Lysosomal mediated apoptosis of Imatinib resistant leukemia cells by an herbal reactive oxygen species (ROS) nanoscavenger

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ABSTRACT SUMMARY

Chronic myeloid leukemia (CML) has been the epicentre of exciting advances in cancer therapy with the discovery of the Bcr-Abl gene fusion and the subsequent development of imatinib mesylate, a small molecule tyrosine kinase inhibitor, to target the kinase activity of the Bcr-Abl protein product. However, therapeutic selectivity and drug resistance are two major issues in imatinib based leukemia therapy prompting development of innovative strategies to overcome imatinib resistance and to eradicate CML. Growing evidence suggests that cancer cells exhibit increased intrinsic reactive oxygen species (ROS) mediated stress, due in part to oncogenic stimulation, increased metabolic activity, and mitochondrial malfunction. Since ROS activates Bcr-Abl kinase, we hypothesized that Bcr-Abl induced endogenous ROS may counteract the activity of imatinib and thus a natural herbal molecule having ROS scavenging property may therefore enhance imatinib-induced cell death in drug resistant cells. In the present study, we document an approach to simultaneously deliver two drugs (imatinib, a anticancer drug and curcumin a ROS scavenger) at target sites (i.e. Bcr-Abl oncoprotein) using poly (lactide-co-glycolide) (PLGA) nanoparticles. Varied in vitro studies indicate that co-treatment with curcumin and imatinib in nanoformulations significantly enhanced imatinib mediated cytotoxicity of Bcr-Abl+ resistant cells. Moreover, the mode of induction of death i.e. induction of lysosomal membrane permeabilization by our formulation was studied with a view to re-sensitize multidrug resistant (MDR) cells to classical chemotherapy.

INTRODUCTION

Chronic myeloid leukemia has been a model for the development of molecularly targeted therapies. Despite the unprecedented success of imatinib for CML, up to one third of patients with CML require alternate therapy due to development of either drug resistance or intolerance. Excessive production of ROS (and/or a deficiency in antioxidant pathways) can lead to oxidative stress, a state that has been observed in several hematopoietic malignancies including acute and chronic myeloid leukemias (AML and CML). Currently it is unclear what the cause of oxidative stress might be and whether oxidative stress contributes to the development, progression, or maintenance of these diseases. Persistent ROS stress may induce adaptive stress responses including activation of redox-sensitive transcription factors such as nuclear factor κB (NF-κB) and Nrf2. This redox adaptation generally enables the cancer cells to survive under increased ROS stress and also provides a mechanism of resistance to many anticancer agents. Thus, the call of the hour is to establish a direct relation between enhanced ROS and MDR in CML cell lines.

Lysosomes are intimately associated with apoptotic cell death and several anticancer drugs can trigger lysosomal membrane permeabilization (LMP) in different cell types. The aim of this study was also to analyze the complex antileukemic effect of imatinib in drug resistant CML cell lines regarding the induction of different cell death modes including lysosomal.

In this regard, the objectives of this studies was to formulate dual drug loaded PLGA nanoparticles loaded with two drug which will act independently of each other on different pathways (Schema 1) but in the long run may show synergistic action by enhancing the effect of each other thereby overcoming resistance in CML cell lines.

EXPERIMENTAL METHODS

Single and dual drug loaded NPs were prepared by oil-in-water single emulsion solvent evaporation method. The prepared NPs were characterized by measuring the size and zeta potential using Malvern zetasizer. Surface morphology and uniform size distribution were determined by doing SEM and AFM analysis. The encapsulation efficiency of these NPs was determined by using HPLC. The in vitro efficacy and the MDR inhibitory potential of these drugs loaded NPs was evaluated in two different CML cell lines i.e. K562 (sensitive) and K562R (resistant) counterpart by MTT assay and RT PCR. Moreover, the therapeutic efficacy of dual drug loaded nano formulations was evaluated by studying lysosomal membrane potential using lysotracker.
RESULTS AND DISCUSSION

To first confirm the rational of the study that the drug resistant leukemia cells experience more enhanced ROS stress in comparison to their wild type counterpart we studied the ROS expression in both cell lines by confocal microscope and found that resistant cells have more ROS production (Figure 1a) and as a result multidrug resistance and Bcr-Abl is more up regulated in the resistant model CML cell line (Figure 1b).

After confirmation of rationale, drug loaded nanoparticles were formulated and physicochemical characterization of the formulated NPs showed that they were of in nanometer range with smooth and spherical surface as confirmed by microscopic analysis (Figure 2a and b). Both the drugs were efficiently loaded in the particles and the entrapment efficiency of both curcumin and imatinib was more than 70 % (as confirmed by RP-HPLC).

In vitro cytotoxicity results showed that dual drug loaded formulations were not only effective in wild type CML cell line but also in drug resistant counterpart in the long run due to sustain release phenomena(Figure 3 a and b).

RT-PCR results depicted that the dual formulation were capable of overcoming not only drug resistance in imatinib resistant cell line but also acted upon Bcr-Abl thereby increasing the therapeutic efficacy of both the drugs at a lower concentration (Figure 4).

The reason for increased cytotoxicity of dual formulation may be due to the higher accumulation of both the drugs in the cells leading to further toxicity.

Lysosomal permeabilization study using lystotracker (a specific dye for tracking lysosomes) confirmed that combination of two drugs in nanoformulations were able to induce apoptosis in more proportion as compared to single alone either in solution or in nanoformulation (Figure 5).

CONCLUSION

Our study demonstrates that use of ROS modulator in combination with an anticancer drug increases the therapeutic efficacy of the drug in drug resistant cancer cell line. Inclusion of curcumin along with imatinib in PLGA nanoparticles resulted in significantly enhanced cellular accumulation of both the drugs where the former acts upon the resistance pumps while the later acted upon Bcr-Abl gene synergistically showing better efficacy and greater cytotoxicity in drug-resistant tumor cells. Molecular analysis of the modality of death induced by both the drugs may give a better idea regarding the way in which such a modality re-sensitize multidrug resistant cells to classical chemotherapy.

REFERENCES


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