Studies on Molecular Insights into Amorphous Systems of Riluzole for Improving Stability, Dissolution and Pharmacokinetic Profile

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By

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6 Summary and conclusion

The idea of these studies was to establish a rationale for polymer selection which would impart stability to the amorphous solid dispersion throughout its shelf-life. For this purpose, Rilizole was chosen as a model compound which belonged to BCS class II and had low bioavailability. This drug also possesses a serious problem of hepatotoxicity. Hence, there was a requirement to increase its dissolution, which would ultimately lead to increased bioavailability of the drug. Due to increase in the bioavailability, to achieve the same therapeutic effect as of the marketed formulation, dose can be reduced hence the toxicity can be managed. For this purpose, PAA, PVP VA and HPMC AS was chosen as polymeric carriers. Polymers play the most significant role in stabilizing any drug in its amorphous form. Since, pure RLZ had very low T_g of 28^{o}C only, hence maintaining its stability was a challenging task. To understand the mechanism of drug and polymer interaction which results into a stable ASD, computational studies were performed. For this study 3D structure of polymers were modelled and then docked with RLZ. Later on interaction energy between RLZ and the respective polymers were calculated and found that RLZ is making the most stable complex with polymer PAA. To confirm these findings, ASDs of RLZ with these three polymers were prepared in different drug and polymer ratio and then various thermal, microscopic and spectroscopic analysis were done. In XRD studies it was found that, in comparison to PVP VA and HPMC AS, PAA is able to inhibit the crystallization of RLZ even at a weight ratio of 30%. Similarly in DSC studies it was found that PAA is contributing towards higher values of T_g with respect to the polymers. While studying the crystallization tendency of the prepared ASDs enthalpy of crystallization was observed for HPMC AS at a weight ratio of 30%, which indicates the instability of amorphous RLZ with this polymer. In TEM studies it was found that after exposing the supersaturated ASDs to higher temperature, the ASD of RLZ

with PAA doesn't change its form, while evidences of drug demixing was observed in case of ASDs prepared with PVP VA and HPMC AS. This mechanism of how stability was imparted by these polymers to the amorphous state of RLZ, spectroscopic studies like FTIR and ssNMR was performed. While FTIR couldn't give any evidence of drug polymer interaction, ssNMR provided strong evidences of the formation of molecular level dispersion of RLZ with all the three polymers. The intensity of interaction was highest among RLZ and PAA, followed by PVP VA and HPMC AS which was observed from the gaussian resonances of the spectra. As, a part of this interaction study, the miscibility between drug and polymer were also evaluated using Flory-Huggins theory. This study suggested optimum miscibility of RLZ with PVP VA and HPMC AS but the stability temperature was low. However, with PAA the miscibility was relatively lower, but the stability temperature was high. Hence, considering all the factors, polymers were ranked as; PAA> PVP VA> HPMC AS based on its suitability to provide stability to the amorphous form of RLZ.

For further studies the HPMC AS was not used because it was showing lesser chemical shift in drug-polymer interaction studies conducted using ^{1}H-NMR study as compared to PAA and PVP VA. The dissolution and wettability studies were carried out with the remaining two polymers. In these studies, the relationship between the surface properties with wettability and dissolution behaviour was established. Since crystallization is mainly affected by the surface rather than bulk properties, hence this study was helpful in understanding the surface properties imparted due to incorporation of polymers and their effect on the dissolution. In the dissolution study conducted with different RLZ ASDs prepared with different drug and polymer ratio it was found that, though every ASD is showing improved dissolution as compared to the crystalline RLZ, the dissolution profile of ASDs improves with higher polymer ratio. This observation was supported by

comparison of different curve fitting profile of the wetting behaviour of ASDs with RLZ. Further, the calculation of total surface free energy was done. This is a sum of dispersive energy and polar energy. It was seen that the polar component of energy was increasing with increase in the weight ratio of PAA and PVP VA in the ASD, which indicates that higher hydrophilicity is achieved with high polymer weight ratio. XPS was used to evaluate the surface chemistry of the ASDs which will support the previous observations of hydrophilicity. In XPS study it was found the pure crystalline RLZ have higher atomic percentage of fluorine, which is the main reason behind the hydrophobic nature of this API. However, in case of ASDs the atomic percentage of fluorine was very low. When the predominant planes of RLZ crystals were observed in mercury software, abundance of fluorine was seen hence, supporting the XPS results. While ASDs will have higher conformational flexibility, the chances of atoms which are capable of forming hydrogen bonds with the surrounding aqueous media increases hence they show improved wettability and dissolution. In this study as well, RLZ:PAA ASD showed more promising result as compared to RLZ:PVP VA ASD. This study helped in understanding the transition of dissolution behaviour from being drug-controlled to carrier-controlled and was supported by surface energy study and surface chemistry studies as well.

In all the previous studies, superior properties of RLZ:PAA ASD were observed. Hence, they were selected to conduct further studies. RLZ:PAA ASDs were prepared in 30:70, 20:80 and 10:90 weight ratio and solid-state characterization with XRD, DSC and TEM studies were done. Saturation solubility of the drug in different media namely, pH 1.2, pH 6.8, FaSSGF and FaSSIF were done. Higher solubility of RLZ was found at acidic pH and in FaSSGF. Hence, dissolution study was done in pH 1.2 and FaSSGF media and compared with the marketed tablet Rilutor™. 10:90 RLZ:PAA ASD was showing maximum drug dissolution hence was selected for conducting pharmacokinetic studies

on rats. The concentration of drug in plasma and brain tissue was analysed using a validated HPLC method. The ASD formulation showed superior pharmacokinetic properties as compared to the marketed formulation Rilutor™. Hence this data was used to estimate the drug-concentration profile in the human brain which is the target organ for this drug. The drug possesses neuroprotective action, hence estimation of its concentration in human brain could be very useful in adjusting its dose in future. For this purpose, a PBPK model was developed as a starting point to explore mechanistic pharmacokinetic for Rilutor™ and RLZ ASD. The model was developed for both rats and humans and were validated utilizing the published literature data on both the species. After establishing the suitability of the PBPK model the drug concentration-time profile was predicted for brain and it was found that there is increase in the drug concentration in human brain for RLZ ASD as compared to Rilutor™.

Considering the neuroprotective efficacy of the drug, cognitive function test was done with the ASD formulation at an equivalent dose of 5 mg/Kg and 10 mg/Kg of RLZ and was compared with its marketed formulation and the standard drug donepezil. Morris water maze test was done to evaluate the cognitive function of the scopolamine-induced memory impaired male wistar rats and it was found that the rats treated with the ASD formulation were showing shorted escape latency as compared to those which were treated with Rilutor™ at the same dose. After this study the plasma of the rats were evaluated to check the levels of ALT and AST as a marker of hepatotoxicity induced by RLZ. In this study, higher levels of liver enzymes were detected in rats administered with ASD formulation. This observation along with the pharmacodynamic test results can be used as a guide to decrease the dose of the drug for achieving similar therapeutic effect as of Rilutor™ but with decreased toxicity.

As a future prospective of this work, there are number of studies which could be done to gather deep insights. The effect of compression, high temperature and humidity effect can be checked to this developed ASD formulation. The scalability of this formulation can be checked utilizing methods like spray drying. However, in case of pharmacokinetic studies a lower dose study could be done since no data except for 10 mg/Kg dose of RLZ is available anywhere in the literature. The pharmacodynamic studies showed promising results at a lower dose than 10 mg/Kg. Hence, integrating the PBPK model, exposure estimation could be done by reverse dosimetry and hence exact dose which will have lesser toxicity and similar efficacy can be developed.