Nifedipine Does Not Inhibit Ciclosporin A Uptake into Human Cultured Proximal Tubular Cells

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Dear Sir,

Calcium channel blockers are reported to ameliorate ciclosporin A (CS)-induced renal impairment in both experimental and clinical studies [1–3]. The protective mechanism afforded by these agents is unclear, although most workers favour a haemodynamic action, with calcium channel blockers ameliorating CS-induced vaso-constriction [1, 2]. Recently, an alternative mechanism has been proposed after the uptake of CS into renal proximal tubular cells (PTC) was shown to be inhibited by both inorganic and organic calcium channel blockers [4, 5]. Using isolated renal PTC obtained from New Zealand white rabbits the uptake of CS was significantly blocked by these agents, including verapamil and diltiazem. This led them to speculate that the portal in the PTC plasma membrane by which CS enters the cytosol is located at or close to the voltage-sensitive calcium channel, so that conformational changes induced by occupation of the voltage-sensitive calcium receptor impede the transmembrane passage of CS. We have recently investigated the effect of nifedipine, a dihydropyridine calcium channel blocker, on the uptake of ^3H -CS into cultured human renal PTC.

PTC were cultured using standard techniques, after isolating from renal cortical tissue taken from 8 patients undergoing investigation for microscopic haematuria [6]. The cells were characterized as PTC by both morphological criteria (electron microscopy: dense microvilli, junctional complexes, abundant rough endoplasmic reticulum and free polysomes, cytoplasmic filaments, basal cell membrane interdigitations and well-developed endocytotic apparatus) and monoclonal antibody staining (keratin-negative, factor VIIIIRAg-negative, fibronectin-negative).

After preliminary experiments to document the time course of ^3H -CS uptake into confluent monolayers of PTC, uptake studies with ^3H -CS were performed in the presence or absence of nifedipine. Uptake studies were undertaken by incubating the cells in 0.5 ml of 10% fetal calf serum and 90% physiological saline solution (PSS) containing 0.0185 MBq ^3H -CS and cold CS (10 $\mu\text{g/ml}$). At the end of the study period the medium was removed and the cells rinsed with cold PSS containing CS (1 $\mu\text{g/ml}$) at 4°C five times. Intracellular radioactivity was extracted with 1 ml of sodium dodecyl sulphate (SDS) in an alkaline milieu (0.1% SDS, 2% Na_2CO_3 , 0.1 N NaOH) and measured by liquid scintillation counting, and the results expressed as cpm/mg protein.

Uptake of ^3H -CS into cultured PTC was shown to be rapid with 80% of total uptake achieved after 10 min, reaching a plateau after 30 min (fig. 1). Thus, uptake studies with nifedipine were

performed over 30 min. The PTC were initially preincubated with 0.5 ml of nifedipine (10^{-6} M) or vehicle (0.1% ethanol in PSS) for 5 min at 37 °C before adding ^3H -CS. Incubation with nifedipine did not influence uptake of ^3H -CS into cultured PTC (fig. 2).

Thus, these observations suggest that the protection afforded by nifedipine in rodents and man receiving CS is not due to a reduction in uptake of CS into PTC. Similar conclusions were also reached by Scoble et al. [7], after demonstrating that verapamil did not ameliorate CS-induced injury of LLC-PK₁ cells, an epithelial cell line which expresses proximal tubular characteristics. The mechanism postulated by Nagineni et al. [4, 5] to account for the reduced uptake of CS by calcium channel blockers into PTC is uncertain, as PTC do not possess voltage-sensitive calcium channels [8].

Whether CS is directly toxic to tubular cells is controversial. Although several groups have documented

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Fig. 1. Time course for the uptake of ^3H -CS into human cultured PTC (cpm/mg protein).
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Fig. 2. The effect of nifedipine (10^{-6} and 10^{-5} M) on ^3H -CS uptake into human cultured PTC (cpm/mg protein).

both overt biochemical and histological evidence of injury [9], others report normal tubular morphology and functional characteristics, despite continuous CS administration to both man and animals [1, 2, 10–12].

In summary, these findings suggest that nifedipine does not influence CS uptake into PTC. It seems likely that the nephroprotection associated with nifedipine and other calcium channel blockers is mediated predominately via a vascular effect, presumably by counteracting CS-induced renal arteriolar vasoconstriction [2].

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