ICMR-International Fellowship for Young Biomedical Scientists-2013

Report

1. Name and designation of ICMR-IF : Dr. N. Rajendra Prasad
   Assistant Professor
2. Address : Department of Biochemistry and Biotechnology
   Annamalai University
   Annamalainagar – 608 002. Tamilnadu.
3. Frontline area of research in which Research was carried out : P-glycoprotein and Cancer Multidrug Resistance
4. Name and address of Professor and Host Institute : Dr. Suresh V. Ambudkar
   Senior Investigator
   Chief, Transport Biochemistry Section,
   Deputy Chief, Laboratory of Cell Biology, CCR,
   NCI, NIH
   Building 37, Convent Drive,
   Bethesda, MD 20892-4256.
5. Duration of Fellowship : Six months (11 February 2013 – 10 August 2013)
6. Highlights of work conducted :

i. Techniques/Expertise acquired:

Multidrug resistance (MDR), caused by multidrug transporter P-glycoprotein (P-gp) that extrudes anticancer drugs out of the cancer cells, is a major cause of failure of cancer chemotherapy. Previously, selenazole containing cyclic peptides were reported as P-gp inhibitors and these were also used for co-crystallization with mouse P-gp, which has 87% homology to human P-gp. In this study, varying lengths of linear and cyclic derivatives of (S)-valine-derived thiazole derivatives were investigated to identify optimal structural requirements for potent P-gp inhibition, previously an unexplored concept. Major highlights of the present findings are gieven below.

i. The ability of QZ derivatives to inhibit the transport function of P-gp was analyzed using flow cytometry. BacMam-P-gp baculovirus transduced HeLa cells were incubated with various concentrations of QZ derivatives and calcein-AM (0.5 μM) for 10 min. The % inhibition of calcein-AM

efflux in the presence of QZ derivatives was calculated by using flow cytometer and Cell Quest program.

ii. Crude membranes from P-gp expressing High-Five insect cells were photolabeled with [^{125}I]-IAAP in the presence and absence of QZ-59S-SSS derivatives. Incorporation of IAAP into P-gp band was determined using phosphorimager and % inhibition of IAAP incorporation was determined. Present results indicate that QZ derivatives compete with IAAP for drug binding site. This indicates that QZ derivatives irrespective of their size interact with P-gp at drug binding site.

iii. To understand the effect of QZ-derivatives on ATPase activity of P-gp, crude membranes from P-gp expressing High-Five cells were incubated with 2.5 μM QZ derivatives in the presence and absence of 0.3 mM sodium orthovanadate and the vanadate-sensitive ATPase activity of P-gp was determined. Interestingly, most of the the QZ derivatives stimulated the ATPase activity 2 to 3-fold when compared to basal activity. This further strengthens the interaction of QZ derivatives with this multidrug transporter.

iv. The present study clearly indicates although all the QZ derivatives interact with P-gp, those molecules having molecular weight between 530 to 650-Da like dimer and trimer derivatives show greater inhibition of transport function. These compounds might form a greater hydrophobic and aromatic interaction at the drug-binding pocket in P-gp. We further found that both linear and cyclic hexamers interact with P-gp but unable to inhibit the activity of this protein even though the size of these hexamers is much similar to cyclosporine-A, a known P-gp inhibitor. Transport function assays with other P-gp fluorescent substrates rhodamine-123, and Bodipy-prazosin also confirmed our present findings.

v. Development of fourth generation P-gp inhibitors by insertion of privileged chemical fragments from the reported potent analogues into chemically modified natural products seem to be a novel approach for high P-gp selectivity and potency. Analysis of the homology modeled

human P-gp bound to QZ59S resulted in the design of a series of (S)-valine-derived bis-thiazole acid, bis-thiazole amine, mono-thiazole acid, mono-thiazole amine and substitutions at both ends of mono—thiazole zwitter ion with various privileged scaffolds. Among 23 analogues studied, four derivatives comprised of the bis-thiazole acid coupled with methoxy substituted aryl/arylakylamines showed greater than 90% inhibition of P-gp at 10 μM. Moreover, five other compounds belonging to mono-thiazole acid coupled with arylamines, showed P-gp inhibition ranging from 54 to 72%. Furthermore, studies of binding interactions of these fourth generation inhibitors within the large drug binding cavity of P-gp will pave the scope for future therapeutic drug development.

ii) **Research results, including any papers prepared/submitted for publication**

Two research manuscripts are being prepared based on the above results for submission to ChemBioChem and Medicinal Chemistry Research journals.

1. Satyakam Singh, Nagarajan Rajendra Prasad, Khyati Kapoor, Eduardo E. Chufan, Suresh V. Ambudkar, Tanaji T. Talele. Design, synthesis and biological evaluation of (S)-valine thiazole-derived cyclic and non-cyclic peptidomimetic oligomers as inhibitors of human P-glycoprotein (ABCB1) (manuscript under preparation).

2. Satyakam Singh, Nagarajan Rajendra Prasad, Bhargav A. Patel, Suresh V. Ambudkar, Tanaji T. Talele. Synthesis and biological evaluation of fourth generation P-glycoprotein inhibitors by peptide coupling of privileged chemical scaffolds carboxyl and amino termini of (S)-valine-derived bis-thiazole acid (manuscript under preparation).

iii) **Proposed utilization of the experience in India**

Multidrug resistance in cancer caused by the overexpression of ABC drug transporters is a major obstacle in modern chemotherapy. Even though many ABC-modulators are available, finding a selective, low toxicity inhibitor/ modulator of ABC drug transporters still appears to be the most likely way to resolve this problem. As a result of some unfavorable clinical outcomes from the first three generations of inhibitors, we are now at

a stage of searching for non-toxic inhibitors with alternative and novel scaffolds from natural sources. The progress of discovering Fourth Generation/ Natural Product Inhibitors is still in the early stages of exploring various extracts/ active components ranging from plants, fungi to marine organisms. Many more herbal extracts or traditional Indian medicinal plants have great potential to be developed into potent chemosensitizers, since they appear to be non-toxic and biologically active. Systematic high-throughput screening of traditional Indian natural products and their semi-synthetic derivatives should be the first step in the discovery of non-toxic, potent and selective inhibitors. QSAR studies and manipulations utilizing combinatorial chemistry must then follow if we are to see any success in using modulators to overcome ABC drug transporter-associated multidrug resistance in clinical settings. The training acquired under ICMR-International Fellowship at National Cancer Institute on the systematic study of QZ59S-SSS derivatives for P-glycoprotein inhibition using different experimental models viz., BacMam baculovirus-HeLa cell expression system, High-Five Insect cell membranes and study on human cancer drug resistant cell lines will be utilized to identify potential ABC-modulator from Indian natural sources. The development of non-toxic fourth generation modulators will also be helpful to increase bioavailability blood—brain penetration of orally given drugs.

REPORT OF HOST INSTITUTE

1. Name of Professor/Investigator under whom training was carried out:

Dr. Suresh V. Ambudkar
Senior Investigator
Chief, Transport Biochemistry Section,
Deputy Chief, Laboratory of Cell Biology

2. Name and address of host institute: Laboratory of Cell Biology, CCR,
National Cancer Insitute, NIH
Building 37, Convent Drive,
Bethesda, MD 20892-4256.

3. Duration of Fellowship :Six months (11 Feb. 2013 to 10 Aug. 2013)

4. Brief highlights of the ICMR-IF achievements:

P-glycoprotein has been a long sought target for drug development in the hope of circumventing MDR in clinical oncology. More than three decades of biochemical studies have indicated that P-gp might possess several distinct substrate-binding sites within a large, flexible region located between the two transmembrane domains; these sites recognize hundreds of chemically unrelated compounds, including traditional anticancer drugs. Our collaborator, Dr. Tanaji T. Talele (St. Johns University) has developed homology modeled human P-gp based on mouse P-gp structure and synthesized number of QZ59S-SSS derivatives. Dr. N. Rajendra Prasad, ICMR-IF investigated these QZ derivatives for their membrane transport function inhibition using BacMam transduced P-gp overexpressing HeLa cells He also investigated the interaction of these QZ derivatives on drug binding site in P-gp using High-Five insect cell membranes. The following findings were observed by Dr. Rajendra Prasad while on the ICMR-IF.

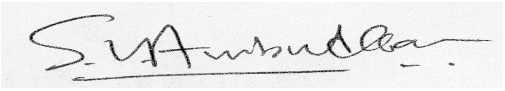
1. QZ-59S-SSS is a P-gp inhibitor and P-gp can accommodate 2 molecules of QZ-59S-SSS molecules at drug binding site. The present study in vinblastine selected KB-V-1 and its parent cell line KB-3-1 indicates that human P-gp does not confer resistance to QZ59S-SSS, suggesting that it may not be a transport substrate.
2. In this present study, various QZ derivatives differing in size, from monomer to hexamer, were studied for their P-gp transport inhibition function. Although, most of the QZ derivatives investigated in this study interact with the binding site of P-gp only dimer and trimer derivatives show maximum inhibitory activity. The present results revealed that size of the QZ59S-SSS derivatives plays major role in P-gp inhibition. The more potent QZ-derivatives identified in this study might be useful for co-crystallization of human P-gp.
3. The ICMR-IF also investigated the P-gp inhibitory potential of newly synthesized thiazole structures that contains natural products core and active scaffolds from the synthetic compounds. Four derivatives were identified as potent P-gp inhibitors. This present findings may lead to generation of novel fourth generation P-gp inhibitors.

5. Your assessment of the ICMR-IF

Dr. N. Rajendra Prasad during six months in the lab acquired training in cell biology, biochemical and pharmacological aspects of MDR-linked ABC transporters. He became proficient in use of baculovirus based mammalian and insect cell transient expression system, use of flow cytometry for detection of cell surface proteins with antibody and to assay drug transport function of ABC transporters. In addition, he gained experience in biochemical assays including photolabeling of P-gp and assay of ATP hydrolysis by P-gp and ABCG2. Dr. Rajendra Prasad will be able to establish these techniques in his department in India and use them for not only basic but also translational studies of resistance to chemotherapy in cancer patients. Dr. Rajendra Prasad during his stay our laboratory learned firsthand about how to provide training to post-doctoral fellows, graduate and under graduate students to foster their independence and creativity. Dr. Rajendra Prasad actively participated in our weekly data club meetings and presented his work at these meetings regularly. He also gave a formal seminar on his work at the MDR group meeting in LCB. Dr. Rajendra Prasad was sincere, willing to learn all techniques, enthusiastic about his work and his productivity is evident from being a co-author on two publications in such a short (six months) duration.

6. Any other comments about ICMR-International Fellowship Program

The ICMR-IF program provided an excellent opportunity to a mid-career young scientist, Dr. N. Rajendra Prasad to not only learn new and cutting edge research techniques but also to firsthand witness training of young researchers. The ICMR-IF program allowed Dr. N. Rajendra Prasad to concentrate full time on bench research without having any teaching duty. I am very confident that the time he spent in our laboratory has helped him to formulate concrete research proposals to carry out research work in the area of ABC transporters and chemo-resistance in cancer patients. Based on my experience with Dr. N. Rajendra Prasad, I will be very happy to mentor another ICMR-international fellow in near future. The ICMR may want to consider providing one to two year break from teaching to ICMR-IF when they return to their home institution/university in India. This break from teaching will allow faculty members to establish techniques/research areas in their home institution without any delay.



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