Lack of Effect of Catheterization on the Pharmacokinetics of (-)-Carbovir in Rats

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Several studies have been published regarding the effect of chronic catheterization on the pharmacokinetics of certain drugs in rats. Terao and Shen reported (1) a decreased volume of distribution and terminal half-life of propranolol in rats after catheterization. Chindavijak et al. (2) also reported that the placement of indwelling catheters had an effect on the pharmacokinetics of antipyrine and propranolol. Torres-Molina et al. (3) demonstrated that chronic cannulation was associated with a decreased total body clearance and steady-state volume of distribution of amoxycillin. These studies suggested that crossover studies should not be done in rats with chronically implanted cannuli due to time-dependent changes in pharmacokinetics.

Several studies were published by our group on the pharmacokinetics of (-)carbovir (CBV), a novel carbocyclic nucleoside with activity against HIV, and one of its prodrugs, (-)6 amino-carbovir (DAC). A crossover design was used (4,5). The first study was to investigate the bioavailability and pharmacokinetics of (-)-carbovir (4). The male Sprague-Dawley rats were cannulated in both the jugular and the femoral veins with cannuli made of PE-50 (Clay-Adams, Parsippany, NJ) and silastic tubing (Dow Corning). The silastic tubing was connected to the PE-50 with a length of 23 g needle, and only the silastic tubing was introduced into the vein. The length of the silastic tubing was 3 cm for the jugular vein and 6 cm for the femoral vein. The rats were randomized to receive CBV following a three-way crossover design, as either a single iv bolus of 18mg/kg, a single oral dose of 54mg/kg, or a single iv infusion of 18mg/kg infused over 5–9 hours into the jugular vein. Blood samples were withdrawn from the femoral vein cannula. The rats were rested for 24 hours after surgery and at for least 48 hours between each treatment. The result of this study was that CBV had an oral bioavailability of approximately 20% when concentrations of CBV were in the linear range. The second study (5) was designed to evaluate DAC as a prodrug of CBV in male Sprague-Dawley rats with a randomized three-way crossover design. The cannuli and surgical procedure were the same as described above. Rats were randomly assigned to receive the following treatments: a 20mg/kg DAC infusion, 40mg/kg DAC orally, and a 20mg/kg CBV infusion. The rats were rested for at least 24 hours after surgery and for at least 48 hours between each treatment. This study indicated that DAC improved the bioavailability of CBV from oral dosing.

Table 1. Two-way ANOVA: Effect of Sequence of Treatments and Routes on CLr and AUC of CBV (from reference 4)

Source	*p*-value for CLr	*p*-value for AUC
treatment order	0.730	0.606
route of administration	0.244	0.027
interaction	0.637	0.802

After reviewing those studies concerning the effect of chronic catheterization in rats (1–3) we were concerned that the results of our studies of DAC and CBV had been influenced by the crossover study design. Therefore, a statistical analysis was conducted to determine if the pharmacokinetics of CBV or DAC were influenced by the order in which the treatments took place. The area under the blood concentration-time profile (AUC) and the renal clearance (CLr) were chosen as parameters of interest for two reasons: they were obtained in all three treatments in each study, and the change in AUC was used by previous investigators as an indicator of the effects of catheterization (1–3).

For the analysis of the CBV bioavailability study (1), we included the five rats that had completed all three treatments. On average, the rats received their first treatment 1.6 days post-surgery, the second treatment 3.6 days post-surgery and the third treatment 6.2 days post-surgery. A two-way analysis of variance (ANOVA) was used to examine two effects on the AUC and CLr of CBV: route of administration (iv infusion or oral gavage) and treatment order. A p value of less than 0.05 was considered to be statistically significant. Because of a nonlinearity in CLr of CBV at high concentrations (as generated by the iv bolus dose), data from the IV bolus dose were not included in the statistical analysis. Table 1 lists the p-values for AUC and CLr values by route of administration and treatment order. As expected, there was a significant effect of route of administration on the AUC of CBV. There was no effect of treatment order on CLr or the AUC.

In the second study, care had been taken to maintain the blood concentrations of CBV in the linear range after all routes of administration. Two factors were again examined with two-way ANOVA: the routes of administration (DAC infusion, DAC by oral gavage and CBV infusion), and the treatment order. On average, the rats received their first treatments 1.7 days post-surgery, the second treatment 3.8 days post-surgery and the third treatment 6 days post-surgery. Table 2 lists the p-

Table 2. Two-way ANOVA: Effect of Sequence of Treatments and Routes on CLr and AUC of CBV (from reference 5)

Route of Administration	*p*-value for CLr	*p*-value for AUC
treatment order	0.430	0.745
route of administration	0.824	0.0008
interaction	0.099	0.277

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ABBREVIATIONS: AAG, α_1-acid glycoprotein; CBV, carbovir; DAC, (-) 6-aminocarbovir; AUC, area under the curve; CLr, renal clearance; ANOVA, analysis of variance.

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Table 3. Two-way ANOVA: Effect of Sequence of Treatments and Routes on CLr and AUC of DAC (from reference 5)

Source	*p*-value for CLr	*p*-value for AUC
treatment order	0.813	0.379
route of administration	0.193	0.011
interaction	0.324	0.817

values for CBV AUC and CLr values by route of administration and treatment sequence. Table 3 lists similar data for DAC. As expected, there was a significant effect of route of administration on the AUC of both DAC and CBV. However, there was no effect of treatment order on either AUC or CLr of CBV or DAC. We concluded that there was no effect of chronic catheterization in the prodrug study.

Terao and Shen had reported (1) a decreased unbound fraction of propranolol in the serum of rats after catheterization. This was accompanied by a decreased volume of distribution and terminal half-life. Since propranolol is highly bound to α_1-acid glycoprotein (AAG), they proposed that the indwelling catheter was responsible for an inflammation-induced increase in AAG, leading to a change in propranolol pharmacokinetics. In reporting the effects of cannulation on the pharmacokinetics of antipyrine and propranolol, Chindavijak et al. suggested that the indwelling catheters caused both an increase in serum binding and a decrease in hepatic metabolism (2). Carbovir has a free fraction in blood of 0.802 ± 0.048 (at 1 μg/ml), and is mainly cleared renally, with a fraction excreted unchanged in the urine of 0.67 ± 0.11 (4). Therefore it differs from propranolol from a protein binding standpoint, and differs from propranolol and antipyrine in terms of its route of elimination.

Carbovir is more comparable to amoxycillin, which is poorly bound and mostly renally cleared (3). In contrast to results with carbovir, Torres-Molina et al. demonstrated that chronic cannulation was associated with a decreased total body clearance and steady-state volume of distribution of amoxycillin (3). One possible explanation for the discrepancy is that the renal clearance of carbovir is almost three times larger than that of amoxycillin (14.4 ml/min vs. 5.5 ml/min for a 300 g rat). It is possible that the effects of chronic catheterization were too weak to have a noticeable effect on the large renal clearance of carbovir. Alternatively, the difference in the strain of rat (Wistar vs. Sprague-Dawley) could be responsible for the difference in the results.

In summary, our previous work does not appear to be invalidated by the use of chronic catheterization with a randomized crossover design. There is no doubt that these techniques should be used with caution for compounds that are highly bound to plasma proteins and/or metabolized by the liver. It is less clear that these approaches should be avoided for compounds that are highly renally cleared.

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