In vitro FK506 kidney tubular cell toxicity


Departments of Urology and Pathology, Osaka University, Osaka, Japan

Abstract. Nephrotoxicity is the most prominent side effect of the new immunosuppressive drug FK506. Some of the histopathological changes associated with cyclosporine (CyA) nephrotoxicity such as tubular vacuolization and glomerular thrombosis have also been reported with FK506 therapy. In this study we used kidney tubular cells in culture to address the issue of FK506- and CyA-induced tubular damage. Exposure of tubular cells to high concentrations of FK506 or CyA (10, 50, and 100 \( \mu \)M) induced a time- and dose-dependent cell injury in vitro characterized by a direct cytotoxic effect on tubular cells as expressed by release of \( ^{3}H \)-thymidine from prelabelled cells, N-acetyl-\( \beta \)-D-glucosaminidase (NAG) release and cell detachment. Ultrastructural changes (vacuolization, swelling and mitochondrial enlargement) and inhibition of the growth (DNA and RNA synthesis) of cultured tubular cells were also observed at high concentrations of FK506 and CyA. These concentrations are higher than those reached in clinical situations, but close to the concentrations that may be reached by FK506 or CyA in tissues. Low concentrations of FK506 and CyA (1, 0.1 and 0.01 \( \mu \)M) were not cytotoxic and induced only a minimal inhibitory effect on the growth of tubular cells in vitro. At the same concentration CyA induced more cell detachment, more NAG release and a stronger inhibitory effect on cell growth than FK506 (\( P < 0.01 \)). Since an evident cytotoxic effect was observed only at high concentrations, we can speculate that tubular toxicity is due to the accumulation of drug in the cells inducing cell disruption and death.

Key words: FK506 – Cyclosporine – Kidney tubular cell – Drug toxicity

FK506 is a newly development immunosuppressive drug which has been used successfully in kidney [9] and liver transplantation [7]. Recently, a number of side effects have been described. Nephrotoxicity appears to be the major adverse effect of this valuable immunosuppressive drug [6]. Some of the features commonly associated with cyclosporine (CyA) nephrotoxicity, such as tubular vacuolization and glomerular thrombosis, have also been observed in patients treated with FK506 [8]. The mechanism by which FK506 and CyA exert their tubular toxicity is not clear, but it has been suggested that the accumulation of the drugs in the cells causes delayed regeneration, morphological changes, and cell death [8]. In the present study, we report an in vitro assessment of kidney tubular cell sensitivity to FK506 compared with CyA, morphological changes induced in tubular cells treated with FK506, and the comparative effect of both these drugs on the growth of tubular cells in vitro.

Materials and methods

Preparation of tubular cells

The LLC-PK1 cell line was used in the present study. This porcine kidney tubular cell line has the characteristics of renal proximal tubular cells [5]. Tubular cells were cultured in medium M199 (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS) (Hyclone Laboratories Inc., Utah, USA). Cell subcultures were obtained by incubating a washed confluent tubular cell culture with a solution of 0.25% trypsin and 0.02% EDTA (Gibco) for 5 min at 37°C.

Drug preparations

FK506 (Fujisawa, Osaka, Japan) was dissolved in absolute methanol. CyA (Sandoz, Basel, Switzerland) was dissolved in absolute ethanol before being added to the media.

Morphometric examination and electron microscopy

Tubular cells were scraped off the culture dish with a rubber spatula and centrifuged at 300 \( \times \) g for 20 min. The cell pellets were fixed by immersion for 4 h at 4°C in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). After washing three times in cacodylate buffer, the samples were postfixied for 90 min in 1% osmium at 4°C, dehydrated through ascending grades of alcohol, and embedded in Epon.
Fig. 1A–C. FK506-induced cell detachment under light microscopy.
A cultured tubular cells were cultured with medium containing appropriate concentration of vehicle (methanol).
B Tubular cells were cultured with 50 μM FK506 for 5 h. Cell detachment is clearly visible.
C Tubular cells were cultured with 10 μM FK506 for 24 h. Many cells show vacuoles of different sizes.