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In-vitro Interaction of a Novel Immunosuppressant, FK 506, and Antacids

M. STEEVES, H. Y. ABDALLAH, R. VENKATARAMANAN, G. J. BURCKART, R. J. PTACHCINSKI, K. ABU-ELMAGD, A. K. JAIN, F. FUNG, S. TODO AND T. E. STARZL

Schools of Pharmacy and Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, USA

Abstract—The effect of selected antacids on the amount of FK 506 in solution in simulated gastric juice has been studied. FK 506 (2·5 mg) was incubated in 100 mL simulated gastric fluid (SGF) with the equivalent of 500 mg of various antacids. The addition of Mylanta and Tums resulted in 14 and 30% loss of FK 506, respectively, in 24 h; 98% loss was observed in 12 h in the presence of Mag-Ox; 100% loss was observed in the presence of magnesium oxide powder in 2 h. The loss of FK 506 from these solutions appears to be due to a pH mediated degradation of FK 506. The addition of aluminium hydroxide gel USP (Roxane) to the FK 506 solution resulted in a 35% loss within 2 min but no further loss was noted for 24 h, indicative of adsorption of FK 506. These results suggest that until additional in-vivo studies are carried out, it is prudent not to dose FK 506 and antacids at the same time to avoid potential interactions.

FK 506 (Fig. 1) is a macrolide with a molecular weight of 822 isolated from the cultures of the fungus *Streptomyces tsukubaensis* (Sawada et al 1987). It is highly lipophilic, poorly soluble in water and alkanes, and freely soluble in ether, chloroform and ethyl acetate (Tanaka et al 1987). FK 506 is nearly 400 times more potent than cyclosporin (CsA) in inhibiting lymphocyte proliferation in mixed lymphocyte cultures (Zeevi et al 1987). The drug has been shown to reverse or prevent the rejection of heart, liver, kidney, pancreas, lung, intestine and skin grafts in mice, rats, dogs, monkeys and baboons (Fung et al 1990; Todo et al 1990). FK 506 is currently undergoing clinical investigations at the University of Pittsburgh. Initial results indicate FK 506 to provide better immunosuppression in liver transplant recipients than CsA (Todo et al 1991).

FK 506 is administered either orally or intravenously (i.v.) to transplant patients. Following oral administration, FK 506 is poorly and incompletely absorbed, with low bioavailability of FK 506 in transplant patients. Absorption is also highly variable among patients (Venkataramanan et al 1990). The large variability in the oral absorption of FK 506 may be related to physiological factors associated with the transplant patients, formulation factors, or interactions with other drugs. Transplant patients routinely receive a large number of concurrent medications including prophylactic antacids. Drug interactions with antacids are well documented (Hansten & Horn 1989; American Society of Hospital Pharmacists 1990). Antacid-mediated alterations in the extent of absorption of FK 506 may lead to the rejection of the transplanted organ or FK 506-related toxicity. This study was designed to determine if representative antacid preparations or their active ingredients chemically or physically interact with FK 506 in-vitro.

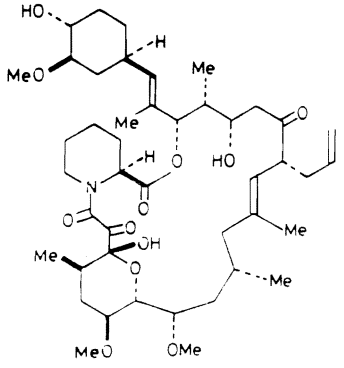

FIG. 1. Chemical structure of FK 506.

Correspondence: R. Venkataramanan, 718 Salk Hall, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261, USA.

Materials and Methods

Materials

Table 1 describes the products and chemicals used in this study. Chemicals were analytical grade and were purchased from Fisher Scientific, Pittsburgh, PA. Antacids were selected on the basis of their use in transplant patients at our institution. Also included in this study is Mag-Ox tablets, commonly given to transplant patients for magnesium replacement. Simulated gastric fluid was prepared without pepsin (USP 1980).

Study design

Simulated gastric fluid (SGF) without pepsin, 100 mL, was warmed and maintained at 37 C in a Waters (Division of Millipore Corp., Milford, MA.) Isotemp Incubator. FK 506, 0·25 mL of a 10 mg mL^{-1} solution (FK 506 i.v. injection, Fujisawa Pharmaceuticals, Kyoto, Japan) was added to the SGF to achieve a concentration of 25 μg mL^{-1}. The equivalent of 500 mg of the antacid in the form of liquid formulation, crushed tablet, or chemical was then added and mixed. Samples (1 mL) were taken before and at 2, 5, 10, 15, 20, 30 min, 1, 1·5, 2, 12 and 24 h after the addition of the test substance. Samples were acidified with 0·2 mL of 1 M HCl immediately after collection. Previous studies have shown that FK 506 is stable for at least a week under these conditions (Abdallah et al 1990). The samples were centri-

Table 1. Summary of products and chemicals.

Product/chemical	Active ingredient(s)		Amount tested in 100 mL	pH* Water	pH* SGF initial	pH* SGF 24 h
Simulated gastric fluid		100 mL		1·3	1·3	1·3
FK 506 for injection		10 mg mL^{-1}	2·5 mg	5·7	1·4	1·3
Aluminium hydroxide			500 mg	6·2	1·5	1·3
Aluminium hydroxide gel dried			500 mg	4·8	3·5	3·6
Aluminium hydroxide gel USP (Roxane)	$Al(OH)_3$ gel	450 mg/5 mL	5·5 mL	6·7	3·3	3·5
Amphogel suspension	$Al(OH)_3$ gel	320 mg/5 mL	8 mL	6·6	3·8	3·6
Calcium carbonate			500 mg	9·4	5·1	6·9
Tums	$CaCO_3$	500 mg	1 tab	4·8	4·6	7·1
Magnesium chloride			500 mg	5·5	1·4	1·3
Magnesium oxide			500 mg	9·6	9·6	9·1
Mag-Ox	Mg	400 mg	1 tab	10·5	6·0	9·3
Maalox suspension	$Al(OH)_3$ gel $Mg(OH)_2$	225 mg/5 mL 200 mg/5 mL	11 mL	7·8	6·4	6·5
Mylanta suspension	$Al(OH)_3$ gel $Mg(OH)_2$	200 mg/5 mL 200 mg/5 mL	12·5 mL	7·9	3·9	7·1

*pH of water or simulated gastric fluid after the addition of liquid antacid, dry powder or crushed tablets.

fuged at 4000 *g* for 10 min. FK 506 in the sample was measured by HPLC.

Assay

A sample of the clear supernatant (100 μL) was injected, with a WISP 712 automatic sample injector (Waters Associates, Milford, MA), onto a Supelco C-18, 5 μ, 4·6 × 150 mm column maintained at 70°C with a Waters TCM heater. The mobile phase consisting of 70% acetonitrile in water was pumped through the column at a flow rate of 1·2 mL min^{-1}. The column effluent was monitored at 214 nm with a Waters 440 detector. Under these conditions, the retention time for FK 506 was about 5 min. Peaks were integrated using a Hewlett Packard model 3390A integrator. Injections were made in triplicate and each experiment was repeated twice. Mean peak area of FK 506 was compared with the value obtained for the zero time sample. All samples were analysed within 48 h of collection. The calculations were corrected for the changes in volume when liquid antacids were tested. The specificity of the assay was tested by intentionally degrading FK 506 under acidic (6 M HCl) and basic (pH 10) conditions.

The pH of the SGF was measured immediately and 24 h after the addition of the test product (Table 1). An additional pH measurement was made immediately after adding the test substance to 100 mL of water.

Statistical methods

Linear regression was used to calculate the slopes of the mean percentage of FK 506 remaining vs time curves. An F test was used to determine if the slopes were significantly different from zero. A difference was considered to be significant for $P \leq 0{\cdot}05$.

Results and Discussion

Fig. 2 shows chromatograms of (A) pure FK 506 solution in mobile phase; (B) FK 506 in simulated gastric juice; (C) a sample of FK 506 that was intentionally degraded with 6 M HCl and elevated temperature (60°C) and (D) FK 506 degraded at pH 10. The absence of any peaks at the retention time corresponding to FK 506 in Fig. 2C and D indicates that the assay is specific for unchanged FK 506. The minimum limit of detection was 10 ng of FK 506 injected on the column, with a signal to noise ratio of 5 to 1. The standard curve was linear over a range of 10 to 3000 ng of FK 506 injected on the column. The slope of the calibration curve was (mean ± s.d.) 0·275 ± 0·015, the intercept 1·5 ± 2·9 and the correlation coefficient 0·993 or more. The intra-day coefficient of variation for injections (n = 6) ranging from 12 to 1200 ng was no more than 3%. The inter-day coefficient of variation was no more than 8% for injections (n = 6) ranging from 400 to 1200 ng. The percent deviation of estimated concentration from actual concentration did not exceed 10% over a range of 100 to 1500 ng injected onto the column.

The percentage of FK 506 remaining in solution as a function of time in the presence of various antacids is illustrated in Figs 3 and 4. Of the products and chemicals tested, aluminium hydroxide powder, aluminium hydroxide gel dried, Amphogel, calcium carbonate, Maalox, and magnesium chloride did not affect the concentration of FK 506 over 24 h. The slopes of the percent remaining vs time curve in the presence of these compounds were not significantly different from the control slope.

Significant loss of FK 506 from solution was observed by 2 h in the presence of aluminium hydroxide gel USP (Roxane, Fig. 4) or magnesium oxide powder. With aluminium hydroxide gel USP (Roxane), there was nearly 35% FK 506 loss within 2 min; the slope of this portion of the curve was 20·5, compared with a control slope of 0·004. Interestingly, no further loss of FK 506 was seen for the remainder of the 24 h. FK 506 was completely lost in 2 h in the presence of magnesium oxide powder (Fig. 3). This loss appears to follow a first-order profile, with a reaction rate constant of 0·052 min^{-1}. Significant decrease in the percent FK 506 remaining in solution was also observed between 2 and 24 h for Mag-Ox, Tums and Mylanta (Slopes −0·136, −0·015 and −0·009, respectively; Fig. 4).

Most of our observations can be explained by considering

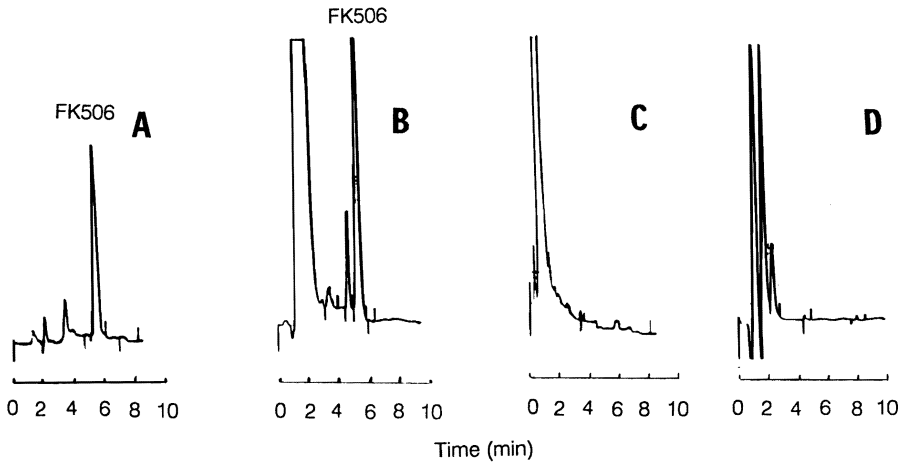

FIG. 2. Chromatograms of FK 506 solutions; (A) pure FK 506 solution in methanol (5 μg mL^{-1}); (B) FK 506 solution in simulated gastric fluid (25 μg mL^{-1}); (C) FK 506 solution degraded by acid and heat and (D) FK 506 degraded at pH 10.

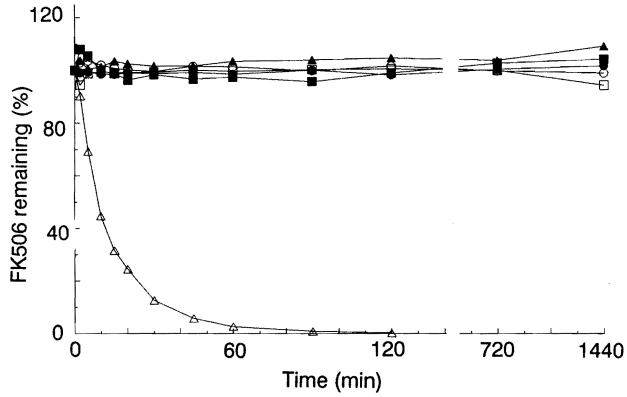

FIG. 3. Mean % FK 506 remaining vs time over 24 h (○) FK 506 control SGF solution; and FK 506 SGF solution with (▲) aluminium hydroxide; (■) aluminium hydroxide dried gel; (□) calcium carbonate; (●) magnesium chloride; and (△) magnesium oxide.

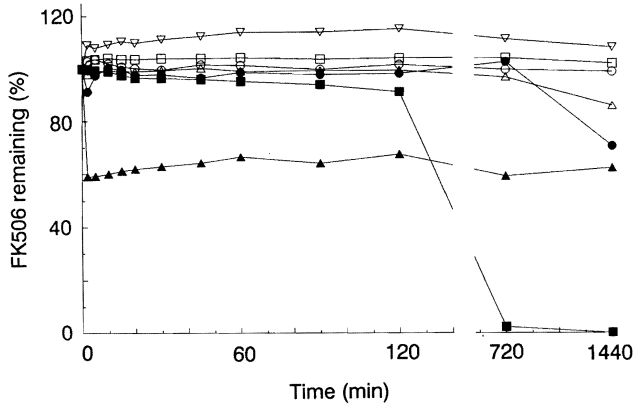

FIG. 4. Mean % FK 506 remaining *vs* time over 24 h for (○) FK 506 control SGF solution; and FK 506 SGF solution with (▲) aluminium hydroxide gel USP (Roxane); (□) Amphogel; (■) Mag-Ox; (●) Tums; (△) Mylanta; (▽) Maalox.

the effect of pH on FK 506 stability. FK 506 was found to be unstable under basic conditions; more than 30% of the drug is lost in 24 h at pH 7·4 (37°C), while total decomposition occurs in 2 h at pH 10 (25°C). At pH values below 7, no appreciable loss was observed in 24 h (Abdallah et al 1990). The acidification of samples immediately after collection prevented any potential change in FK 506 concentration in the solution with time. The pH of the FK 506 solution in the presence of aluminium hydroxide powder, aluminium hydroxide gel dried, Amphogel, or magnesium chloride was 3·6 or less while in the presence of Maalox, the pH was 6·5. Correspondingly, there was no loss of FK 506 from the solution when these compounds were present.

The pH of FK 506 solution in the presence of calcium carbonate also increased over time (5·1 to 6·9); however, the 5% loss FK 506 over 24 h was not significantly different from control values. The pH of FK 506 solution in the presence of Mylanta changed from 3·9 to 7·2 over 24 h. No significant loss of FK 506 was observed at 12 h; however, after 24 h 14% of FK 506 was lost (Fig. 4). The pH of FK 506 solution in the presence of Tums increased over time (4·6–7·1) with the slow dissolution of crushed powder into the SGF. This may explain the 30% loss of FK 506 seen between 2 and 24 h (Fig. 4).

Magnesium oxide (MgO) in SGF had an initial pH above 9 and remained so over 24 h. The high pH may explain the significant degradation of FK 506 (loss of 100% in 2 h) in the presence of MgO (Fig. 3). Mag-Ox tablet, when crushed to a fine powder, did not evenly disperse throughout the SGF. This may explain the slow rise in pH (6–9·3) from 5 min to 24 h as the powder slowly wetted and dissolved and the slower rate of loss of FK 506 (Fig. 4). A 98% loss of FK 506 was observed after 12 h. Conversely, magnesium chloride, which is acidic, did not have any significant effect on FK 506 (Fig. 3). This observation supports the role of pH on the stability of FK 506 rather than a specific interaction between the compounds.

The percent remaining vs time profile obtained with aluminum hydroxide gel USP (Roxane) was different from the other products tested. Although the addition of this product to the SGF did not raise the pH significantly (Table 1), approximately 35% of FK 506 was lost in 2 min and the FK 506 concentration remained constant for the remainder of the 24 h period (Fig. 4). This experiment was repeated with 30 s sampling intervals over 5 min and a 35% loss of FK 506

was noted within the first half min. Aluminium hydroxide powder, Amphogel (a brand of aluminium hydroxide gel) as well as other antacids containing aluminium hydroxide gel, namely Mylanta and Maalox, did not show a similar profile. This suggests that either an inactive ingredient in the suspension or the form of aluminium hydroxide gel used by Roxane may be adsorbing the FK 506 from the solution.

In order to test this hypothesis, the solid pellet of aluminium hydroxide gel USP (Roxane) remaining after centrifugation of the 5 min sample was washed twice with 1 mL of SGF followed by a final wash with 1 mL acetonitrile. These washes were analysed for FK 506 as described above. The first wash contained approximately 15% of the original concentration of FK 506, the second wash 8%, and the last wash of acetonitrile contained 17% of FK 506. This represented a total recovery of 40% of the original amount of FK 506, approximately equal to the observed loss. This observation supports the hypothesis that FK 506 is adsorbed by aluminium hydroxide gel USP (Roxane) and that this process is reversible.

Antacids can alter drug absorption due to physical adsorption, changes in gastric pH, or their effect on gastric motility. Antacid-induced increase in the gastric pH may affect the disintegration, dissolution (captopril), solubility (iron), or ionization (salicylates) of enteric coated preparations and weakly acidic or basic drugs, and thereby alter their absorption (Gugler & Allgayer 1990). Antacids can also alter gastrointestinal (GI) motility. Aluminium-containing antacids and calcium carbonate slow GI motility and are constipating (American Society of Hospital Pharmacists 1990; Gugler & Allgayer 1990). Aluminium salts may also delay gastric emptying. On the other hand, magnesium salts may increase GI motility (American Society of Hospital Pharmacists 1990). The potential effect of antacids on GI motility and therefore on FK 506 absorption could not be assessed in this in-vitro study.

Antacids can also alter drug elimination by increasing the urinary pH, thereby enhancing or decreasing the tubular reabsorption of weakly basic or acidic drugs (American Society of Hospital Pharmacists 1990; Gugler & Allgayer 1990). Since FK 506 is completely eliminated by metabolism (Venkataramanan et al 1990) and is known to be a neutral compound (Kino et al 1987; Tanaka et al 1987) this is not a likely mechanism by which FK 506 and antacids may interact.

The only other macrolide that is commercially available and commonly used is erythromycin. Antacids do not appear to alter the bioavailability of erythromycin significantly (Yamreudeewong et al 1989). Even though FK 506 and erythromycin are in the same class, their chemical structures and properties are quite different and one cannot predict in-vivo FK 506 antacid interactions from this study. The results of this study suggest that, until in-vivo data are available, FK 506 should be dosed separately from antacids in general and from Mag-Ox. This would avoid any potential interaction between these agents.

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