rat lens and renal inner medulla, with glyceraldehyde substrate. For both tissues, 5 µM Zopolrestat inhibited AR activity by 92–94% ($P<0.002$); 10 mM AA by 16–20% ($P<0.02$); and 10 mM aminoguanidine by 22–24% ($P<0.03$). We conclude that AMG and AA are not readily usable as inhibitors of renal AR.

Key words. Aldose reductase – Aminoguanidine – Ascorbic acid – Kidney Sorbinil – Sorbitol – Zopolrestat

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Organic osmolytes are small solutes widely used by cells to reduce water loss, such as that caused high extracellular NaCl concentrations (Yancey, 1994). Sorbitol is one such osmolyte used by the mammalian kidney, along with betaine, myo-inositol, glycerophosphorylcholine (GPC), and taurine (Burg, 1988; Sizeland et al., 1995). Sorbitol, myo-inositol and taurine are thought to be basic “compatible“ osmolytes that do not perturb cell macromolecules, while the methylamines betaine and GPC are proposed to counteract the destabilizing effects of urea and salt ions on proteins (Yancey, 1994). Sorbitol is converted from glucose by the enzyme aldose reductase (AR); under normal conditions in mammals, this occurs significantly only in the kidney medulla (Burg, 1988) and bladder (Mähler et al., 1998). However, sorbitol may rise to damaging levels in other AR-containing tissues, such as the eye, erythrocytes and nerves, during diabetic hyperglycemia (Burg and Kador, 1988; Lee et al. 1995; Van Gerven and Tjon-A-Tsien, 1995). Aldose reductase inhibitors (ARIs) such as Sorbinil and Zopolrestat (Pfizer) have been developed to reduce this conversion (Burg and Kador, 1988; Oates, 1994). While ARIs may protect tissues that normally do not contain sorbitol, they could upset osmotic balance in the kidney since sorbitol is a dominant osmolyte in the inner medulla (Yancey et al., 1990a). However, our previous work on rats in vivo has shown that the concentration of betaine, a methylamine renal osmolyte, increases when the sorbitol concentration decreases, in apparent osmotic compensation (Yancey et al., 1990b). While this appeared to maintain cell volume effectively, the effects of replacing a compatible polyol osmolyte with a putative stabilizing methylamine could not be determined. Therefore, we have sought an in vitro system for examining the health of cells subjected to a shift in these osmolyte concentrations.

Although ARIs have produced mixed results in human clinical trials, there is still considerable interest in seeking effective means of inhibiting detrimental sorbitol accumulation (Pfeifer et al., 1997; Yabe-Nishimura, 1998). Other compounds not specifically developed as ARIs have been examined, including the antioxidant vitamin ascorbic acid and aminoguanidine (AMG). These have been reported to inhibit sorbitol accumulation, but not consistently. AMG inhibits nitric oxide synthase and the nonenzymatic glycosylation of proteins by high glucose concentrations (Clark and Lee, 1995; Soulis et al., 1997). AMG has also been reported to act as an ARI in the rat kidney (Kumari et al., 1991a) and lens (Kumari et al., 1991b). However, several recent studies found that AMG has little effect as an ARI in nerves (Cameron et al., 1992; Frank et al., 1998), erythrocytes (Frank et al., 1998), or the retina (Chibber et al., 1994; Frank et al., 1998). Ascorbic acid (AA), which may become depleted in hyperglycemia (Lindsay et al., 1998), has been reported to act as an ARI with moderate effectiveness in guinea pig lens (Bates et al., 1992) and human erythrocytes (Cunningham et al., 1994), and weakly in brain extracts (Cunning-