P-glycoprotein (P-gp) is one of the important factors that affect the disposition and pharmacokinetics of drugs in humans. However, there are few reports on species differences in terms of P-gp mediated transport. The purpose of the present study was to elucidate species differences in P-gp mediated transport in vitro. The transcellular transports of diltiazem or cyclosporin A by monolayers of human multidrug resistance 1 (MDR1), monkey MDR1, canine MDR1, rat MDR1a, rat MDR1b, mouse mdr1a, or mouse mdr1b overexpressing LLC-PK1 cells were measured. Net transport at 1 hr after the addition of the substrate was used to calculate the kinetic parameters. For the transport of diltiazem, the Km value of human P-gp was 10 µM, which was higher than that of monkey P-gp and mouse P-gp encoded by mouse mdr1a and was lower than that of canine P-gp and mouse P-gp encoded by mouse mdr1b. For the transport of cyclosporin A, the Km value of human P-gp was 9 µM, which was the lowest among these species. The cyclosporin A transport was not saturable in canine P-gp and rat P-gp encoded by both rat MDR1a and rat MDR1b below 20 µM cyclosporin A. It was demonstrated that species differences exist in P-gp mediated transport. The Vmax values may reflect the expression levels of P-gp. We will discuss the procedures for evaluating species differences with consideration of the total protein content of the monolayer in a well and the expression levels of P-gp measured by Western blot analysis.