Transplant Int (1992) 5 [Suppl 1]: S514–S515

TRANSPLANT
International

FK506 and rapamycin: differential sensitivity of human, baboon, cynomolgus monkey, dog and pig lymphocytes

S. Metcalfe, R. Svvennsen, and R. Y. Calne

Department of Surgery, Addenbrookes Hospital, Cambridge, United Kingdom

Abstract. It has recently been suggested that there are species differences in the sensitivity of T lymphocytes to the immunosuppressive effect of FK506 [3]. We explore this phenomenon further and compare FK506 with rapamycin in lymphocytes from dog, pig, baboon, cynomolgus monkey and man. We fond that the relative sensitivity of T cells to FK506 did not necessarily correlate with their sensitivity to rapamycin, further emphasising the different mode of action of each drug. The serum may alter results of dose-response curves, and thus autologous serum may introduce an uncontrolled variable when different species are being compared in vitro. We conclude that the differences in sensitivity of lymphocytes from various species to FK506 and rapamycin are independent of each other, and these differences are likely to reflect variation in the overall interactive pathways involved in T-cell mitogenesis.

Key words: Immunosuppression – FK506 – Rapamycin

The species differences in sensitivity to a given drug may result from intrinsic species differences between target cell types. Alternatively, the species differences in pharmacokinetics and metabolic pathways may result in the target cell being exposed to different concentrations of the active drug, giving an apparent, rather than real, difference in cellular sensitivity to the drug in vivo. Recent data suggest that baboon lymphocytes are relatively insensitive to the immunosuppressive effect of FK506, both in vitro and in vivo, when compared with those of rats, dogs and humans [3]. The implication that there are intrinsic species differences in the control of lymphocyte activation led us to explore this phenomenon further, and here we have compared FK506 with a second immunosuppressive macrolide, rapamycin, in vitro using five different large animal species. These drugs are known to act on either early (FK506) or late (rapamycin) pathways of lymphocyte activation [2, 5].

Offprint requests to: S. Metcalfe, Department of Surgery, Addenbrookes Hospital, Hills Road, Cambridge, United Kingdom

Materials and methods

Drugs. FK506 (kindly donated by Fujisawa Pharmaceuticals), and rapamycin (kindly donated by Wyeth Research Laboratories) were each prepared from stock solutions at 10^{-3} *M* in ethanol and stored at + 4°C. Concanavalin A (conA; Sigma) was stored at 1 mg/ml in phosphate buffered saline (PBS) at + 4°C.

Cells. Peripheral blood lymphocytes (PBL) were obtained from blood taken from the following: adult human volunteers, adult baboons (*Papio anubis,* kindly provided by Huntingdon Research Centre); adult cynomologous monkeys (*Macaca fascicularis,* kindly provided by Huntingdon Research Centre); outbred mongrel dogs; and pigs (Large White). Each species pair gave a good two-way mixed lymphocyte response (MLR). All PBL were preserved in liquid nitrogen using medium containing 50% autologous serum and 10% dimethyl sulphoxide (DMSO), at 10^7 cells/ml, until use. Autologous serum was collected from each blood sample and stored at – 20°C until use. Heat-inactivated fetal calf serum was obtained from Advanced Protein Products.

Cultures. Cells were washed and made up to 5×10^7 cells/ml in RPMI 1640. These were diluted tenfold into appropriate control or drug stocks for conA-activation experiments, with or without serum. DNA synthesis was measured at 2 days (conA) or 7 days (MLR). All detailed comparisons were made within a given experiment to avoid any variation due to the preparative procedure.

Results

In serum-free growth medium there were some differences in the order of sensitivity to both FK506 and to rapamycin between the different species. Moreover, the order of sensitivity to FK506 did not correspond to that for rapamycin, further emphasising the different modes of drug action (Table 1).

The use of serum in the culture medium could reduce the apparent drug sensitivity, presumably by sequestering drug molecules (Fig. 1 a). Autologous serum could similarly cause a reduction in the dose-response curve. Thus, if autologous serum is to be used for the comparison of cells between species, then it would also be advisable to include experiments using a common source of serum (FCS), to

Table 1. FK506 and rapamycin: drug concentration resulting in 50% inhibition of mitogenesis in conA-stimulated peripheral blood lymphocytes (PBL) from dog, pig, cynomolgus monkey, baboon and man

Species	FK506 ($x10^{-10}$ *M*)	Rapamycin ($x10^{-8}$ *M*)
Dog	2	5
Pig	4[a] (1, 7)[b]	50 (10, 100)
Human	4 (0.9, 4, 4, 20, 20)	1 (0.5, 1, 1)
Baboon	9 (3, 3, 9, 12, 30)	5 (0.7, 0.9, 11, 11)
Cynomolgus monkey	20 (11, 20, 20, 40)	8 (1, 1, 8, 10, 15)

[a] Median ID_{50} is given for repeat experiments
[b] Each experimental ID_{50} is shown to illustrate interexperimental variation
These experiments were in serum-free medium. Each species gave a stimulation index (SI) of 50 or more, with the exception of the dog, which responded poorly under serum-free conditions with a SI of 10. For this reason, the dog experiments were not repeated in this series, although other experimental series in the presence of serum (not shown) supported the relative data presented here

allow variation due autologous serum components to be identified. This will be especially relevant to drugs of high potency, such as FK506, where the molar concentration in culture is already low and thus particularly sensitive to sequestration by serum components.

When replicate experiments from conA-activated cultures (baboon, serum-free) were compared to an MLR, the dose-response curves were similar in their sensitivity to both FK506 and rapamycin (Fig. 1 b). This supports the relevance of conA-activation studies for assessing these immunosuppressive drugs.

Discussion

Our data demonstrate some variation in the drug sensitivity between PBL from different species. Since these occur in serum-free experiments, this implies that the differences are at the cellular level and may relate to variations in the generation of activation signals between species. It is known that the activation pathway of lymphocytes is multifactorial and includes "cross-talk" between pathways [1, 4] and that the relative contribution of each pathway may differ between species. Any variation in the concentration of a putative target protein associated with a given pathway would result in a similar variation in the concentration of the drug required to reach immunosuppressive levels.

We were interested to note the differential order of sensitivities to FK506 compared with rapamycin. This indicates that any interspecies variation in cross-talk which culminates in activation (involving FK506-sensitive, early "activation genes" including c-*myc*, interleukin 2 and its receptor [2] is independent of the other interspecies variation which occurs later, that is in the rapamycin-sensitive signalling pathways for cell cycle progression (requiring further gene activation, including that of c-*myb* [1].

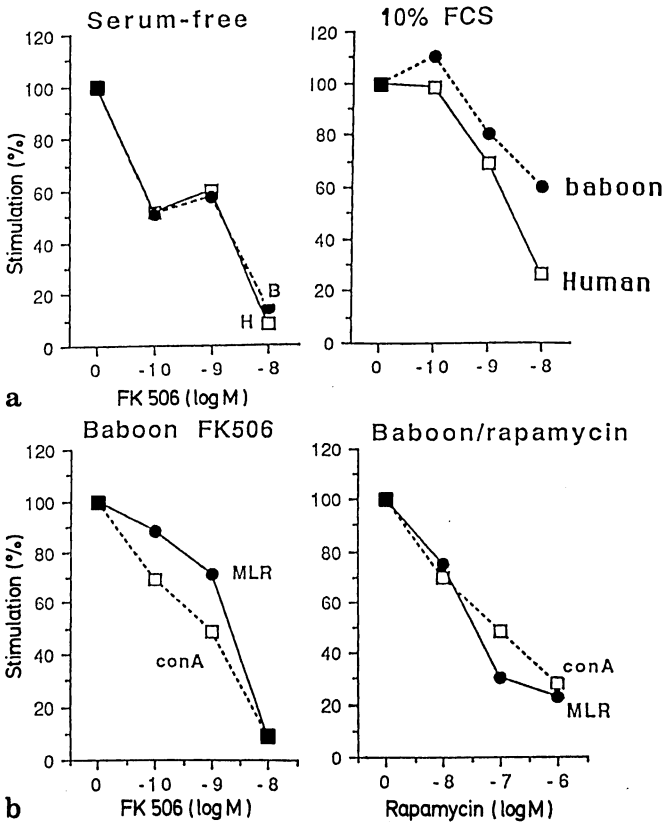

Fig. 1. a Dose-response curves to FK506 in concanavalin (conA)-stimulated cultures of baboon *(B)* or human *(H)* PBL in a single experiment. Cells and drugs were from respective common stocks, and the only variable was the presence or absence of 10% fetal calf serum (FCS). Control levels of DNA synthesis for baboon (human) were 24179 (37829) cpm under serum-free conditions and 81328 (46660) in 10% FCS. **b** FK506 and rapamycin. Dose-response curves for baboon PBL after 2 days of conA stimulation (in serum-free medium). This is the mean of the 5 experiments; in addition, a 7-day MLR (in 2% FCS) between the two baboons was measured. The MLR control gave 119903 cpm (background 2800), whilst the representative cpm for the conA control was 65106 cpm (background 439)

Acknowledgement. This work was funded by the British Medical Research Council.

References

1. Crabtree GR (1989) Contingent genetic regulatory events in T lymphocyte activation. Science 243: 355–361
2. Dumont FJ, Staruch MJ, Koprak SL, Melino MR, Segal NH (1990) Distinct mechanisms of suppression of murine T cell activation by the related macrolides FK506 and rapamycin. J Immunol 144: 1418
3. Eiras G, Imventarza O, Murase N, et al (1990) Species differences in sensitivity of T lymphocytes to immunosuppressive effects of FK506. Transplantation 49: 1170–1172
4. June CH, Ledbeter JA, Linsley PS, Thompson CB (1990) Role of the CD28 receptor in T cell activation. Immunol Today 11: 211–216
5. Metcalfe S, Milner J (1991) Evidence that FK506 and rapamycin block T cell activation at different sites relative to early reversible phosphorylation involving the protein phosphatases PP1 and PP2A. Transplantation 51: 1318–1320