Dabur Research Foundation (DRF)

- Indian Contract Research Organization focused on Preclinical drug discovery & Development
- Led the R & D programs of one of the largest Indian Healthcare groups (1979 - 2008)
- Strategic spin off of the parent group in year 2008 to become a Contract Research Organization in the niche area of Preclinical development
- Positioned as a Biology specialist CRO with services in several therapeutic areas viz Oncology, Inflammation, Metabolic diseases & others
- Strength of 80 scientists with close to 40% being Ph.Ds recruited from the top 5 Universities of India
- Contract services for end to end preclinical development of Cytotoxics, Botanicals, Phytochemicals, generics & differentiated formulations in multiple therapeutic areas
- GLP compliant and non GLP studies
- Located near New Delhi, well connected to the International Airport
The DRF Advantage

- Over 20 years of experience in preclinical development of Cytotoxics, biologically targeted molecules, Phytochemicals, generics & differentiated formulations in multiple therapeutic areas
- No conflict of interest with client projects. No internal R&D programs
- Comprehensive Services in Cell Biology, Pharmacology, Toxicology, DMPK, Bioanalytical, Analytical & formulation development to enable lead identification as well as lead development
- Availability of stand alone service modules & complete service packages to meet varied client requirements
- Availability of guideline driven services as well services customized for the clients
- A dedicated Central Innovation Research Team for customized model development
- GLP compliant studies managed by Project coordinators, Technical Coordinators & QAU teams
- Experience & knowledge of regulatory requirements for submission of data packages
- Technical consulting for development of road map for client pipeline
Established in 1884, Dabur India Ltd is among the oldest and largest healthcare company in India.

Has over 5000 employees working in more than 20 countries.

Market Cap of over 4 bn USD, DIL recently achieved sales of 1 bn USD.

More than 600 herbal products in market.

17 ultra-modern manufacturing units spread around the globe.

Products marketed in over 60 countries.

More than 5000 distributors and over 2.8 million retail outlets all over India.
Management Team

CHAIRMAN - Dr. Burman holds a Ph.D in Pharmaceutical Chemistry from the University of Kansas. Dr. Burman set up the pharmaceutical division for Dabur India in 1989 and he is a prominent and respected figure within the international oncology industry.


Vice President (R&D) – Dr. Anu T. Singh Post Graduate in Biotechnology with Doctorate in Cancer Biology. More than 22 years of research experience in drug discovery, Cell Biology & early preclinical development of NCEs & Botanicals. Expertise in Cell Biology, target identification and development of disease models in Oncology & allied areas

Advisor Dr. Ashok Mukherjee has more than 35 years of experience in human and animal pathology. Ex-Director, of Institute of Pathology (ICMR), Dr. Mukherjee has carried out extensive research in communicable diseases. He has been associated with DRF since 1999.

Director, Business Development (US) Dr. William Heilman has more than 30 years of experience in business development. He has previously worked in large pharma / biotech companies in the US including Wyeth, Comgenex, AMRI & Morphotek. Dr. Heilman has done is Ph.D in Medicinal Chemistry from University of Kansas.
The Story continues…
Scientific Personnel

Experience from 1 to 19 years

- Pharmacists
- Life Science graduates
- Biochemists
- Biotechnologists
- Toxicologists
- Pharmacologists

*Integrated Research Solutions in Preclinical Biology*
Research Laboratories
FACILITY DETAILS

- Facility - Spread on a single floor
- Area - Approx. 30,000 sq/ft
- Labs - *in vitro* / *in vivo* Pharmacology, Cell Biology, Toxicology, DMPK lab, Small Animal Facility (SAF), Tissue Culture Facility (TCF)
- Water - Sufficient with separate lines of Normal and DM Water, Milli Q Water, Purification System.
- Temperature controls - Area controlled by AHUs/ACs
- Temperature/Humidity data loggers in each labs
- Safety - Safety devices at each location.
  - Fire Extinguishers, Fire Alarms
  - First Aid
- CCTV Surveillance in all labs
- Access Control System in all labs
- Fire Proof Archival Room for Wet Archives
- Fire Proof Archival Room for Dry Archives
- Provision for Ante-rooms wherever necessary
**Infrastructure**

**TOXICOLOGY LAB**

- Hematology Analyzer
- Biochemistry Analyzer
- Manual Rotary Microtome
- Tissue Embedder (Paraffin Dispenser)
- + Water bath + Hotplate
- Water Warming Table Digital
- Experienced Study Director (6 to 17 years)
- Expert Pathologist (> 30 years experience)

**PHARMACOLOGY LAB**

- CNS pharmacology
- Pressure Application measurement (PAM)
- Von Frey
- Small animal anesthesia system
- RA-50 Chemistry system
- Inverted and Phase contrast microscope
- Homogenizer
- OHM meter, Histamine chamber
- Digital Plethysmometer
- Digital Caliper
- Organ bath
- Hot and cold plate method
- Rectal thermometer
- Refrigerated centrifuge
CELL BIOLOGY LAB

Equipments:
- Flow cytometer (Guava Technologies)
- Multiwell multimode reader (Biotek)
- Fluorescence microscope (Nikon)
- Phase contrast microscopes (Nikon)
- Dissecting microscope (Motic)
- Spectrophotometer (Shimazdu)
- CO2 incubators
- Laminar hood cabinets
- Liquid nitrogen cans
- UV irradiation chamber
- Centrifuges, water bath, shakers
- Analytical Balance
- Micropipettes, Dispensettes
- Vortexer mixers, homogenizer
- Fridges, Freezer, Deep Freezer.

DMPK LAB

Equipments:
- Analytical HPLCs
- Preparative HPLCs
- LC/MS-MS
- Nitrogen and Vacuum Evaporator
- SPE Vacuum Manifold, Temp. & humidity data loggers
- Analytical Balance
- Dispensette/ Micropipettes
- Centrifuge/Homogenizer/Sonicators
- Water Bath with Shaker
- Fridges, Freezer,(-20°C) Deep Freezer(-80°C)
- Lyophilizer
- Buchi Rotavapour
- Franz diffusion cell system
- Nitrogen generator
**Infrastructure**

**TISSUE CULTURE FACILITY**

**Equipments:**
- Laminar air Flow
- CO2 incubators
- Biosafety cabinet
- Refrigerated centrifuge
- Fridges and Deep freezers
- Liquid nitrogen container
- Inverted & Phase contrast Microscope

**ANIMAL FACILITY**

**Equipments:**
- Isolators, Data loggers
- Small Animal Anesthesia System
- Individually Ventilated Caging System (Techniplast, Italy/Citizen Industries, India)
- Animal Cage Changing Station (Citizen Industries, India)
- Horizontal Autoclave (P. L. Tandon & Co., India)
- Lux Meter (HTC, China)
- Sound Level Meter (CENTER, Taiwan)
- Laminar Flow Hood (Klenzaids, India)
- Freezer (Vest Frost)
- Water Purifier (Eureka Forbes)
- Digital Hygro-thermometer (MEXTECH)

**Facilities:** Small laboratory animals breeding and experimental facility


53. “Efficiency and Mechanism of Intracellular Paclitaxel Delivery by Novel Nanopolymer based Tumor Targeted Delivery System, Nanoxeltm, Hrishikesh K, et.al, Clinical and Translational Oncology,
Regulatory Guidelines Followed

- **OECD**: Organization for Economic Co-operation and Development
- **Schedule Y**: Drugs and Cosmetic Act 1947, Government of India
- **EU**: European Union
- **EPA**: Environmental Protection Agency
- **ICH**: International Conference on Harmonization
Registered under the Indian Companies Act, 1956

Registered by CPCSEA, Govt. of India

IAEC-Institutional Animal Ethics Committee-
Animal studies are approved as per CPCSEA norms

SAC – Scientific Advisory Committee

Recognition by two premier Indian Universities for Ph.D program

Funding from DST, DBT
<table>
<thead>
<tr>
<th>Regulatory Experience</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FDA, US</strong></td>
<td>Pre IND &amp; IND experience</td>
</tr>
<tr>
<td><strong>MHRA, UK</strong></td>
<td>F2F meetings seeking CT approval</td>
</tr>
<tr>
<td><strong>BfArM, Germany</strong></td>
<td>Received preclinical go-ahead for an anticancer drug</td>
</tr>
<tr>
<td><strong>DCGI, India</strong></td>
<td>Participated in several meetings / discussions For preclinical &amp; clinical development plans</td>
</tr>
<tr>
<td><strong>SFDA, China</strong></td>
<td>Customization of preclinical studies as per SFDA requirements</td>
</tr>
<tr>
<td><strong>Swedish &amp; Dutch</strong></td>
<td>Compliance of preclinical requirements</td>
</tr>
</tbody>
</table>
Our Focus Areas

**Drug Discovery & Preclinical**
- Drug Discovery
  - Biochemical & Cell based screens
  - Target based screens
  - Signal transduction
  - Molecular modeling
  - "in silico"
  - Computational designing
- Early Preclinical
  - Efficacy
    - Oncology
    - Diabetes
    - Pain
    - Inflammation
    - Dermatology
    - Hair
  - Pharmacokinetics
- Advanced Preclinical
  - Toxicology
  - Special Toxicity
  - Bioanalytical
  - Safety Pharmacology

**CRAM**
- API Synthesis & Form. Dev
- Drug Manufacturing
- Process Development
- Scale up
- Characterization
- GMP Synthesis

**Clinical**
- Phase I
- Phase II
- Phase III
- Clinical Support
- Data Management Plan
- Database Design
- CRF Management
- Double Data Entry
- Central Lab Data Import
- Medical / AE Coding
- Query Management
- Manual Data Quality Control
- Bioavailability
- Bioequivalence
- Tissue banking

**Integrated Research Solutions in Preclinical Biology**
Therapeutic Areas

INFLAMMATION

ONCOLOGY

IMMUNOMODULATION

IMMUNOGENICITY

HEPATOPROTECTIVES

HAIR BIOLOGY

INNOVATION RESEARCH TEAM

DIGESTION

DERMATOLOGY

METABOLIC DISEASES

PAIN
Service Modules

- In vitro Screens
- Target Based Screens
- Efficacy in Animals
- Mechanistic Profiling

- Vascularization
- Systemic Safety
- Dermal safety
- Clinical Toxicities

- Hypersensitivity
- ADME and PK
- Bioanalytical Techniques
- Skin and Hair Biology

- Immunomodulation
- Diabetes
- Pain and Inflammation
- Technical Consultation
What Do We Offer

**SMART Package**
- Screening
- Module for Accelerating Research and Therapy

A comprehensive package from Discovery to Pre-IND selection

<table>
<thead>
<tr>
<th>in vitro screens</th>
<th>Mechanism</th>
<th>Tumor Models</th>
<th>ADMET Studies</th>
<th>Lead Selection</th>
<th>Pre formulation Dev</th>
<th>Special Studies</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Rapid ADME Profiling of Investigational New Drugs (RAPID Screen)

A comprehensive package for ADME profiling

**RAPID Package**
- Rapid
- ADME
- Profiling of
- Investigational new
- Drugs

**Components:**
- Solubility
- Metabolic stability
- Plasma protein binding
- Permeability
- CYP 450 phenotyping
- CYP 450 Inhibition
A comprehensive package for clinical toxicity screening

- Normal cell toxicity
- Cardiotoxicity
- Alopecia
- Neuropathy
- GI Toxicity
- Neutropenia

Screens Available For Frequently Encountered Toxicity (SAFETY Screen)
Cellular screens for Mechanism of Action Profiling (CellMAP Screen)

A comprehensive screening platform for Mechanism of action profiling

CellMAP Package

Cellular screens for
Mechanism of
Action
Profiling

Drug Uptake
Intracellular Tracking
Signal Transduction
Target Expression
Cell Cycle
Angiogenesis
Apoptosis
Our National & Overseas clients

- AYURVET
- Actinobac Biomed, Inc.
- advanced cancer therapeutics®
- Akaal Pharma Pty Ltd
- ALLOSTEM THERAPEUTICS
- ARNO THERAPEUTICS
- Aspyriar Therapeutics Inc
- AURIGENE Accelerating Discovery
- avance
- BioConcept
- BIOLINEAX
- CIPLA Caring for life
- CTCI International Center for Cell Therapy & Cancer Immunotherapy
- RANBAXY LABORATORIES LIMITED
- CURADEV PHARMA PRIVATE LIMITED
- DEBIOPHARM GROUP™
- CLAIMS Synergy for Success Clinical Aesthetics and Investigative Management Service Pvt Ltd.
- Emcure®
- ENovate Biolife
- Epi Therapeutics
- FOLLICUM AB
- Fresenius Kabi
- Galaxy
- HQL PHARMACEUTICALS

Integrated Research Solutions in Preclinical Biology
Our National & Overseas clients

- SAMI LABS LIMITED
- Dabur
- Orion Pharma
- Emami
- UNIVERISTY OF OXFORD
- Celleron Therapeutics
- Fresenius Kabi
- Lee’s Pharmaceutical Holdings Ltd
- CendR Inc
- Glenmark
- Sirbal Ltd.
- Innoveda Biological Solutions Pvt Ltd
- Research and Development Institute
- Genentech (A Member of the Roche Group)
- premas biotech
- JNU
- Folllicum (Treatment of Hair Loss)
- Dabur (Celebrating 125 Years of Health & Well Being 1884 - 2009)
Our National & Overseas clients
Case Studies
**DISCOVERY AND DEVELOPMENT OF BETULINIC ACID DERIVATIVES FOR THE TREATMENT OF CANCER**

Manu Jaggi, MIA Siddiqui, Praveen R, Anand Vardhana, Rama Mukherjee, Anand C.Burman
Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad. Uttar Pradesh. INDIA

**Abstract**

Betulinic acid is a potentially interesting pentacyclic triterpenoid that has demonstrated selective cytotoxicity against melanoma and glioblastomas. It is in development in several countries as an anticancer agent. Betulinic acid has shown promising results in animal and cell culture studies. In this study, we have investigated the potential of betulinic acid and its derivatives as potential anticancer agents, focusing on their anti-cancer activity and anti-angiogenic potential.

**Materials and Methods**

**Cell lines**
ENVR cell line was procured from Dr. Takahashi (Tokyo University, Tokyo, Japan). All other cell lines were procured from NCCS, Pune, India. Cell lines were grown in DMEM, containing L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, buffered with 4 mM sodium bicarbonate, and 25 mM HEPES and 5% FBS. All cells were differentiated in DMEM (without hormones).

**Reagents**
Drugs used were obtained from Sigma-Aldrich, Cathepsin D and CD44 antibodies were obtained from Cell signalling technology (USA). The Selectin antibodies were obtained from Abcam (USA). The rabbit polyclonal antibodies were obtained from Santa Cruz Biotechnology, Inc. (USA). The secondary antibodies were obtained from Jackson ImmunoResearch Laboratories (USA). The cell culture plates were obtained from Nunc, ThermoFisher Scientific (USA). The HPLC-grade solvents were obtained from Sigma-Aldrich (USA). The protein assay kit was obtained from Bio-Rad Laboratories (USA). The nuclear factor kappa B (NF-kB) translocation assay kit was obtained from Cell Signaling (USA). The Mycobacterial DNA was obtained from Sigma-Aldrich (USA).

**Cell viability assay**
Cell viability was determined using the methyl thiazole tetrazolium (MTT) assay. The cells were seeded in 96-well plates at a density of 5 x 10^3 cells/well. After 24 hours, the cells were exposed to various concentrations of the test compounds for 24 hours. The MTT solution was added to each well and incubated for 4 hours. The absorbance at 570 nm was measured using a microplate reader.

**Effect of betulinic acid on growth of tumor xenografts**
Male BALB/c nude mice, age 6-8 weeks, weighing between 20-25 gms were selected for the study. 3 animals per group were used. The tumors were allowed to grow for 3 weeks before treating with the test compounds.

**Effect of betulinic acid on NF-kB activity**
The NF-kB activity assay kit was used to measure the NF-kB activity. The cells were lysed and the NF-kB activity was determined using the assay kit.

**Effect of betulinic acid on CD44 expression**
The CD44 expression was measured using an antibody against CD44. The cells were washed and fixed with 4% paraformaldehyde. The cells were permeabilized and stained with the primary antibody. The secondary antibody was used to detect the primary antibody. The cells were then analyzed using a flow cytometer.

**Effect of betulinic acid on cathepsin D expression**
The cathepsin D expression was measured using an antibody against cathepsin D. The cells were washed and fixed with 4% paraformaldehyde. The cells were permeabilized and stained with the primary antibody. The secondary antibody was used to detect the primary antibody. The cells were then analyzed using a flow cytometer.

**Effect of betulinic acid on Mycobacterial DNA levels**
The Mycobacterial DNA levels were measured using a real-time PCR assay. The cells were lysed and the DNA was extracted using a DNA extraction kit. The Mycobacterial DNA was measured using real-time PCR.

**Effect of betulinic acid on cancer stem cell population**
The cancer stem cell population was measured using an antibody against CD44. The cells were washed and fixed with 4% paraformaldehyde. The cells were permeabilized and stained with the primary antibody. The secondary antibody was used to detect the primary antibody. The cells were then analyzed using a flow cytometer.

**Effect of betulinic acid on tumor microenvironment**
The tumor microenvironment was measured using an antibody against VEGF. The cells were washed and fixed with 4% paraformaldehyde. The cells were permeabilized and stained with the primary antibody. The secondary antibody was used to detect the primary antibody. The cells were then analyzed using a flow cytometer.

**Effect of betulinic acid on tumor vascularization**
The tumor vascularization was measured using an antibody against CD31. The cells were washed and fixed with 4% paraformaldehyde. The cells were permeabilized and stained with the primary antibody. The secondary antibody was used to detect the primary antibody. The cells were then analyzed using a flow cytometer.

**Effect of betulinic acid on tumor invasion**
The tumor invasion was measured using a transwell invasion assay. The cells were seeded in the upper chamber and incubated for 24 hours. The cells that invaded to the lower chamber were stained with crystal violet and counted.

**Effect of betulinic acid on tumor metastasis**
The tumor metastasis was measured using a Matrigel invasion assay. The cells were seeded in the upper chamber and incubated for 24 hours. The cells that invaded to the lower chamber were stained with crystal violet and counted.

**Effect of betulinic acid on tumor angiogenesis**
The tumor angiogenesis was measured using a tube formation assay. The cells were seeded in the upper chamber and incubated for 24 hours. The cells that formed tubes were stained with crystal violet and counted.

**Effect of betulinic acid on tumor migration**
The tumor migration was measured using a wound healing assay. The cells were seeded in a 6-well plate and incubated for 24 hours. The cells were then wounded with a pipette tip and incubated for 24 hours. The cells that migrated to the wounded area were stained with crystal violet and counted.

**Effect of betulinic acid on tumor proliferation**
The tumor proliferation was measured using a BrdU incorporation assay. The cells were seeded in a 96-well plate and incubated for 24 hours. The cells were then treated with BrdU for 24 hours. The cells that incorporated BrdU were measured using a microplate reader.

**Effect of betulinic acid on tumor apoptosis**
The tumor apoptosis was measured using an antibody against caspase 3. The cells were washed and fixed with 4% paraformaldehyde. The cells were permeabilized and stained with the primary antibody. The secondary antibody was used to detect the primary antibody. The cells were then analyzed using a flow cytometer.

**Conclusion**
Betulinic acid has broad-spectrum anti-cancer activity. The derivatives have better potency and varying degree of specificity in cancer cells.

**Acknowledgements**
This work was financially supported by the National Biotechnology Development Division of Dabur Research Foundation.
A Novel Nanopolymer Based Tumor Targeted Delivery System for Paclitaxel

Anna A Singh, Manu Jogi, Dileep Khatkar, Vandana Jadhav, U.B. Khedkar, Surendra Tapke, Anil Chhunna Dabur Research Foundation, 223, B. Shah, Sahlabad, Mulund, 421, 7, INDIA

ABSTRACT: Dabur has developed a novel amphiphilic copolymer based delivery system (Polymeric delivery system-DONIPD) that targets the breast tumor and can deliver paclitaxel (PTX) in the tumor microenvironment. The PTX payload is released in the microenvironment owing to the presence of a trigger and an effector moiety in the polymer. The DONIPD is comprised of a copolymer of N-vinylpyrrolidone and 2-(methacryloyloxy) ethyl phosphorylcholine (MPC) that is sensitive to both pH and proximity to the tumor. The DONIPD has been shown to target and deliver PTX to the breast tumor and is currently undergoing Phase I clinical trials.

I. Introduction

Paclitaxel (Taxol®) is a widely used chemotherapy agent that is approved for the treatment of breast, ovarian, and other cancers. However, the efficacy of paclitaxel is limited by its poor solubility in water, low oral bioavailability, and the requirement for intravenous administration. Dabur Research Foundation has developed a novel amphiphilic copolymer based delivery system (Polymeric delivery system-DONIPD) that targets the breast tumor and can deliver paclitaxel (PTX) in the tumor microenvironment. The PTX payload is released in the microenvironment owing to the presence of a trigger and an effector moiety in the polymer. The DONIPD is comprised of a copolymer of N-vinylpyrrolidone and 2-(methacryloyloxy) ethyl phosphorylcholine (MPC) that is sensitive to both pH and proximity to the tumor. The DONIPD has been shown to target and deliver PTX to the breast tumor and is currently undergoing Phase I clinical trials.

II. Preformulation

Preparation of DONIPD: The DONIPD was prepared by free radical polymerization of N-vinylpyrrolidone and 2-(methacryloyloxy) ethyl phosphorylcholine in the presence of a water-soluble initiator. The polymer was characterized by size exclusion chromatography, nuclear magnetic resonance, and Fourier transform infrared spectroscopy. The results showed that the polymer had a narrow molecular weight distribution and was compatible with paclitaxel.

III. Formulation

Aqueous Solutions: The DONIPD was formulated as an aqueous solution for intravenous administration. The solution was prepared by dissolving the polymer and paclitaxel in water and adjusting the pH to 7.4. The solution was sterile and stable at room temperature for at least 24 hours.

IV. Stability

Stability of DONIPD: The DONIPD formulation was stable for at least 2 years at 4°C. The formulation was tested for changes in particle size, zeta potential, and drug content over time. The results showed that the formulation was stable and the drug content was constant.

V. In Vivo Studies

In Vivo Studies: The DONIPD formulation was evaluated in vivo in a murine breast tumor model. The formulation was administered intravenously to tumor-bearing mice and the efficacy of the formulation was compared to that of paclitaxel administered intravenously. The results showed that the DONIPD formulation was effective in reducing the size of breast tumors and was well-tolerated by the mice.

VI. Conclusions

The DONIPD formulation is a novel delivery system for paclitaxel that shows promise for the treatment of breast cancer. The formulation is stable and effective in vivo and may provide a new treatment option for breast cancer patients.
**ABSTRACT**

Adriamycin (ADR) and its metabolites induce dose-dependent cardiotoxicity in various animal models. In this study, NDR/NCE-25 has been evaluated to investigate its cardioprotective effects in Adriamycin (ADR)-induced cardiotoxicity in rats. The cardiotoxic activity was measured by measuring the activities of creatine phosphokinase (CPK)-MB, lactate dehydrogenase (LDH), and superoxide dismutase (SOD) in the hearts of rats.

**INTRODUCTION**

Cardiotoxicity is the major limitation in the use of doxorubicin (Dox, BB, R0, summits Oncol. 169 – 666, 1992). The risk of developing cardiotoxicity becomes unacceptable higher than the cumulative dose of 550 mg/kg (Elsalama et al., Cancer Res. 1973, 33: 394). In addition to clinical heart failure, cardiotoxicity encompasses clinical cardiotoxicity such as congestive heart failure and/or cardiac arrhythmia, and subclinical cardiotoxicity as detected by pathologic changes in cardiac biopsy or decrease in ventricular junction function. Thus, it has been reported that the cardiotoxicity onset time must be determined before the maximum effective cumulative dose has been administered to a patient bearing a neoplastic disease, because of the development of late-onset cardiotoxicity. Hence, while doxorubicin-induced cardiotoxicity is an effective and anti-tumor effect, the aggressiveness is significantly reduced by the concurrent cardiotoxicity encountered with the use of the drug. Thus, doxorubicin cardiotoxicity is closely associated with other chemotherapeutic agents like Mitomycin at doses >100-140mg/kg. These studies show that cardiotoxicity is not a part of the multi-drug resistance phenomenon. This evidence indicates that Adriamycin-induced cardiotoxicity is not completely understood. These intracellular mechanisms are observed with doxorubicin binding to cell nucleic acid and altering membrane functions, and antioxidant Na+ / K+ concentrations and stimulation of lipid peroxidation to form reactive radicals, (Young, R.B.et al., Br. J. Cancer 1991, 64: 1057-1067). The molecular mechanisms of Adriamycin appear to be an integral part of the biochemical mechanisms of its toxicity. Chronic administration of Adriamycin modulates the myocardial substrate, calcium and AMP-activated protein kinases. Adriamycin and thioglycolic acid are effective at reducing the severity of cardiac tissue damage caused by Adriamycin.

**MATERIALS & METHODS:**

Quantitative assays for radical scavenging activity by DPPH, I. DPPH (1-p,2-phenylenediamine)

Methods: DPPH has a blue color, which in the presence of free radical scavenger undergoes a one-electron transfer and becomes colorless.

In its radical form (DPPH) is absorb at 517nm, but upon reduction by an antioxidant or radical scavenger (such as NDR/NCE-25) DPPH exhibits a yellow color and absorbs at 416 nm. DPPH radical concentrations were determined using the absorbance at 517 nm and 416 nm respectively. Thus, DPPH was selected for the evaluation of NDR/NCE-25 in scavenging the DPPH radical.

Conclusions: NDR/NCE-25 was incorporated into CLB and CLB levels were monitored for the first time. The results indicate that NDR/NCE-25 is effective in reducing the production of CLB.

**ELECTRON MICROSCOPIC IMAGES OF CARDIAC TISSUES FROM Wistar Rats Treated with ADRIAMYCIN or NDR/NCE-25**

**CONCLUSIONS**

- NDR/NCE-25 is a potent free radical scavenging molecule in vitro.
- NDR/NCE-25 increases SOD enzyme activity in animals treated with Adriamycin in vivo study model.
- NDR/NCE-25 reduces Lipid peroxidation in animals treated with Adriamycin in vivo study model.
- NDR/NCE-25 increases CK-MB and LDH levels in animal treated with Adriamycin in acute study model.
- NDR/NCE-25 reduces myocardial cardia markers study model was observed.
- NDR/NCE-25 increases free radicals activity myocard treated with Adriamycin in acute study model.
- NDR/NCE-25 decreases basal cardiac oxidative enzymes treated in cardia muscle.
- NDR/NCE-25 decreases cardial oxidative enzymes treated in cardia muscle.
- NDR/NCE-25 reduces the severity of cardiac tissue damage caused by Adriamycin.
ANTICANCER ACTIVITY OF DRF7295: 
A PEPTIDE COMBINATION TARGETING MULTIPLE NEUROPEPTIDE RECEPTORS IN COLORECTAL CANCER

Manu Jagee, Anu T. Singh, Sudhanand Prasad, Praveen Rajendran, Sarjana Dutt, Anand C. Burman, Rama Mukherjee
Dabur Research Foundation, 22, Site 4, Sahibabad, Ghaziabad-201010, Uttar Pradesh, India www.daburpharma.com

ABSTRACT
Peptide combinations of particular importance in drug discovery are neurotrophic factors. The interaction of neuropeptides and neuropeptide receptor signaling with specific agonistic/antagonistic effects on neuropeptide receptor signaling, a hallmark of the new therapeutic approaches to the treatment of cancer. [1] Neuropeptides and their analogues are expected to find a high specificity toward the target tissue and a potential for treatment of cancer [2]. The aim of the present study was to evaluate the potential of DRF7295, a new neurotrophic factor as a novel therapeutic approach to cancer treatment, in vitro and in vivo. The study involved the evaluation of the cytotoxic effects of DRF7295 on various human cancer cell lines, including colon cancer cells. The results showed that DRF7295 had significant cytotoxic effects on colon cancer cells, with an IC50 value of 0.15 µM. The study also demonstrated that DRF7295 had a synergistic effect when used in combination with other neurotrophic factors. The results suggest that DRF7295 could be a promising therapeutic agent for the treatment of colon cancer.

INTRODUCTION
Peptide hormone function is often involved in various physiological processes and acts as neurotransmitters and neuromodulators. In many cases, the receptors mediating peptide hormones are an important component of the G protein-coupled receptor signaling network. [1] Neuropeptides have been explored to play a role in cancer. [2] The interaction of neuropeptides with specific neuropeptide receptors is a hallmark of the new therapeutic approaches to the treatment of cancer. [1] Neuropeptides and their analogues are expected to find a high specificity toward the target tissue and a potential for treatment of cancer [2]. The aim of the present study was to evaluate the potential of DRF7295, a new neurotrophic factor as a novel therapeutic approach to cancer treatment, in vitro and in vivo. The study involved the evaluation of the cytotoxic effects of DRF7295 on various human cancer cell lines, including colon cancer cells. The results showed that DRF7295 had significant cytotoxic effects on colon cancer cells, with an IC50 value of 0.15 µM. The study also demonstrated that DRF7295 had a synergistic effect when used in combination with other neurotrophic factors. The results suggest that DRF7295 could be a promising therapeutic agent for the treatment of colon cancer.

CONCLUSIONS
Peptide hormones such as neuropeptides are known to possess therapeutic potential for the treatment of various diseases, including cancer. The present study evaluated the cytotoxic effects of DRF7295, a novel neurotrophic factor, on human colon cancer cells. The results showed that DRF7295 had significant cytotoxic effects on colon cancer cells, with an IC50 value of 0.15 µM. The study also demonstrated that DRF7295 had a synergistic effect when used in combination with other neurotrophic factors. The results suggest that DRF7295 could be a promising therapeutic agent for the treatment of colon cancer.

REFERENCES

MATERIALS & METHODS
Peptide combinations of particular importance in drug discovery are neurotrophic factors. The interaction of neuropeptides and neuropeptide receptor signaling with specific agonistic/antagonistic effects on neuropeptide receptor signaling, a hallmark of the new therapeutic approaches to the treatment of cancer. [1] Neuropeptides and their analogues are expected to find a high specificity toward the target tissue and a potential for treatment of cancer [2]. The aim of the present study was to evaluate the potential of DRF7295, a new neurotrophic factor as a novel therapeutic approach to cancer treatment, in vitro and in vivo. The study involved the evaluation of the cytotoxic effects of DRF7295 on various human cancer cell lines, including colon cancer cells. The results showed that DRF7295 had significant cytotoxic effects on colon cancer cells, with an IC50 value of 0.15 µM. The study also demonstrated that DRF7295 had a synergistic effect when used in combination with other neurotrophic factors. The results suggest that DRF7295 could be a promising therapeutic agent for the treatment of colon cancer.
Development of a Dendritic-Cell Based Assay to Screen Molecules for Potential Anti-Inflammatory Activity

Alka Madan, Manu Jaggi, Rama Mukherjee

Dabur Research Foundation, 22, Site-IV, Sahibabad, Ghaziabad, Uttar Pradesh - 201010

INTRODUCTION

Anti-inflammatory cytokines, such as Tumor Necrosis Factor (TNF-α) and Interleukin (IL)-1β have been explored as potential targets in therapeutic interventions for various inflammatory disorders (1). Currently available TNF-α inhibitors include monoclonal antibodies (infliximab, D3E7) to neutralize TNF-α activity, TNF-α specific combinator receptor construct (Enanetcet), and CTLA4 fusion protein (Abatacept) to block TNF-α activity by inhibiting T cells (2,3). Dendritic cells (DC), which recognize invading pathogens and process the processed antigens to secondary lymphoid organs for cells activation, have been qualified as the target cells to investigate pharmacological role of various immunomodulatory agents (4-8). In an in vitro septic shock assay was developed with murine bone marrow-DCCs to screen new molecules for potential anti-inflammatory activity. Extent of modulation in pro-inflammatory cytokines and chemokines was taken as an indicator of immunomodulatory activities. A naphthyridine class of molecules has previously been reported as potential anti-inflammatory agents (9,10). We investigated the potential anti-inflammatory activity of novel derivatives of this class of molecules using in DCC based assay.

METHODOLOGY

1. Generation of Molecular Molecules: Primary DC cultures were generated from mouse bone marrow. Bone marrow progenitor cells were cultured in presence of mGM-CSF at 37°C. 5%CO2. Immature DCs were preincubated with LPS, resulting in elevated levels of pro-inflammatory cytokines and chemokines.

2. Screening of molecules: 18 naphthyridine derivatives were screened from an in-house library of NCEs synthesized at DFR. Primary screening of new molecules was done at two specific concentrations 0.1ug/ml and 1ug/ml. Molecules with potential anti-inflammatory activity were subjected to screening over a multiple dose range of concentration of 0.001 µg/ml to 10 µg/ml. Superantigens were collected for analysis.

Cytokine and chemokine panels: The panel of pro-inflammatory cytokines and chemokines was analyzed using specific enzyme linked immunoassay kits for modulatory activity of test compounds comprised of:

- Tumor Necrosis Factor-α
- Interleukin-1β
- Interleukin-6 (IL-6)
- Macrophage-Inflammatory Protein (MIP-1α)
- MIP-1β
- IL-8

Animals: Animals were taken from the Institutional Animal Ethics Committee (IAEC) facility of Dabur Research Foundation. All animals were approved by the Institutional Animal Ethics Committee of Dabur Research Foundation.

RESULTS AND DISCUSSION

- BMDC based assay was developed to screen new molecules with potential anti-inflammatory activity.
- Culture conditions were optimized to obtain reproducible DC yields and minimum inter and intra assay variations.
- Assay system was validated with known anti-inflammatory agents, such as NS-398 and Indomethacin for downregulation of TNF-α and IL-6.

- A library of 100 NCEs belonging to naphthyridine group was evaluated for anti-inflammatory activity by modulation of pro-inflammatory cytokines and chemokines.
- The extent of inhibition of elevated levels of pro-inflammatory cytokines by test molecules was indicative of their anti-inflammatory properties. The downregulation of chemokine and cytokine levels by ≥ 25% was considered as significant.

TNF-α activity:
- Significant down regulation of TNF-α activity was observed for compound no. #59, #62, #66, #68, and #71.
- Screening was carried out over a concentration range of 0.001-10 µg/ml for selected compounds no. #59, #62, #66, #68, and #71.
- IC50 values were found to be <0.001 µg/ml for these compounds.

CONCLUSIONS

- This DC based in vitro screening can be used as a mechanism based assay for early identification of molecules with potential anti-inflammatory activity.
- Screening molecules using DC assay has resulted in early identification of potential anti-inflammatory candidates. These molecules can be further evaluated in an in vivo model for inflammatory disorder. Hence less number of animals are required for early screening studies.
- Action of biological drugs is mediated by targeting molecular markers on cell surface, for e.g. Abatacept competes with CTLA4 on T-cells to block T cells activation. This model presents DC as an excellent cellular target for evaluation of anti-inflammatory activity and offers scope for evaluating and targeting other relevant DC markers

ACKNOWLEDGEMENTS:

The support of Dr. Anand Burman, Chairperson, Dabur Research Foundation is gratefully acknowledged.
Thanks