RESEARCH PAPER

Effect of Amiodarone on the Pharmacokinetics of Gliclazide in Rabbits

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ABSTRACT

Gliclazide is a widely used sulfonylurea. Amiodarone, prescribed for arrhythmias, forms metabolites that inhibits CYP enzymes and may precipitate pharmacokinetic drug-drug interactions. This study assessed whether amiodarone alters the gliclazide pharmacokinetics in rabbits. In a four-period, two-phase, paired crossover study, healthy albino rabbits (n=6) received single doses of gliclazide alone (period-1), amiodarone alone (period-2), a combination of amiodarone prior to gliclazide (period-3) in phase A, and multiple doses of amiodarone for 8 days and on day 9, amiodarone dose before gliclazide (perod-4) in phase B, with 7-day washouts. Serial blood samples (0-24 h) after gliclazide were assayed using HPLC; non-compartmental analysis pharmacokinetics was performed in R (PK-NCA), and one-way repeated-measures ANOVA followed by Dunnett post-hoc test was used for comparisons ($p<0.05$). Amiodarone increased gliclazide exposure (C_{max}, AUC_{0-24}, $AUC_{0-\infty}$) in both single and multiple dose studies, with a more prominent effect after multiple dosing. T_{max} delayed from 4 to 6 h following repeated dosing, and decreased clearance and volume of distribution. Amiodarone significantly increases gliclazide bioavailability with a stronger effect following repeated dosing consistent with metabolite-mediated inhibition. Coadministration warrants glucose monitoring and potential dose adjustment.

Keywords: gliclazide, amiodarone, drug-drug interaction, rabbits, pharmacokinetics

How to cite this article: Gadiko C, Kilari EK. Effect of Amiodarone on the Pharmacokinetics of Gliclazide in Rabbits. Int J Drug Deliv Technol. 2026;16(18s): 82-86. DOI: 10.25258/ijddt.16.18s.9

INTRODUCTION

Type 2 diabetes is often known to coexist with cardiac arrhythmias, making polypharmacy routine and increasing likelihood of clinically significant drug-drug interactions (DDIs).[1-3] Among insulin secretagogues, gliclazide remains a widely prescribed drug owing to its glucose lowering efficiency and its ability to exhibit a lower risk of severe hyperglycemia when compared to some of the commonly prescribed sulfonylureas.[4,5] Gliclazide is metabolized majorly by CYP2C9 and lesser contribution from CYP3A4 and CYP2C19. Any drug that may inhibit or induce these enzymes are expected to affect the pharmacokinetics of gliclazide.[6,7] Amiodarone, on the other hand is a highly lipophilic class III antiarrhythmic agent used across atrial and ventricular rhythm disorders. It has a long terminal half-life, extensive tissue binding, low clearance and forms active metabolites like desethylamiodarone which accumulates with repeated dosing. Amiodarone and its metabolites inhibit multiple CYP enzymes most notably CYP2C9 and CYP3A4 in both reversible and time-dependent manner, raising the chances of pharmacokinetic interactions with the drugs that are metabolized with these enzymes.[8-12] This creates a scope for drug-drug interactions and warrants dose adjustments for the drugs affected.

Clinically, at pharmacological doses concomitant sulfonylureas-amiodarone therapy has been associated with severe hypoglycemia compared with other antiarrhythmics. This implies a clinically relevant interaction which could be pharmacokinetics driven. However, the pharmacokinetic-pharmacodynamic linkage has not always been systemically studied in preclinical models.[12,13] Our previous work,[14] showed that amiodarone alone has a negligible hypoglycemic action but it can increase and prolong the gliclazide-induced hypoglycemic effect in rats and rabbits, especially after multiple-doses. Building on prior pharmacodynamic work in rats and rabbits,[14] we aimed to evaluate how single-dose and multiple-dose amiodarone exposure alters gliclazide pharmacokinetics in rabbits over 24 h interval. This would be a mechanistic study to establish the pharmacokinetic interaction between amiodarone and gliclazide in the context of known CYP2C9 and CYP3A4 involvement in gliclazide metabolism.

MATERIALS AND METHODS

Drugs and Chemicals

Gliclazide ((Micro Labs, Bangalore, India) and amiodarone (Aurobindo Pharma Ltd., Hyderabad, India) were obtained as gift samples. Analytical grade reagents were used.

Animals and ethics

Six healthy albino rabbits (either sex, 1.38–1.68kg; 3months) were purchased from Tina Bio Labs, Hyderabad (regd. no.: 177/99CPCSEA). Rabbits were maintained under CPCSEA-approved conditions. Animals were given standard pelleted diet (VRK Nutritional Solutions, Pune, India) and water ad libitum. The study was approved by Institutional Animal Ethics Committee (1533/PO/a/11/CPCSEA).

Study design

This was a four-period, two-phase, paired crossover study in healthy albino rabbits (n=6), with 7-day washouts between periods to prevent carryover effect of the treatment administered in subsequent periods. Prior to each dosing period, animals were fasted ~18 hours (h) with water ad libitum.

- **Phase A (Single-dose evaluation):** Peroral administration of the following treatments: gliclazide alone (period1), amiodarone alone (period2)*, and amiodarone administered 30-minutes prior to gliclazide (period3).
- **Phase B (Multiple-dose evaluation)**: After an additional 7-day washout, rabbits were administered amiodarone once daily for 8 days. On Day 9, amiodarone was administered followed 30-min prior to gliclazide (period4).

*Note: Blood samples collected in period2 were used exclusively to measure percent blood glucose reduction (pharmacodynamic (PD) interaction) which has been previously published.[14] No gliclazide sampling is required in this period.

Doses, sampling and bioanalysis

Gliclazide was administered at a dose of 5.6mg/1.5kg (optimized standard rabbit test dose),[15] and amiodarone 100 mg/kg orally in all relevant periods.[16] At each of the dosing period, marginal ear vein samples were collected at pre-dose (0 h), and 1, 2, 3, 4, 6, 8, 10, 12, 16, 18 and 24 h post gliclazide dosing in period1, 3 and 4.

Serum gliclazide concentrations were quantified using reverse-phase high-performance liquid chromatography with ultraviolet detection (RP-HPLC-UV) assay employing liquid-liquid extraction and an internal standard. Samples were extracted into an organic phase, evaporated, reconstituted, and analyzed isostatically on a C18 column. The method was in accordance with previously published procedures.[17,18]

Pharmacokinetic and Statistical analysis

Pharmacokinetic evaluation of gliclazide was executed using non-compartmental analysis (PK NCA package) in R (version 4.4.2). PK parameters include the maximum observed concentration (C_{max}), time to reach that peak concentration (T_{max}); area under the concentration versus time to the 24h (AUC_{0-24}) using linear trapezoidal rule; area under the curve from time zero (0) to infinity ($AUC_{0-\infty}$), area under the first moment curve to 24 h ($AUMC_{0-24}$); elimination rate constant (K_{el}); extrapolated area under the curve ($\%AUC_{extrap}$). Mean residence time (MRT_{0-24} and $MRT_{0-\infty}$); apparent volume of distribution (V_d/F) and apparent systemic clearance (Cl/F) were also determined.

All pharmacokinetic parameters were summarized as mean ± standard error of mean (SEM).

Because the same animals were studied across all periods, statistical comparisons were done using one-way repeated-measures ANOVA followed by Dunnett-type post-hoc approach with significance at $p<0.05$. All statistical analysis were performed using R statistical software (version 4.4.2).

RESULTS

The Mean±SEM concentration-time profiles and summary pharmacokinetic parameters for gliclazide are shown in Figure 1 and Table 1. Following administration of gliclazide alone (period1), the drug was absorbed rapidly, achieving a median T_{max} of 4 h, followed by a gradual decline in concentration towards 24 h sampling interval.

Figure 1: Mean±(SEM) serum gliclazide concentration-time profiles for control (gliclazide alone), single-dose amiodarone+gliclazide, and multiple-dose amiodarone + gliclazide (n=6)

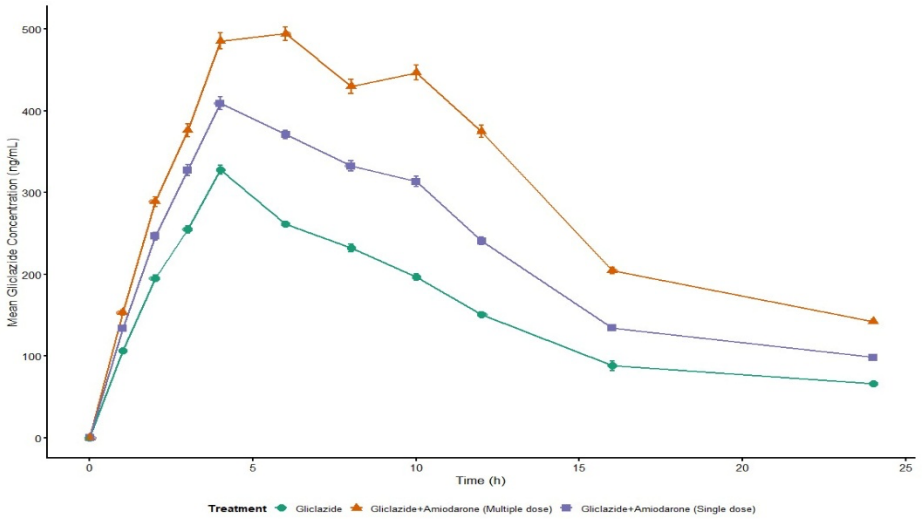

Table 1: Pharmacokinetic parameters of gliclazide (Mean±SEM, n=6)

Parameter	Gliclazide (G)	G + Amiodarone (Single-dose)	G + Amiodarone (Multiple-dose)
C_{max} (ng/mL)	328.19±5.46	409.33±8.17***	495.94±8.47***
T_{max} (h)#	4.00 (4.00 – 4.00)	4.00 (4.00 – 4.00)	6.00 (4.00 – 6.00)
AUC_{0-24} (ng.h/mL)	3759.00±55.18	5420.44±37.00***	7442.12±46.83***
$AUC_{0-\infty}$ (ng.h/mL)	4581.97±89.07	6671.32±52.89***	9214.02±126.13***
$\%AUC_{extrap}$	17.92±0.60	18.73±0.72	19.18±0.78
$AUMC_{0-24}$ (ng. h^2/mL)	35509.11±834.19	53257.51±533.90***	76840.18±678.92***
MRT_{0-24} (h)	9.44±0.09	9.83±0.07***	10.33±0.06***
$MRT_{0-\infty}$ (h)	14.29±0.31	14.87±0.29	15.35±0.31**
$t_{1/2}$ (h)	8.65±0.33	8.80±0.23	8.61±0.30
K_{el} (h^{-1})	0.08±0.00	0.08±0.00	0.08±0.00
V_d (mL)	16553.04±161.23	11610.50±374.29***	8202.42±215.94***
Cl/F (mL/h/kg)	816.35±16.03	559.79±4.45***	405.55±5.35***

Significance: *p< 0.05; **p<0.01; ***p<0.001
#Tmax: median (range); non-parametric method used.
V_d reported as absolute mL per rabbit

Coadministration of amiodarone produced a marked increase in gliclazide bioavailability. After a single dose of amiodarone (period3), both C_{max} and AUC increased substantially compared to gliclazide alone (C_{max}: 328.19±5.46 to 409.34±8.17 ng/mL; AUC_{0-24}: 3759.00±55.18 to 5420.44±37.00 ng.h/mL; $AUC_{0-\infty}$: 4581.97±89.07 to 6671.32±52.89 ng.h/mL). The increase in exposure was even more pronounced after multiple-dose amiodarone pretreatment (period4) with C_{max} increasing to 495.94±8.47 ng/mL, AUC_{0-24} to 7442.12±46.83 ng.h/mL, and $AUC_{0-\infty}$ to 9214.02±126.13 ng.h/mL.

The T_{max} was unchanged after a single amiodarone dose (4 h), but shifted to 6 h following multiple dose amiodarone pretreatment, indicating a modest delay in rate of absorption, however $\%AUC_{extrap}$ remained below 20% for all periods.

AUMC increased in parallel with exposure in both interaction phases A & B (single-dose and multiple-doses). MRT_{0-24} showed a steady increase across treatments (9.44±0.09 to 9.83±0.07 to 10.33±0.06 h), whereas $MRT_{0-\infty}$ remained relatively consistent (Mean values ~ 14.29 to 15.35 h), reflecting stable overall disposition despite higher systemic exposure.

Amiodarone coadministration led to a reduction in apparent clearance (Cl/F) and apparent volume of distribution (V_d/F), consistent with increased systemic exposure. The K_{el} values remained unchanged, resulting

in a minor change in $t_{1/2}$ values.

DISCUSSION

In clinical practice, it's common for patients to take multiple medications, but these drugs don't always work well together. To see how they interact, researchers often use animal models to map out the biological interactions that can occur namely, pharmacokinetic or pharmacodynamic interactions. We specifically looked at how amiodarone affects the way the body processes gliclazide. By using healthy albino rabbits-a commonly used model for this type of research,[19] we were able to study how amiodarone might change the gliclazide concentration in the blood, helping to predict and prevent potential adverse effects in humans.

Our data showed that amiodarone increases gliclazide exposure (both C_{max} and AUC) after a single dose and increased further after multiple-dose pretreatment, while T_{max} remained 4 h with single dosing and delayed it to 6 h following repeated dosing. Despite this delay in absorption phase, $\%AUC_{extrap}$ was below 20% in all periods, indicating adequate characterization of the terminal phase. The progressive increase in MRT_{0-24} along with relatively stable $MRT_{0-\infty}$ and minimal change in K_{el}, $t_{1/2}$ together suggest that the interaction is primarily driven by absorption or clearance processes rather than by a transformation of terminal elimination. Additionally, simultaneous decrease in V_d/F indicate a shift in gliclazide distribution possibly resulting from metabolite-mediated inhibition of CYP enzymes, a major elimination pathway of gliclazide. Evidence from human microsomal studies has shown that these metabolites can inhibit multiple CYP isoforms, most notably CYP2C9, CYP1A2, CYP2D6 and CYP3A4 both reversibly and time-dependent manner.[20] With repeated dosing, tissue accumulation of amiodarone and increased formation of its primary metabolites (N-desethylamiodarone - DEA) are expected, which can intensify the CYP inhibitory effect and may result in pronounced pharmacokinetic effect observed during multiple-dose treatment phase.[8,11]

The outcome of this study is consistent with earlier interaction studies in rabbits, where amiodarone coadministration increased the exposure of orally administered sulfonylureas.[16] A similar trend was observed in the present study, with a gradual increase in gliclazide exposure across treatments (multiple-dose>single-dose>gliclazide control). This progression aligns with the known inhibitory effect of amiodarone and its primary metabolite (DEA) on CYP2C9 (major) and CYP3A4 (minor) metabolic pathways of gliclazide.[11] Clinical database analysis showed higher heart rate and an increased risk of hypoglycemic events after starting amiodarone in patients using sulfonylureas,[12,13] supporting the pharmacokinetic changes observed in this study.

CONCLUSION

Amiodarone increases gliclazide bioavailability and delays the time to peak, with the effect more pronounced after multiple-dose pretreatment. These findings indicate a potential pharmacokinetic interaction at metabolic level that may result in enhanced gliclazide's glucose-lowering activity. When these drugs are used together, careful monitoring of blood glucose and dose adjustments may be necessary to prevent hypoglycemia.

ACKNOWLEDGEMENTS

The authors are thankful to Mr. Raj Bharath R for assistance with animal experiments.

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