CHAPTER 4

BIOCHEMISTRY

Doctoral Theses

01. ARYA (Richa)
Biochemical and Biophysical Characterization of Some Key Proteins of Fatty Acid Metabolism Pathway of Leishmania Major.
Supervisor: Prof. Suman Kundu
Th 22814

Abstract
(Verified)

Leishmania major encodes a type II fatty acid biosynthetic pathway for which limited information exists. As a first step towards understanding the pathway in Leishmania, we characterized the central players of the pathway, acyl carrier protein (LmACP) and phosphopantetheinyl transferase (LmPPT). Using the solution structure of LmACP, we established that LmACP is a type II ACP but surprisingly its cognate PPT (LmPPT) displayed no enzymatic activity towards it. However, LmPPT readily converted other type I and II ACPs into their holo-forms. E. coli AcpS (group I PPT) did not convert LmACP to its holo-product while N35D/F44M LmACP (similar to E. coli ACP) was converted to holo-form by AcpS. Sfp (a prototype group II PPT) displayed slow catalysis towards wt LmACP as compared to other type II ACPs but its catalysis rate enhanced in case of N35D LmACP. Mutagenesis studies underscored the importance of the residues present at the interaction interface of LmACP in modulating the activity of PPTs. All the acyl-CoAs synthesized in the parasite or scavenged from the host are ferried by acyl-CoA binding proteins (ACBPs). Leishmania encodes six ACBPs, of which three are free standing, while other three are part of long polypeptides. $ACBP_{89}$, with one ACBP domain, was found to be constitutively expressed and localized in the mitochondria and nucleus. It binds long chain-acyl-CoAs, with highest affinity towards C_{14}-CoA, which is required for GPI linker biosynthesis. ITC and NMR titration studies suggested relatively weaker binding between the two as compared to the already reported ACBP-acyl-CoA interactions, which might be a reason for the rapid release of the ligand at the site of action by ACBP. To combat leishmaniasis, we screened 330 small molecules against LD promastigotes and the best hits were tested against the in vitro activity of LmPPT to identify anti-leishmanial small molecule inhibitors.

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02. CHANDRA (Sunandini)
Regulation of Membrane Fusion Mediated Entry of Sendai Virus by Host Cell Signaling.
Supervisor: Prof. Debi P. Sarkar
Th 22818

Abstract
(Verified)

Reconstituted Sendai viral envelopes (Virosomes) have shown substantial potential for membrane fusion mediated targeted cytosolic delivery of bioactive molecules in liver cells. Membrane fusion is thought to be a highly regulated process involving cellular machinery in addition to viral factors, but mechanisms of this process has remained elusive. Here, we used proteomics tools involving two-dimensional DIGE (Differential In-Gel Electrophoresis) to analyze the early response of HepG2 to virosome-induced membrane fusion. Quantitative mass spectrometry and biochemical analysis revealed that villin, an actin-modifying protein, is upregulated and threonine-phosphorylated and enriched in insoluble fractions as a result of membrane fusion. In contrast, fusion of virosomes with CHO cells co-expressing villin and c-src leads to villin phosphorylation at tyrosine. We found that phosphorylated villin interacts with actin and influences its dynamics which, in turn, promotes membrane mixing mediated by Sendai viral glycoproteins in both cell types. Modulating villin in host cells perturbed the progression of membrane merging, and in consequence also led to a discernible effect on the budding of Sendai viral progeny. This study has long term implications, both in elucidating novel mechanism of viral entry and identifying potential targets for antiviral therapy.

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03. DEY (Sanjay Kumar)
Characterization of Dopamine Beta Hydroxylase and Cytochrome B5 Reductase3 and their Interactions with Small Molecule Inhibitors Identified by Structure-based Methods and their Validation in Animal Models of Hypertension and Cardiac Hypertrophy .
Supervisor: Prof. Suman Kundu
Th 23192

Abstract
(Not Verified)

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide. Hypertension (HTN) and cardiac hypertrophy (CHT) are two of the most important risk factors associated with CVDs with many fatal consequences. To counter HTN and CHT, a wide variety of anti-hypertensive drugs are available. Nevertheless, there have been no new classes of drugs in the last several years which can utilize body's own blood pressure (BP) lowering mechanism thus probably causing fewer side effects with desired BP. Human dopamine β-hydroxylase (hDBH), expressed in noradrenergic nerve terminals as well as in adrenal medullary chromaffin cells, converts dopamine into norepinephrine and controls BP significantly. Inhibition of DBH has been implicated for the treatment of HTN and CHT. However, only a relatively few mechanism based DBH-inhibitors with moderate efficacies are available till date. DBH was thus purified and characterized biochemically and biophysically. Small molecule libraries were then screened using structure-based-rational drug design methods against DBH three-dimensional model built in our lab and lead inhibitors against DBH were identified and validated in vitro, ex vivo and in vivo. Few of the lead compounds identified were found to be anti-hypertensive and anti-cardiac hypertrophic in L-NAME and isoproterenol-induced animal models, respectively. Cytochrome-b5-reductase3 (CYB5R3) was investigated as another non-conventional target to combat HTN. Inhibition of CYB5R3 activity may keep the NO signaling "ON" in the myo-endothelial junctions to cause vasodilation in smooth muscle cells thus lowering BP. Unfortunately, very few inhibitors are reported against CYB5R3 probably due to lack of knowledge about its anti-hypertensive role earlier. Small molecule inhibitors against CYB5R3 were identified along similar lines, validated and characterized in vitro and ex vivo. One of the inhibitors was also co-crystallized with CYB5R3 and the structure solved. Few inhibitors were identified for screening in animal models in the near future.

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1. Introduction, review of literature, rationale of the study, hypothesis, aims and objectives 2. Biochemical, biophysical and structural characterization of dopamine β hydroxylase and cytochrome B5 reductase3 3. Structure-based identification of small molecule inhibitors in silico and their validation in vitro 4. Characterization of interaction of lead molecules with DBH and CYB5R3 5. Evaluation of lead inhibitors of DBH in animal models of hypertension and cardiac hypertrophy. Summary. Future perspectives. Appendix. Biography.

04. JHA (Deepa)
Proteome Analysis During Blood Stages of Plasmodium Species After Treatment with Various Anti-Malarials.
Supervisors: Prof. P. C. Ghosh and Dr. T. Adak
Th 22815

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1. Introduction 2. Review of literature 3. Aims and objectives 4. Differential proteome analysis of plasmodium falciparum and plasmodium berghei upon treatment with free monensin and liposomal monensin 5. Differential proteome analysis of plasmodium falciparum and plasmodium berghei upon treatment with artemisinin 6. Differential proteome analysis of plasmodium falciparum and plasmodium berghei upon treatment with combination artemisinin and monensin 7. Differential protein profile of P. berghei infected mice plasma and liver proteins upon treatment with artemisinin and monensin 8. Summary and conclusion 9. Appendix.

05. KANU (Megha)
Study on Neurotransmitters and Expression of Regulating Enzymes in Rat Brain Following Radiofrequency Electromagnetic Radiation (RF-EMR) Exposure.
Supervisors: Prof. A. K. Tripathi and Prof. B. D. Banerjee
Th 22817

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1. Introduction 2. Aim and objectives 3. Review of literature 4. Materials and methods 5. Results 6. Discussion 7. Summary and conclusion 8. References. Appendix. List of publications.

06. KUMARI ASHA
Studies on Emerging Inflammatory Biomarkers and Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphism in Patients of Psoriasis with and without Conventional Cardiovascular Risk Factors.
Supervisors: Prof. Suman Bala Sharma, Prof. Archana Singal, Prof. Vinod Kumar Arora and Dr. Amitesh Aggarwal
Th 22816

Abstract
(Not Verified)

Psoriasis is a chronic immune-mediated inflammatory disease (IMID) of skin. The etiology of the disease is related to combination of environmental, behavioral, biochemical, immunological and genetic factors. Psoriatic patients have also been found to be at increased risk of atherosclerosis. This association may occur due to various conventional and non-conventional risk factors. Thus in the present study, patients of psoriasis without (Group B) and with known CVD risk factors (Group C) were evaluated for various

biochemical, oxidative, inflammatory and genetic parameters. The incidence of dyslipidemia was significantly higher in psoriasis patients when compared to healthy controls. Atherogenic factors (ox-LDL, Apo B/Apo A1, Homocysteine) were associated with increased CIMT in psoriasis patients. Serum leptin and OPN levels were significantly higher ($p < 0.05$) in patients of Group C when compared to Group B. In lesional psoriatic skin, moderate to intense OPN expression was observed in the keratinocytes distributed throughout the epidermis. OPN was also expressed in the inflammatory cells and microvasculature endothelial cells of the dermis, in patients. In the present study, MTHFR C677T gene polymorphism was studied. A significant difference ($p < 0.001$) in homocysteine levels of CC genotype and (CT+TT) mutant variants was observed in each study group. A significant difference in allelic distribution of T among psoriasis patients and healthy controls (odds ratio: 2.55 and $p = 0.004$) was observed. The presence of T allele was significantly associated to hyperhomocysteinemia regardless of presence or absence of conventional cardiovascular risk factors. These findings conclude that psoriasis is associated with skin injury and production of reactive oxygen species (ROS) which produce ox-LDL that leads to atherosclerosis. Conventional CVD risk factors predispose severe psoriasis patients to increased CIMT. These shared pathophysiological conditions between psoriasis and atherosclerosis may explain the progression of CVD in psoriasis patients.

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07. SHARMA (Anju)
Study of Hormonal Profile and Role of MAMLD1 (CXorf6) Gene Polymorphism in Children with Isolated Hypospadias.
Supervisors: Prof. Dr. T. K. Mishra, Dr. Simmi K. Ratan and Dr. Seema Kapoor
Th 22819

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08. SHARMA (Mohini)
Study of NRF2 and NQO1 Gene Polymorphisms and Expression Profile in Diabetic Patients with and without Chronic Kidney Disease.
Supervisors: Dr. Rimi Shukla, Dr. J K Gambhir and Dr. O P Kalra
Th 22820

Abstract
(Not Verified)

Aim: Diabetic nephropathy (DN) is a leading cause of chronic kidney disease worldwide. It is corroborated that hyperglycemia-induced oxidative stress (OS) is a major factor in the development of DN. Nuclear factor erythroid 2 Related Factor 2 (Nrf2) and NAD(P)H quinone oxidoreductase 1 (NQO1) play an important role in pathogenesis of DN. Therefore, this study aimed to find the emerging role of NRF2 and NQO1 gene polymorphisms and expression profile in patients of type 2 diabetes mellitus with and without nephropathy in Indian population. Methods: Subjects were recruited into three groups (200 each) i.e. healthy controls (HC), T2DM without complications (T2DM) and DN. Nrf2 and NQO1 levels were estimated using ELISA. FRAP, GST, MDA and reduced GSH were measured spectrophotometrically. NRF2 and NQO1 gene polymorphisms were analyzed by DNA sequencing and PCR-RFLP respectively. Real Time-PCR was done for the expression profile of NRF2 & NQO1. Results: Nrf2, NQO1 and GST levels were significantly increased in patient groups however these were highest in T2DM group followed by DN and

HC. GSH and FRAP were significantly lowest in DN whereas highest MDA levels were seen in DN as compared to T2DM and HC ($p<0.05$). Nrf2 and NQO1 showed significant positive correlation with GST, GSH and FRAP although negatively correlated with MDA in all study groups. NRF2 -617A allele and NQO1 609T allele are associated with decreased Nrf2, NQO1, GST, GSH and FRAP levels whereas increased MDA levels in all study groups suggesting higher risk towards the development of DN ($p<0.05$). Expression of NRF2 and NQO1 was significantly lowest in individuals carrying A and T allele of the selected NRF2 and NQO1 gene polymorphisms respectively ($p<0.05$). Conclusion: The functional polymorphisms of NRF2 and NQO1 genes have significant diminished effect on Nrf2 and NQO1 expression which may increase susceptibility to DN.

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09. SHARMA (Tusha)
Gene Environment Interaction and Risk of Epithelial Ovarian Cancer.
Supervisors: Prof. B. D. Banerjee, Prof. A. K. Tripathi, Prof. Rafat S. Ahmed, Prof. Kiran Guleria and Prof. V. K. Arora
Th 22821

Abstract
(Not Verified)

Environmental pollutants are increasing at an exponential rate and many of them pose a potential health risk. Pesticides exposure has been found to be associated with a range of human health problems like immune suppression, hormone disruption, adverse reproductive outcomes, neurological disorder, diminished intelligence, cancer etc. Epithelial ovarian cancer (EOC), is one of largest cause of women morbidity and mortality due to cancer. Risk factors known to be associated with EOC are early menarche and late menopause, hormonal imbalance, genetic predisposition, nulliparity, age, oxidative stress etc, which may antedate the pathogenesis of EOC. However, the etiology of idiopathic EOC remains poorly understood because of multiple factors governing EOC. In the present study, significant higher level of OCPs like β-HCH, endosulfan-I, endosulfan-II, p'p'-DDT, p'p'-DDE and heptachlor were found in EOC cases as compared to controls which confers that there is an association of high OCP levels with the disease etiology. While comparing the genotypic variations of gene among cases and controls, it has been observed that the null deletion of GSTM1/GSTT1, heterozygous allele of IL-6 -174G/C, heterozygous allele of TNF-α -1031T/C, heterozygous and homozygous mutant of p53 72 codon arg/pro were significantly higher in EOC cases compared with the controls. mRNA expression of CYP1A1, IL-6, TNF-α, NFκB, COX-2, p53 and BRCA-1 gene were higher in EOC cases while the mRNA expression of GSTM1/T1 genes were 9.38 and 7.83 folds lower in EOC cases. The extent of DNA damage was found to be significantly higher among cases as compared to the controls. Moreover, a positive correlation was observed between olive tail moment and high level of β-HCH and heptachlor. The thesis concluded that there is significant role of gene-environment interaction in the etiology of EOC.

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10. VINOTH RAJENDRAN
Liposome Mediated Delivery of Anti-Malarial Drugs for the Treatment of Malaria.
Supervisor: Prof. Prahlad C. Gosh
Th 22822

Abstract
(Not Verified)

Emergence of drug resistant Plasmodium falciparum malaria parasite is impeding the therapeutic efficacy of existing antimalarial drugs in clinical use. Therefore, there is an urgent need to develop an efficient drug delivery system to circumvent drug resistance and improve the efficacy of existing antimalarials. The anticoccidial drug monensin has been shown to exhibit antimalarial properties. Here, we developed a liposome-based drug delivery of monensin and evaluated its antimalarial activity in different lipid formulations against blood stages of P. falciparum (3D7) in culture and established P. berghei NK-65 and ANKA infection in murine model. The developed liposomal formulations were found to exhibit superior efficacy than a comparable dose of free monensin. The enhancement of antimalarial activity was dependent on the liposomal lipid composition and preferential intracellular uptake by infected red blood cells (RBCs). The enhanced antiplasmodial activity of monensin was observed in stearylamine (SA) liposome and SPC:Chol-liposome exhibited markedly superior efficacy to that of free drug under in vitro and in vivo condition with minimal toxicity to erythrocytes. In addition, we assessed the combinatorial effects of monensin in liposomes in combination with various potent antimalarial drugs like chloroquine, FR900098, or piperaquine in free form against both chloroquine sensitive and resistant strains of P. falciparum in culture and rodent strains in murine model. In another study, chloroquine entrapped in long circulating liposome formulations containing 5mol% (DSPE-mPEG-2000) on liposomal surface markedly reduced the parasite load with improved survival in chloroquine resistant P. berghei infection. Therefore, Polyethylene glycol (DSPE-mPEG-2000) coated liposomes with 5mol% density on liposomal surface containing either monensin or chloroquine showed enhanced clearance of parasite load in blood circulation with improved survival in mice relative to the free drug. This study clearly demonstrates that delivery of antimalarial drugs in PEGylated liposomal formulations has remarkable chemotherapeutic potential against human malaria infections.

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