NANOMEDICINE
Nanotechnologies for novel drug design and delivery, diagnostic, regenerative medicine

Solid Lipid Nanoparticles from warm microemulsion: snapshot on different drug delivery applications

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Outline

Short Company and Technology overview

Oral and transdermal delivery  Melatonin
Intravenous delivery  ANTI-VEGF (VEGF-AS-ODN)
Eye drops ocular delivery  Myriocin

Aim: to show flexibility, versatility and efficacy of proprietary carriers


R. Rezzani et al., Melatonin delivery in solid lipid nanoparticles: prevention of cyclosporine A induced cardiac damage, J. Pineal Res. 46, 255–261, 2009


Enrica Strettoi et al., Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of Retinitis Pigmentosa, PNAS Oct. 11, 2010

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Short History
Nanovector is R&D based company incorporated in 2001 to exploit research results of long term research performed by a team in University of Turin.
Lab is in located in Environment Park in Turin

Technology and IP
Nanovector has specific know-how and expertise on Microemulsions and on Solid Lipid Nanoparticles (SLN) obtained from warm microemulsions by proprietary process.
Nanovector owns ten patent families (pending and granted)

Mission
To research, to develop and to exploit proprietary technical platform on lipid carriers in Pharmaceuticals, Diagnostics and Nutraceutics.
**NANOVECTOR**
Technical Platform

**MICROEMULSIONs and WARM MICROEMULSIONs (µEs)**

- µEs are liquid systems: components are water, oil (room temp.) or melted lipid (warm temp.) phase, surfactants and cosurfactants
- Components are biocompatible (GRAS)

**SOLID LIPID NANOPARTICLES (SLNs)**

- SLNs are solidified nanodrops of warm µEs
- Hydrophobic and Hydrophilic drugs can be loaded in our SLNs, by different approaches (O/W µEs, W/O/W µEs, Ion Pair technique, pro-drug lipid matrix)

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**Solid Lipid Nanoparticles from warm microemulsion: snapshot on different drug delivery applications**
Characterization and Pharma process

- Spherical shape, with average diameters ranging from 40 to 150 nm (depending also on drug loaded into)
- Homogeneous size distribution (low PI)
- Zeta Potential with high values (negative or positive) for dimensional stability
- Washing by Tangential Flow Filtration
- Freeze drying process
- Most final SLNs dispersions can be sterilised by filtration at 0.2 um. Some of them can bear heat sterilization (depending on drug loaded into). As alternative sterilizing filtration can be applied at warm microemulsion process step.
Melatonin is a hormone produced by the pineal gland at night. It is involved in the regulation of sleep and circadian rhythms. The synthesis and secretion of MT is induced by darkness and suppressed by light.

Plasma MT levels
During the day, about 10 pg/ml in young adults, pulse of secretion starting at 9 p.m. After peak of about 70–100 pg/ml between 2 / 4 a.m. levels return to baseline at 7 / 9 a.m.
Pharmacokinetics of exogenous MT is not favorable due to short half-life of elimination.

Excellent drug model with its favorable toxicity profile: tested into healthy volunteers.

Melatonin has been loaded in SLN
Hydrophilic/Hydrophobic drug – simple microemulsion process (O/W)
Invivo test has been performed by Oral Administration and TD Administration

Work with
Prof Fraschini Team (UNIMI – Pharmacology dept)
Prof Mauro Team (IRCCS – Piancavallo, UNITO - Neuroscience dept)
Prof Esposti Team (UNIMI – Physiology dept)

Solid Lipid Nanoparticles Incorporating Melatonin as New Model for Sustained Oral and Transdermal Delivery Systems

Solid Lipid Nanoparticles from warm microemulsion: snapshot on different drug delivery applications
Invivo test
SLN incorporating MT 3 mg (MT-SLN-O) were orally administered at 8.30 a.m. in 7 healthy subjects.

MT 3 mg in standard formulation (MT-S) was then administered to the same subjects after one week at 8.30 a.m. as control.

Freeze dried amounts of MT-SLN (MT = 4.13%) containing 3 mg MT were included in hard gelatine capsules (Scherer) for oral administration (MT-SLN-O); 3 mg of MT was also included in hard-gelatine capsules for oral administration (MT-S).

Melatonin plasma level profiles after ORAL adm.
(3mg - 7 Health Volunteers)
MT (blue) Vs MT-SLN (red)

Solid Lipid Nanoparticles Incorporating Melatonin as New Model for Sustained Oral and Transdermal Delivery Systems

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Invivo test
SLN incorporating MT were administered transdermally (MT-SLN-TD) in 10 subjects
application of a patch at 8.30 a.m. for 24 hours
Freeze-dried MT-SLN (MT = 2%) were dispersed in a mixture of water and propylene glycol (70:30) (w/w)
and successively added of Carbopol 940® (1.2%) obtaining agel (MT-SLN-TD); the melatonin
concentration in the gel was 1.8 mg/g (w/w).
Dosage was 3.6 mg MT on 9 cm² area
As baseline controls, in this group of subjects blood samples were collected after one week and at
the same intervals from 8 a.m. until 7 p.m.
In two subjects blood samples were collected from 8 a.m. to 3 p.m. on 3rd day.

Melatonin plasma level profiles after TD adm.
(3.6 mg - 10 Healthy Volunteers)
MT-basal (blue) Vs MT-SLN (red)

Solid Lipid Nanoparticles Incorporating Melatonin as New Model for Sustained Oral and Transdermal Delivery Systems

Solid Lipid Nanoparticles from warm microemulsion: snapshot on different drug delivery applications
Nucleic acid delivery is one of major problem worldwide in order to achieve applicability of gene therapy.

Vascular Endothelial Growth Factor (VEGF) can be strategic target for antiangiogenetic therapy in high grade gliomas.

VEGF Antisense Oligonucleotide (AS ODN) has been loaded in SLN (Hydrophilic drug – multiple microemulsion process (W/O/W))

Invitro tests
Invivo test has been perfomed by Intraveous Administration

Work with
Prof Mauro Team (IRCCS – Piancavallo)
Prof Mauro Team (UNITO - Neuroscience dept)
Dr Zara Team (UNITO – Anatomy Dept – Pharmacology and Forensic Medicine)

**INTRAVENOUS administration - Nucleic acid delivery**

*In vitro:*

rat C6 glioma cells under hypoxic conditions were treated different concentrations for 24 and 48 hours with

- free AS-ODN solution
- AS-ODN-SLN, SLN carrying AS-ODN as water dispersion

At Western blot analysis cellular VEGF expression was significantly reduced (p < 0.01) after 48 hours treatment with AS-ODN-SLN compared to both hypoxic and normoxic conditions, while remained stable after free AS-ODN treatment.

![Graph showing VEGF expression](image)

**VEGF expression**

- VEGF 120
- VEGF 164
- VEGF 188

Statistical analysis and semi-quantification of western blot data. VEGF isoforms (120, 164, and 188) expression (Adjusted Volume OD*mm^2) were measured under normoxic (hours) and hypoxic conditions at 24 and 48 hours, in the absence (-) or presence (+) of AS-ODN-SLN. Bars represent mean ± s.d. of the triplicates; (*) and (•) indicate respectively 95% and 99% significance at Student's t-test (calculated comparing the hypoxic and the corresponding treated samples).

**INTRAVENTOUS administration - Nucleic acid delivery**

*In vivo:*

Wistar rats underwent C6 cell orthotopic intracerebral stereotactic implant after 14 days, were treated i.v. for three (3) days with either free AS-ODN or AS-ODN-SLN solutions.

At day 17th a great VEGF reduction was found in central and peripheral tumor regions of AS-ODN-SLN treated rats while any appreciable result was obtained after free AS-ODN treatment.

VEGF immunohistochemistry (brown spot)

xenografted tumor sections (40x)

A control rats  
B corresponding negative control rats  
C animals treated with 2mg/Kg AS-ODN free solution  
D animals treated with 2mg/Kg AS-ODN-SLN

INTRA VENOUS administration - Nucleic acid delivery

In vivo:
In present rat glioma models AS-ODN-SLN showed high effectiveness in reducing VEGF expression suggesting that SLN can be regarded as a good carrier for gene therapeutical agents delivery in the Central Nervous System.

![Immunofluorescence images](image)

VEGF immunofluorescence
xenografted tumor sections (60x)

A, D  VEGF green fluorescence
B, E  red propidium iodide nuclear staining
C, F  merge panel

After AS-ODN-SLN treatment only few cells were still expressing VEGF (F, arrows).
OCULAR administration – Retina delivery

Mechanism of action has been pointed out for a certain drug, but functional tests couldn’t be performed.

Aim was:

To test efficacy of such drug in Retinitis Pigmentosa Animal model by eye topical administration of drug loaded SLN.

To evaluate toxicity of SLN in overtime administration (chronic).

Myriocin has been loaded in SLN.

Hydrophobic drug – simple microemulsion process (O/W).

Invitro tests:

Invivo test has been performed by Eye Drops administration.

Work with:

Prof Ghidoni Team (Osp San Paolo – UNIMI)
Dr Strettoi Team (CNR Pisa)
Prof Gargini (UNIPI)

Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of Retinitis Pigmentosa.

Enrica Strettoi et al. - PNAS Oct. 11, 2010

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**Evidences**

In Retinitis Pigmentosa (RP) Rod photoreceptor death is largely thought to occur by **apoptosis**

**Ceramide** is a well characterised death effector among pro-apoptotic cellular messengers

Several lines of evidences suggest that **ceramide can mediate photoreceptor degeneration**

**To alter sphingolipid metabolism may have therapeutic potential**

**Model: RD10 mouse**

Faithful model of Human RP
In this mouse mutant, pr begin to die from apoptosis during the third week of life, photoreceptor death peak at day 24 post natal (P24)
Rods first, cones subsequently
Vertical section of retina
  - at P10  12-14 rows of photorcp
  - at P30  2-3 rows of photorcp

**Drug: Myriocin**

a fungal metabolite, atypical amino acid, similar to sphingosine
is selective **inhibitor of Serine Palmitoyl Transferase (SPT)**, enzime which catalyse 
*denovo* biosynthesis of ceramide

**Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of Retinitis Pigmentosa**

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**Solid Lipid Nanoparticles from warm microemulsion: snapshot on different drug delivery applications**
First steps
Myriocin DMSO

Ceramide level is similar in RD10 & WT until P16, then starts to increase in RD10

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First steps
Myriocin DMSO

Retinas from Myriocin treated eyes had fewer picnotic nuclei than DMSO treated

Myriocin induced 52.6% reduction in number of picnotic nuclei

Retinas from Myriocin treated eyes had fewer picnotic nuclei than DMSO treated

but ........

...ERG traces from myriocin treated animals overlapped with those from DMSO-treated animal

-> single injection did not have measurable functional effect

A drug delivery problem!

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Solid Lipid Nanoparticles from warm microemulsion: snapshot on different drug delivery applications
Myriocin has been tested by eye-drops administration but ceramide quantification didn’t showed any difference with control.

Myriocin has been loaded in SLN.

Concentration (0.6 mM) was high enough for 3 times a day topical administration for more then 20 days.

MYR-SLN dispersion can be sterilized by filtration at 0.2 um.

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Second step
Myriocin SLN

3 days fluo
Fluo SLN have been given first for 3 days by eye-drops and they have been found in retina as aggregates not present in untreated animals

3 days Myr
Myriocin loaded SLN have been given first for 3 days by eye-drops (P19):
Mean value of Ceramide was 2.49 pmol/nmol Pi, while in untreated animals was 4.19 pmol/nmol Pi

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> 20 days
Myriocin loaded SLN have been given first for 3 times a days by eye-drops (P14 to P35)

Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of Retinitis Pigmentosa

Enrica Strettoi et al. - PNAS Oct. 11, 2010

Increased photoreceptor survival after chronic treatment
Number of photoreceptor row in the outer retina is higher during P24-P30 time window

Solid Lipid Nanoparticles from warm microemulsion: snapshot on different drug delivery applications
Mean amplitude of ERG b wave decreased progressively overtime in concomitance of photoreceptor degeneration
Absolute values of mean amplitudes are higher for MYR-SLN (except P35)
Significant differences in A wave at P30, P35 benefit at bright light

Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of Retinitis Pigmentosa
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Solid Lipid Nanoparticles from warm microemulsion: snapshot on different drug delivery applications
Significant differences in A wave at P30, P35 benefit at bright light (cone)

SAFETY: administration of Myr-SLN showed no toxicity after chronic administration for 3 times/day (clinical examination, histology examination, ERG)

Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of Retinitis Pigmentosa

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Effects of myriocin-SLNs on ERG. The best examples of ERG responses to flashes of light of increasing intensity from two rd10 mice age P24. Traces in the panel corresponding to the lowest light intensity are purely rod-driven, whereas the others represent mixed rod–cone responses. Red traces, responses from a mouse treated with myriocin-SLNs; black traces, responses from a mouse treated with control SLNs. In this instance, the SLNs contained the highest myriocin concentration (1 mM) of this study.
OCULAR administration – Retina delivery

SLN dispersion was perfectly tolerated: there was no evidence of toxic effects based on the clinical examinations, also in chronic treatment.

Electroretinography evaluations showed no functional alterations overtime.

SLN caused no histopathological or ultrastructural damage to the retina or other ocular tissues

Topical administration of MYR-SLN resulted in functional detectable levels to assess efficacy of loaded drug in pathology of back of the eye (POC)

- Pat. WO2004039351
  Pharmaceutical composition suitable for the treatment of ophthalmic diseases
  Granted in EU, China
  Pending in US, India

- Pat. MI2009A000284 (26/02/2009)
  Related to Retinitis Pigmentosa
  (30% Unimi, 30% CNR, 20% Unipi, 20% Nanovector)
  Extension PCT in February 2010

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PROLONGED DRUG RELEASE SYSTEMS

MODIFICATION OF PHARMACOKINETIC PARAMETERS

TARGETING TO LYMPH - ABSORPTION BY INTESTINAL MUCOSA (ORAL ADM.)

STEALTH PREPARATION REDUCES UPTAKE BY MACROPHAGES (RES) AND ENHANCE CIRCULATING TIME

INTERACTION WITH CELLS IN FEW MINUTES

POSSIBILITY TO ADMINISTER DRUGS NOT ABSORBED BY INTESTINAL MUCOSA (FROM IV TO ORAL)

PHYSIOLOGICAL BARRIERS OVERCOMING (Brain and Eye Posterior Globe)

TESTED BY DIFFERENT ROUTES OF ADMINISTRATION (animal model and Human)

Parenteral (IV), Duodenal/Oral, Ocular Topical, Transdermal

Hydrophobic Drugs
Ciclosporine
Hydrocortisone
Paclitaxel
Desoxicortisone
Diazepam
Nifedipine
Steroids

Hydrophilic Drugs
Tymopentine
Tobramycin
Doxorubicin
Idarubicin
LHRH-TRP6
VEGF ODN - AS
Platinum Complexes

Prodrug
Cholesteryl Butyrate proprietary formulation (2 patents) – HDAC, Antinflammatory

Diagnostics
Gadolinium Complexes
Superparamagnetic Iron Oxide Nanoparticles
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Thank you for Kind attention