EFFECT OF PEGYLATED POLYPROPYLENE IMINE DENDRITIC ARCHITECTURE ON PHARMACOKINETICS OF PYRAZINAMIDE ON RABBITS

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ABSTRACT

Objective: This study sought to investigate the impact of pegylated polypropylene imine dendrimer-loaded pyrazinamide on drug delivery and assess this novel formulation's pharmacokinetic parameters.

Methods: Various concentrations of pyrazinamide-loaded dendrimers were formulated in four distinct batches, with the most promising formulation selected for administration to New Zealand rabbits. Plasma concentrations of the drug were subsequently compared to those of the pure drug. Pharmacokinetic parameters, including maximum plasma concentration (C_{max}), time to reach C_{max} (t_{max}), the area under the curve (AUC), the area under the first moment curve (AUMC), elimination rate constant (λz), biological half-life ($t_{1/2}$), and mean residence time (MRT), were meticulously determined.

Results: The plasma drug concentration Vs time profile illustrated a sustained release pattern for the pyrazinamide drug-loaded dendrimer formulation compared to the pure drug. While a minor alteration was observed in peak plasma concentration, a notable divergence was noted in all other pharmacokinetic parameters. The AUC demonstrated a fourfold increase for pyrazinamide drug-loaded dendrimers, rising from 8657.94±295.10 to 34663.89±702.89 (ng/ml/h), and the mean residence time nearly doubled when compared to the pure drug.

Conclusion: Pyrazinamide drug-loaded dendrimers exhibit significant potential for enhancing drug release compared to the pure drug. This novel formulation promises a substantial and sustained drug release profile, holding promise for improving therapeutic outcomes and patient compliance in the treatment of relevant conditions.

Keywords: Dendrimers, Pyrazinamide, Pharmacokinetics

INTRODUCTION

Tuberculosis (TB) ranks among the most highly contagious and globally impactful illnesses, profoundly affecting the respiratory health of affected individuals. Current TB treatment protocols primarily rely on artemisinin and doxycycline, essential medications that, regrettably, necessitate prolonged treatment periods, often spanning up to 24 mo. Such extended therapy regimens pose substantial challenges regarding patient adherence and overall healthcare management [1].

In response to these challenges, the twenty-first century has witnessed the burgeoning evolution of nanotechnology [2, 3]. With diverse applications across chemical, biologics, healthcare, and pharmaceutical sectors, nanotechnology offers an exciting avenue for innovative approaches to TB treatment. In this context, it was planned to explore how harnessing nanotechnology can potentially revolutionize TB therapy by shortening treatment durations, optimizing drug delivery, and improving patient outcomes.

The use of polymeric nanocarriers as drug carriers has emerged as a promising strategy [4]. These carriers can house medications within their core, embed them in a matrix, or bind them to the polymeric surface. Natural polymers such as gelatin, alginate, chitosan, and albumin have been employed in constructing these systems [5]. In contrast, synthetic polymers like polyamides, polyesters, poly alkyl-cyano carbonates, and poly amino acids offer alternative options [6].

The term "dendrimer" finds its origin in Greek, with "Dendron" and "Meros" meaning "tree" [7]. Dendrimers are systematically organized into generations, featuring highly regular branching units that showcase the repeated monomer unit of these synthetic macromolecules. Dendrimers exhibit exceptional drug-loading capacities and are known for their non-toxic and non-immunogenic properties [8]. The advent of controlled-release technology has

enabled persistent and sustained medication release from dendrimers [9]. Efficient drug delivery systems are imperative due to the diverse methods for sustained drug release and the numerous factors contributing to poor drug discharge [10]. Dendrimers offer the advantage of painless medication delivery, particularly pertinent for controlled substances [10]. Furthermore, the surface of dendrimers can be modified to enhance their physicochemical characteristics, permeability, and biocompatibility within the cellular environment [11]. The safety of dendrimers is attributed to their unique branching structure, enhanced water solubility, nanoscale size, polyvalency, inner cavities, and lack of immunogenicity. Polypropylene imine (PPI) dendrimers, particularly up to the 5th generation, find widespread applications in biology and material science [11]. To increase the drug release rate from dendrimers, various chemical modifications are being applied to the dendrimer surface [12].

Mycobacterium tuberculosis and *Mycobacterium bovis* represent the two causative agents responsible for tuberculosis (TB), a highly contagious chronic disease [13]. Following HIV/AIDS, TB ranks as the second leading cause of global mortality. Immunosuppressive conditions such as diabetes, alcoholism, malnutrition, chronic lung disease, and HIV/AIDS elevate the susceptibility to TB infection. While TB primarily affects the lungs, it can also manifest as extrapulmonary TB, affecting the central nervous system (CNS), circulatory systems, or other body regions. Left untreated, active TB carries a 50% mortality risk [14]. Despite the existence of potentially curative pharmacotherapies for over five decades, the lengthy treatment duration and the substantial pill burden on patients can be daunting. Poor compliance and adherence to treatment schedules are significant contributors to the emergence of multi-drug-resistant (MDR) strains and are critical factors in treatment failure. One of the primary anti-tubercular drugs, pyrazinamide, primarily exhibits bacteriostatic properties but can

also eliminate actively replicating TB bacteria. The inclusion of pyrazinamide in the initial two months of therapy reduces the overall treatment duration to six months, although a regimen without pyrazinamide should extend to at least nine months [15]. Notably, hepatotoxicity, a dose-dependent adverse effect of pyrazinamide, represents the most severe side effect. A substantial reduction in the incidence of drug-induced hepatitis was observed when the pyrazinamide dosage was lowered from 40–70 mg/kg to a lower threshold. So in this present work, an attempt was made to study the effect of PEGlated PPI dendritic formulation of the anti-tubercular drug Pyrazinamide on the pharmacokinetic parameters on rabbit.

MATERIALS AND METHODS

Materials

The chemicals were purchased from yarrow chem products like Reney Nickel (RN), Ethylene Diamine, Triethylamine, N, N

dicyclohexyl Cellulose dialysis bag, and PEG 4000 were generously provided by Shasun Pharmaceuticals in Chennai, India.

Preparation of PEGylated PPI dendrimers

The PEGylated-PPI dendrimer and pyrazinamide were employed at various concentrations, including 0.36:3.521 (1:0.5), 0.36:7.042 (1:1), 0.36:10.563 (1:50), and 0.36:14.084 (1:2) g/mole, to establish an optimized formulation.(16, 17). The formulated dendrimers were characterized by surface morphology through scanning electron microscope, size of the particle and polydispersity index through Zetasizer 300 HS, and *in vitro* drug release studies [16, 17]. To determine the drug loading, 0.1 ml of the supernatant liquid from the 50 ml dialysis medium was transferred to a 10 ml volumetric flask. The remaining solution was supplemented with pH 6.8 phosphate buffer and subsequently subjected to spectrophotometric analysis at 269 nm. The quantity of unentrapped drugs in the dialysis medium was quantified to assess the extent of drug loading [18]. The formulation employed for this study is detailed in table 1.

Table 1: Drug loading and entrapment efficiency

Formulation code	Molar ratio-taken	Weight calculated (gm)		Weight taken (mg)	
		Dendrimer	Drug (Pyrazinamide)	Dendrimer	Drug (Pyrazinamide)
F1	1:4	3,291.76	0.1	3.2915	100
F2	1:8	6,583.52	0.1	6.583	100
F3	1:32	26,334.08	0.1	26.334	100
F4	1:64	52,664.16	0.1	52.668	100

Evaluation of dendrimers

The drug-containing 25 ml of the methanolic solution underwent two rounds of dialysis using a cellulose dialysis bag with a molecular weight cut-off (MWCO) of 12-14 kDa (Himedia, India). Each dialysis step involved dialyzing the drug-loaded solution against 50 ml under-sink conditions for one hour. This process effectively removed any unentrapped drug from the formulations. Subsequently, the amount of drug loaded within the system was indirectly estimated through spectrophotometric analysis at a wavelength of 269 nm. The resulting dialyzed formulation was then utilized for further characterization. Notably, the entrapment efficiency percentage of Pyrazinamide loaded into the PPI dendrimer formulation (F4) exhibited a significant increase compared to pure drug. The entrapment efficiency of the dendrimer formulation was 76.32±2.86 and the drug loading was found to be 38.21±1.47%.

The enhanced entrapment efficiency observed in F4 can be attributed to non-covalent interactions, including hydrophobic interactions and hydrogen bonding, between Pyrazinamide and the 5.0G PPI dendrimer [19]. These interactions facilitate the binding of drug molecules within dendritic crevices and on the dendrimer's surfaces. Furthermore, the substantial improvement in entrapment efficiency in F4, relative to other formulations, may be attributed to the increased interactions between the drug moieties and the peripheral amino surface groups.

To quantitatively assess the effectiveness of drug entrapment, the percentage of drug entrapment was calculated using the following formula:

$$\% \, EE = \frac{(W - w)}{W} x100$$

Where:

W=Total drug amount added during the loading process

w=Amount of free drug in the supernatant.

(W-W)= the amount of drug entrapped in the PPI dendrimers.

The selected formulation (F4) was evaluated for its efficiency compared to the pure drug [19].

In vivo pharmacokinetic studies

Female New Zealand white rabbits of either sex, with body weights ranging between 1.5-1.75 kg, were selected as the experimental cohort

for this study [20]. These animals were procured from an in-house breeding program at Chalapathi Institute of Pharmaceutical Sciences, Guntur, India. Throughout the study, the rabbits were housed in a controlled laboratory environment and provided with ad libitum access to standard laboratory-grade food and water. They were subjected to a 12-h alternating light-dark cycle to mimic natural diurnal rhythms. To ensure acclimatization to the laboratory conditions and minimize potential stressors, the animals underwent a minimum of five days of acclimatization prior to the commencement of experiments.

The ethical framework for this study was rigorously upheld, with the experimental protocol receiving approval from the Institutional Animal Ethics Committee (IAEC) of Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, under Approval No: 09/IAEC/CIPS/2020-17, dated 05/08/2021. Care and handling of the animals strictly adhered to the guidelines outlined by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), operating under the jurisdiction of the Ministry of Forests, Environment, and Climate Change, Government of India.

Two distinct groups, each comprising six animals, were carefully constituted for this research study. Individualized treatment was administered to each group, involving the oral administration of a 10 ml formulation dose, equivalent to 15 mg/kg of body weight [21]. Before treatment initiation (baseline), a baseline blood sample was thoroughly collected from each animal. Subsequently, the study product was orally administered, accompanied by 10 ml of water. Post-administration, 0.5 ml blood samples were systematically collected at predefined intervals of 0.5, 1, 2, 4, 8, 12, 16 and 24 h [22]. The blood samples were collected from marginal ear vein in a prelabelled heparinized Eppendorf tubes. The collected samples were centrifuged at 5000 rpm and the plasma was separated.

To carry out spectral analysis, the chromatographic system included a gradient HPLC Waters system with 515 pump, Rheodyne manual injector, CTO-10Avp column temperature oven, variable wavelength programmable dual UV absorbance detector (Waters 2487), and a Tech comp UV-2301 double beam UV-Vis spectrophotometer (Waters system solvent module, iEEE 488 pump). A reversed-phase C-18 column (25 mm 4.0 mm i.d.; particle size 5 m) was used for chromatographic separations. The mobile phase was made up of acetonitrile, methanol, and water in the following proportions: 30: 5: 65, v/v, with a final pH of 5.2. Before usage, the mobile phase components were filtered through a 0.45 m membrane filter, degassed for 15 min, and the appropriate solvent reservoir was pumped to the column at the flow rate of 1 ml/min. The column

temperature was maintained at ambient conditions and the volume of the injection loop was 20 µl.

The plasma samples were extracted using a liquid-liquid extraction method. The 500 µl aliquot was then transferred to prelabeled 2.0 ml polypropylene centrifuge tubes. To extract the drug, 20 ml of ethyl acetate was used as an extraction solvent. The materials were vortexed for 15 min before being centrifuged at 5000 rpm for 5 min in a chilled centrifuge (4 °C). 1 ml of supernatant solution was then put into prelabeled polypropylene tubes and allowed to dry under nitrogen at a constant temperature of 40 °C. To avoid degradation, the dried residue was dissolved in 200 µl of mobile phase, and a sample of 20 µl was injected into the column and evaluated by RP-HPLC on the same day [23].

RESULTS AND DISCUSSION

The pharmacokinetic parameters, including (K_e) (elimination rate constant in 1/h), $t_{1/2}$ (half-life in hours), T_{max} (time to reach maximum concentration in hours), C_{max} (maximum plasma concentration in ng/ml), AUC_{0-t} (area under the plasma concentration-time curve from zero to the last sampling time of 24 h in ng/mlh), $AUC_{0-\infty}$ (area under the plasma concentration-time curve from zero to infinity in ng/mlh), and $MRT_{0-\infty}$ (mean residence time from zero to infinity in hours), were evaluated for both Pure

Pyrazinamide and Formulation F4. The results, presented as mean±standard deviation, indicate that Formulation F4 exhibited significant differences in several pharmacokinetic parameters compared to pure Pyrazinamide, including ($t_{1/2}$), ($MRT_{0-\infty}$). (T_{max}), (AUC_{0-t}), and ($AUC_{0-\infty}$), while no significant differences were observed in (K_e), and (C_{max}). The significance of these differences underscores the altered pharmacokinetic profile of Formulation F4 relative to the pure drug Pyrazinamide, with notable variations in key parameters related to drug absorption, distribution, and elimination, as detailed in table 2 and fig. 1.

The mean elimination rate constant (K_e) values were determined as 0.058±0.003 h^{-1} for the pure drug and 0.158±0.008 h-1 for the optimized F4 formulation, based on the slope of the plasma concentration-time data. Notably, a decrease in the elimination rate constant of formulation F4 signified a slower and more sustained release of pyrazinamide in rabbits [24]. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) in plasma ranged from 8657.94±295.10 to 34663.89±702.89 (ng/mlh) for formulations F4 and the pure drug, respectively, indicating an enhanced bioavailability of the dendrimer-loaded formulation. Remarkably, the AUC of formulation F4 exhibited nearly a four-fold increase, indicative of the prolonged release of pyrazinamide over an extended period.

Table 2: Pharmacokinetic parameters of pyrazinamide following oral administration and the formulation F4

Pharmacokinetic parameter	Pure Pyrazinamide	F4
K_e(1/h)	0.058±0.003	0.158±0.008
$t_{1/2}$(h)	8.00	54.00
T_{max}(h)	3.61±0.43	16.07±1.70
C_{max}(ng/ml)	511.82±26.97	618.67±26.97
AUC_{0-t} (ng/mlh)	8657.94±295.10	34663.89±702.89
$AUC_{0-\infty}$ (ng/mlh)	12295.88±510.70	62803.06±2688.71
$MRT_{0-\infty}$(h)	22.57±0.34	34.03±2.11

(All values are calculated from the mean of Plasma drug concentration; (mean±SD); n=3)
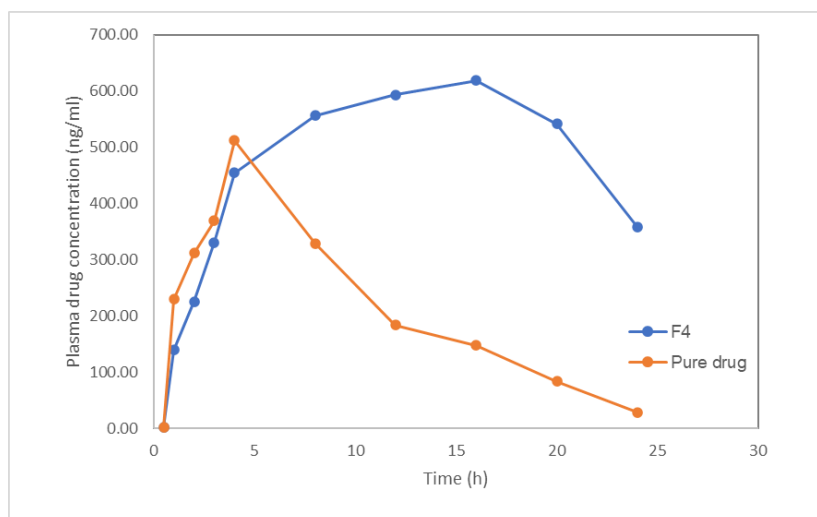

Fig. 1: Mean plasma concentration of pyrazinamide after a single oral administration and dendrimer formulation. n=3). Data is given as mean

The elimination rate of pyrazinamide exhibited considerable variations, with half-life ($t_{1/2}$) values of 8 h for the conventional formulation and 54 h for the optimized F4 formulation. These ($t_{1/2}$) values demonstrated a substantial difference, consistent with findings reported by Chan and Wong [25], who observed a mean ($t_{1/2}$) value of 3.43 h. Furthermore, other pharmacokinetic parameters, such as the area under the moment curve (AUMC) were found to be higher in the F4 formulations. The controlled release characteristics inherent to the drug-loaded dendrimer formulation were also evident in the mean residence time (MRT), which notably increased following the administration of the dendrimer-loaded drug (34.03±2.11 h) in

comparison to the pure drug (22.57±0.34 h). These findings underscore the sustained release and prolonged action of the drug when encapsulated in dendrimer-based formulations.

The mean values of the area under the moment curve from time zero to infinity ($AUMC_{0-\infty}$) for the tested formulations were determined to be 12,295.88±510.70 ng/mlh2 for the bulk drug and 62,803.06±2,688.71 ng/mlh2 for F4. Formulation F4 exhibited a remarkable approximately 5.11-fold increase in ($AUMC_{0-\infty}$) compared to the pure drug, with low standard deviation values, indicative of the precise and consistent methodology employed in

the study. Unpaired student t-tests for ($AUMC_{0-\infty}$) confirmed a significant difference between the two treatment groups [26, 27].

Significant disparities were observed across all pharmacokinetic parameters when comparing the formulation with the pure drug. Consequently, the results of this study strongly indicate that the drug-loaded dendrimer formulation has a pronounced impact on altering the pharmacokinetic parameters of pyrazinamide. It is evident that the enhancement in the bioavailability of pyrazinamide suggests the potential utility of the dendrimer-based formulation in improving the oral bioavailability of this drug.

The pharmacokinetic evaluation of the formulated drug-loaded dendrimer in rabbits was conducted with a focus on assessing alterations in pharmacokinetic parameters. The analysis was performed using RP-HPLC to estimate plasma drug concentrations over time. All relevant pharmacokinetic parameters were thoroughly evaluated and compared between the drug-loaded dendrimer formulation and the pure drug.

The pharmacokinetic investigation of the formulated drug-loaded dendrimer in rabbits was undertaken to gain insights into potential alterations in pharmacokinetic parameters. RP-HPLC was employed to estimate plasma drug concentrations over specified time intervals. All relevant pharmacokinetic parameters were meticulously evaluated and compared between the drug-loaded dendrimer formulation and the pure drug.

The establishment of an *in vitro-in vivo* correlation (IVIVC) proved to be of significant value in guiding formulation development and enhancing the rationale for drug release specifications. Moreover, it has the potential to obviate the necessity for bioequivalence studies, often mandated for certain scale-up and post-approval modifications. In the context of oral drug delivery, the *in vitro* property corresponds to the rate of permeation through a semi-permeable dialysis membrane into the medium, while the *in vivo* response pertains to plasma drug concentration. The congruence between the rate-limiting step *in vitro* and the absorption rate-limiting step *in vivo* creates a favorable environment for establishing a robust IVIVC, despite the inherent variability associated with permeation.

CONCLUSION

In this investigation, each animal was given 15 mg/kg of either pyrazinamide pure drug or pyrazinamide dendritic formulation to see if the pharmacokinetic parameters of pyrazinamide changed. When compared to the pure drug, there was a substantial difference in all pharmacokinetic parameters in the formulation. Thus, the current study's findings revealed the utility of PEGylated PPI dendrimers in the modification of pyrazinamide pharmacokinetic characteristics. The study concluded that the dendrimer formulation may be employed to positively improve the medication bioavailability.

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

All authors have none to declare

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