Nano Innovation 2020
Rome, 15-18 September
Conference & Exhibition

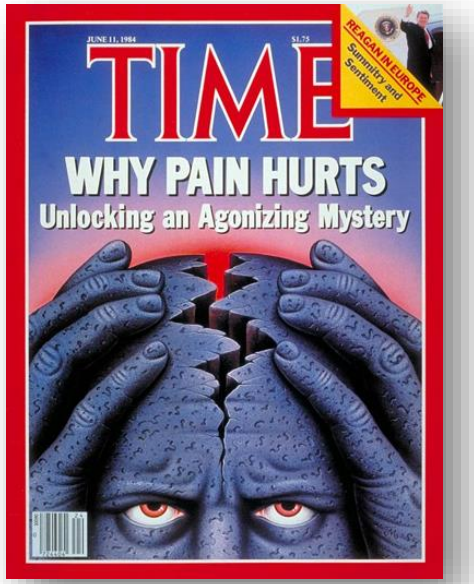
pH-sensitive niosomes and liposomes: Effects on inflammation and pain in murine models

Minosi P[1], Rinaldi F[2], Hanieh PN[2], Marianecci C[2], Marzoli F[1], Ciarlo L[1], Carafa M[2] and Pieretti S[1]

[1] National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, 00161, Rome, Italy.

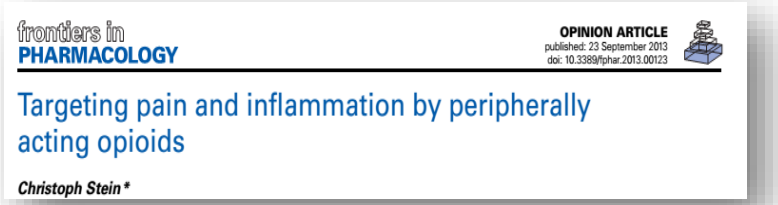
[2] Department of Drug Chemistry and Technologies, Sapienza University, 00185 Rome, Italy.

Introduction



In several diseases and disorders, acute and chronic inflammation is often associated with pain.

Introduction

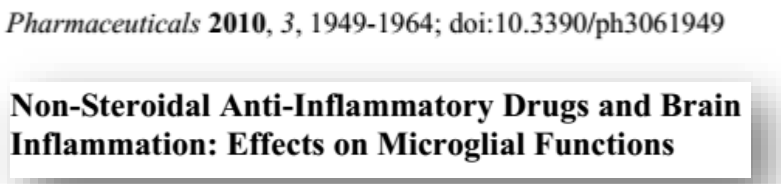

frontiers in PHARMACOLOGY

OPINION ARTICLE
published: 23 September 2013
doi: 10.3389/fphar.2013.00123

Targeting pain and inflammation by peripherally acting opioids

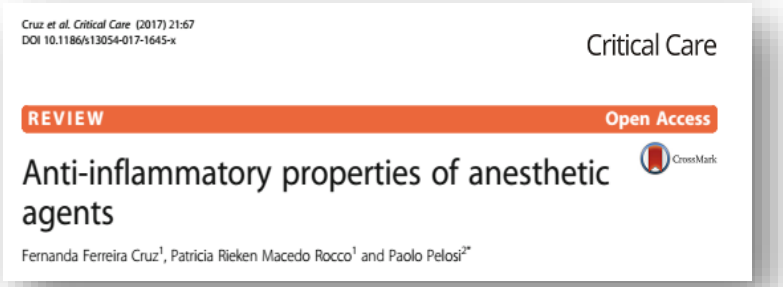
*Christoph Stein**

Opioids can reduce pain and inflammation by activating opioid receptors inside and outside the central nervous system.


Pharmaceuticals **2010**, *3*, 1949-1964; doi:10.3390/ph3061949

Non-Steroidal Anti-Inflammatory Drugs and Brain Inflammation: Effects on Microglial Functions

Non steroidal anti-inflammatory drugs are the therapeutic agents of first choice for the treatment of inflammation, pain, and fever.


Cruz *et al. Critical Care* (2017) 21:67
DOI 10.1186/s13054-017-1645-x

Critical Care

REVIEW Open Access

Anti-inflammatory properties of anesthetic agents

Fernanda Ferreira Cruz[1], Patricia Rieken Macedo Rocco[1] and Paolo Pelosi[2*]

Local anesthetics have also anti-inflammatory activities. By interfering with the inflammatory cascade at different levels, local anesthetics attenuate the inflammation and this effect has been employed to treat some acute and chronic inflammatory diseases.

Introduction

Side effects of opioids

Effect	Manifestation
Mood changes	Dysphoria, euphoria
Somnolence	***Lethargy, drowsiness***, apathy, inability to concentrate
Stimulation of CTZ; Delayed gastric emptying	***Nausea, vomiting***
Respiratory depression	Decreased respiratory rate
Decreased GI motility	***Constipation***
Increased sphincter tone	Biliary spasm, urinary retention
Histamine release	Hives, itching, asthma exacerbation (rare)
Tolerance	Larger doses for same effect
Dependence	Withdrawal symptoms w/ abrupt d/c Adapted from Dipiro et al.

Introduction

Side effects of non-steroidal anti-inflammatory drugs

GENERAL	GASTROINTSTINAL SYSTEM	CARDIOVASCULAR SYSTEM	HEMATOLOGY	RENAL SYSTEM	CENTRAL NERVOUS SYSTEM
Nausea/ Vomiting	Peptic ulcers	Increased risk of heart failure	Prevents coagulation, increasing the risk of bleeding	Sodium and water retention	Aseptic meningitis
Headache/ Dizziness	Gastrointestinal bleeding	Increased risk of myocardial infarction	Anemia	Hypertension	Increased risk of cerebrovascular accident
Diarrhea/ Constipation	Esophagitis/Strictures			Acute kidney injury	Psychosis and cognitive dysfunction in the elderly
Ringing in the ears	Colitis			Acute tubular necrosis	
Allergic skin reactions	Increased risk of exacerbation of inflammatory bowel disease symptoms			Analgesic nephropathy	
Increased risk of asthma attacks in people with asthma	Increased complications of diverticular disease				
	Reyes syndrome in children				
	Liver problems, including liver failure				

Source: Literature review conducted by Kasarla M.

Introduction

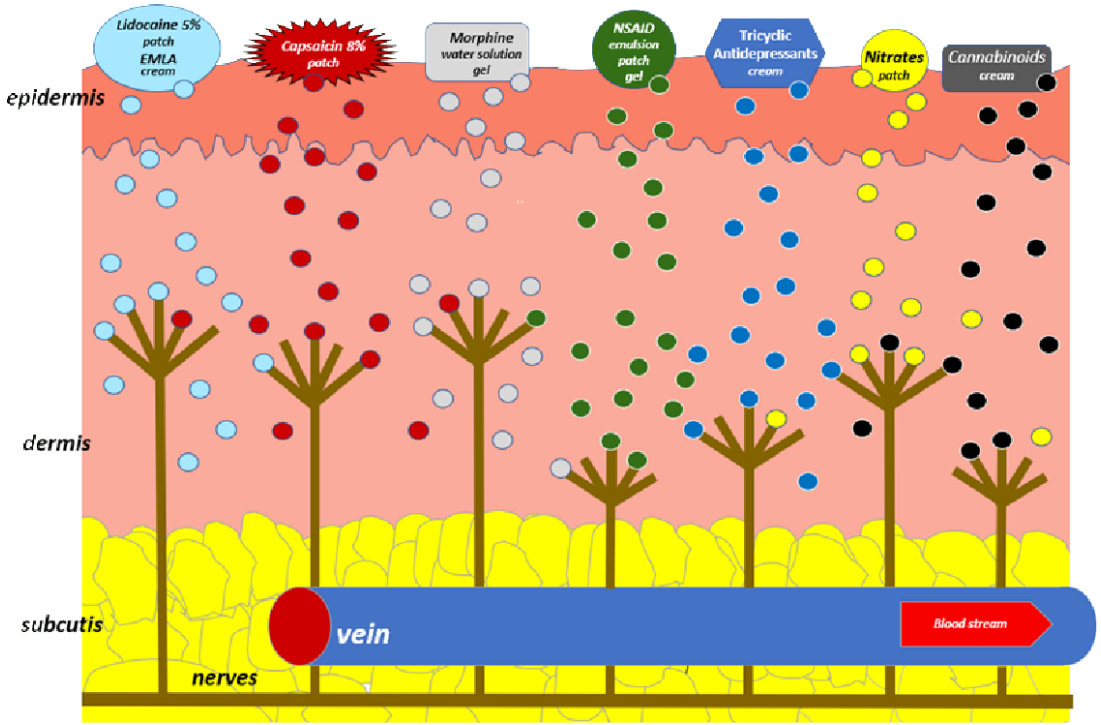
Side effects of local anesthetics

Type of reaction	Characteristic clinical manifestations
Toxic responses in normal individuals	*Central nervous system effects* Stimulation: restlessness, garrulousness, seizures Depression: coma, respiratory arrest *Cardiovascular effects* Myocardial: depression, cardiac arrest Peripheral vascular: hypotension, shock
Drug-unrelated reactions	*Psychomotor responses* Hyperventilation syndrome: dyspnea, paresthesia, dizziness, syncope Vasovagal: syncope, bradycardia *Sympathetic stimulation* Caused by endogenous epinephrine release Caused by administered epinephrine *Operative trauma*
Idiosyncratic responses	Methemoglobinemia (prilocaine)
Allergic reactions	Urticaria, angioedema, anaphylaxis, contact dermatitis, delayed hypersensitivity (?)

deShazo RD, et al. JACI 1979

Introduction

Transdermal and topical routes of analgesics, anti-inflammatory and anesthetic drugs are a non-invasive method of administration.

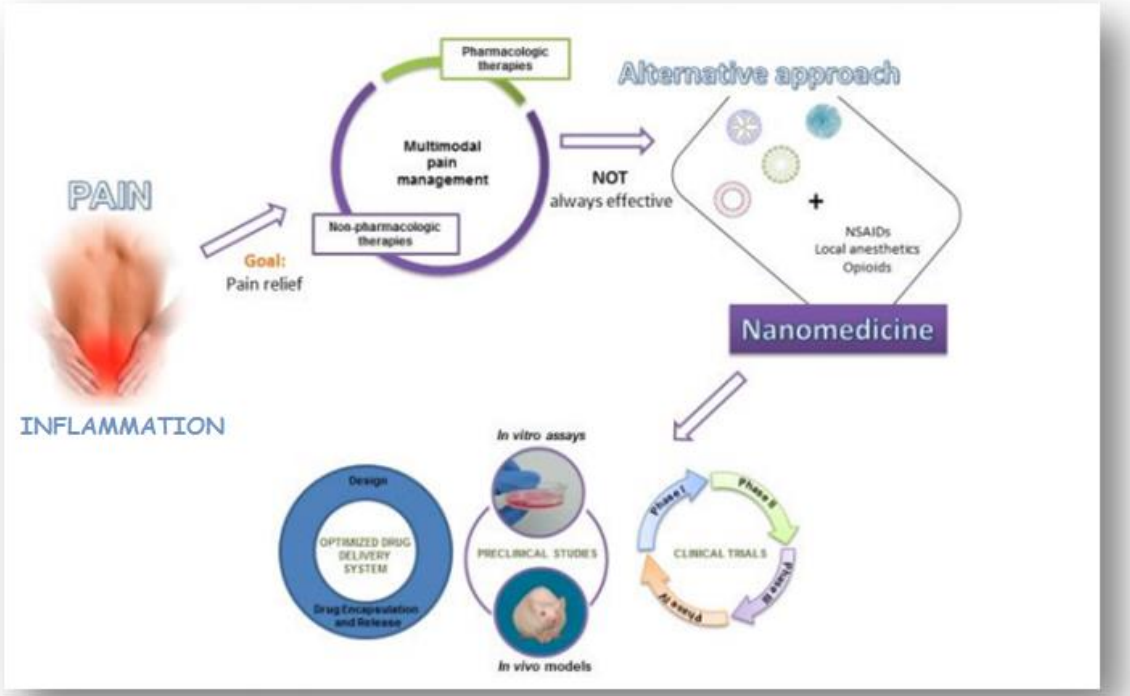


They may provide some more advantages such as high efficacy along with a beneficial profile of side effects.

However, these drugs show limited efficacy on intact skin and need prolonged applications and high drug concentrations.

Leppert W, Malec-Milewska M, Zajaczkowska R, Wordliczek J. Transdermal and Topical Drug Administration in the Treatment of Pain. *Molecules*. 2018;23(3):681.

Introduction



Researchers have been focused on the manufacturing of both organic and inorganic nanoparticle formulations intended for the delivery of a variety of pain drugs including local anesthetics and non-steroidal anti-inflammatory drugs.

Andreu V, Arruebo M. Current progress and challenges of nanoparticle-based therapeutics in pain management. *J Control Release*. 2018;269:189-213.

Introduction

pH PROFILES OF PATHOLOGICAL STATES

Modification of pH derives in several pathological condition and inflamed tissues often show decreased pH levels and activation of inflammatory mediators when compared with healthy tissues.

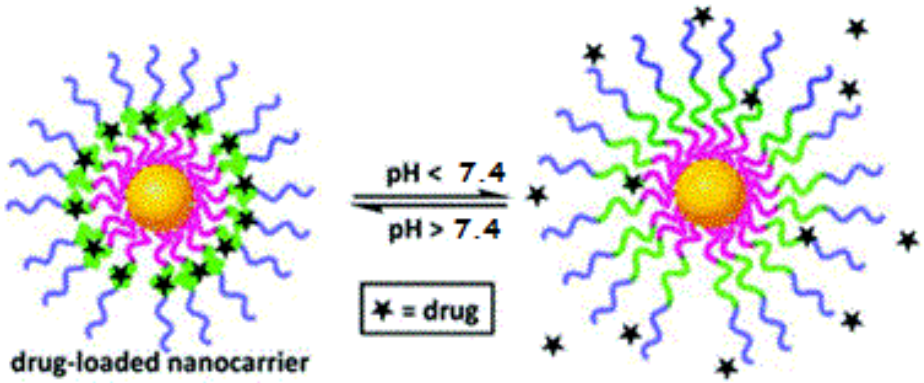
Extracellular pH values ranging from 5.5 to 7.0 have been detected in inflamed tissues associated with bacterial infections, inflammation and cancers.

Casimir GJ, Lefèvre N, Corazza F, Duchateau J, Chamekh M. The Acid-Base Balance and Gender in Inflammation: A Mini-Review. *Front Immunol.* 2018;9:475.

Introduction

pH-RESPONSIVE NANOCARRIERS

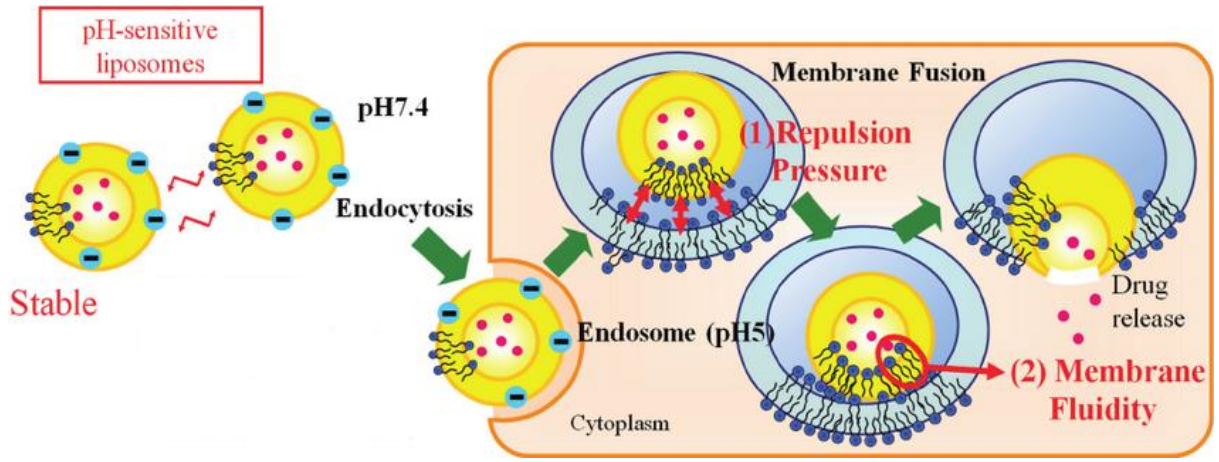
Fusogenic vesicles are stable at physiological pH (pH 7.4), but undergo destabilization under acidic conditions, leading to the release of their contents at the intracellular level or in inflamed sites. pH sensitive drug delivery systems have been designed to deliver drugs at the target site in this way.



This leads to many advantages, such as: decreased dose to be administered and side effects; improved drug utilization and patient compliance.

Lehner R, Wang X, Wolf M, Hunziker P. Designing switchable nanosystems for medical application. *J Control Release*. 2012;161(2):307-316.

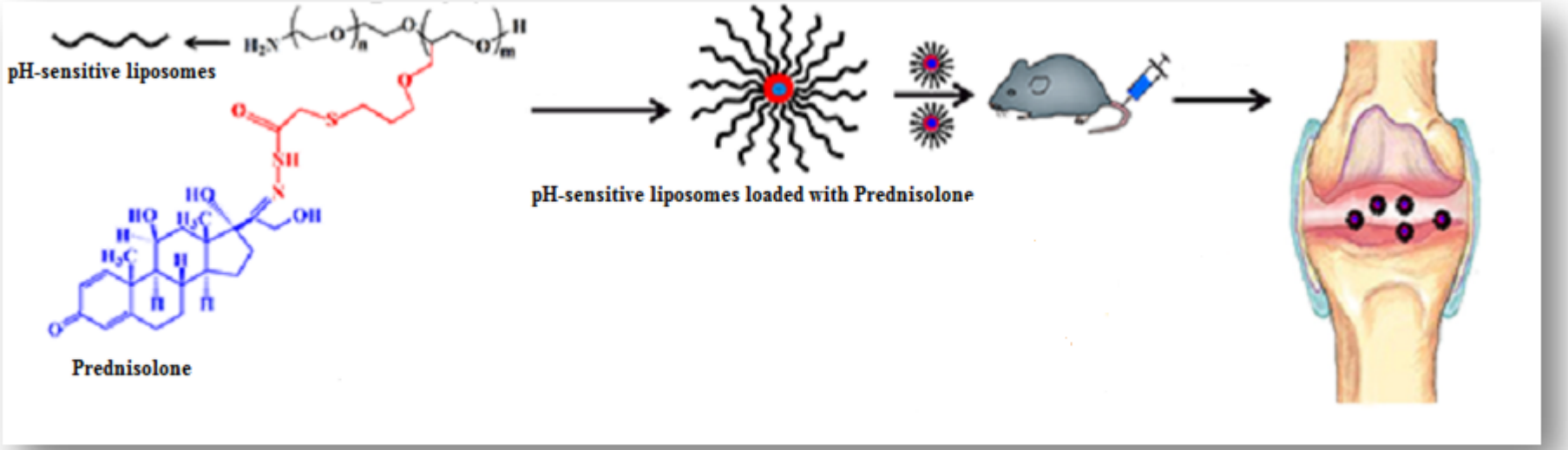
State of art: liposomes drug carriers with pH-sensitivity



pH-sensitive liposomes are stable at physiological pH and they are internalized by endocytosis after binding to cell surface receptors. Following receptor-mediated endocytosis, liposomes will be retained in the early endosomes (internal pH 6.5), which will mature into late endosomes (internal pH 5.5 ~ 6.5). As the pH decreases, pH-sensitive liposomes were destabilized leading to the release of their contents into the cytosol. Besides, the encapsulated compounds can be released directly into the cytoplasm due to the fusion between pH-sensitive liposomes and the endosomal membrane.

Balamuralidhara V, Pramodkumar TM, Srujana N, et al. pH Sensitive Drug Delivery Systems: A Review. *American Journal of Drug Discovery and Development*. 2011;1:24-48.

pH-sensitive liposomes: targeted drug delivery strategy for rheumatoid arthritis



Gouveia VM, Lopes-de-Araújo J, Costa Lima SA, Nunes C, Reis S. Hyaluronic acid-conjugated pH-sensitive liposomes for targeted delivery of prednisolone on rheumatoid arthritis therapy. Nanomedicine (Lond). 2018;13(9):1037-1049. Li C, Li H, Wang Q, et al. pH-sensitive polymeric micelles for targeted delivery to inflamed joints. *J Control Release*. 2017;246:133-141.

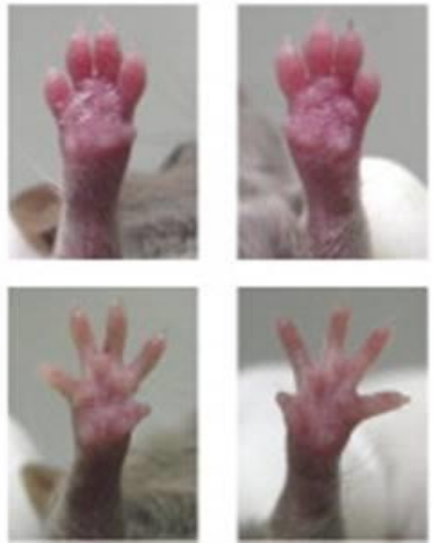
State of art: liposomes drug carriers with pH-sensitivity

Prednisolone loaded with pH-sensitive liposomes

Intravenous injection of prednisolone loaded with pH-sensitive liposomes into rats with collagen-induced arthritis led to prednisolone accumulation in affected joint tissues. In fact, when the pH-sensitive liposomes reached the acidic environment of synovial fluid, they were destabilized leading to the release of free prednisolone.

↓

Photographs of right hind limbs indicated that swelling in the hind legs was significantly milder in the rats treated with pH-sensitive liposomes than in the other animal groups. In fact, no significant difference was observed between the pH-sensitive liposomes-treated group and the healthy, untreated control group.

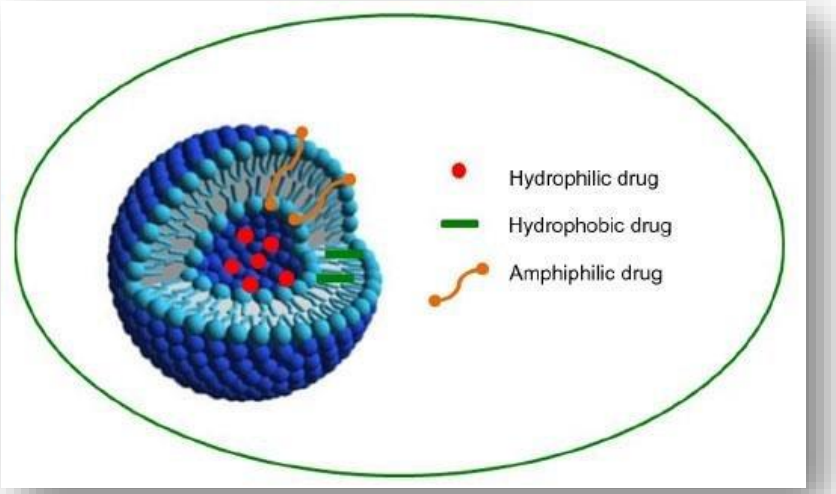


Gouveia VM, Lopes-de-Araújo J, Costa Lima SA, Nunes C, Reis S. Hyaluronic acid-conjugated pH-sensitive liposomes for targeted delivery of prednisolone on rheumatoid arthritis therapy. Nanomedicine (Lond). 2018;13(9):1037-1049. Li C, Li H, Wang Q, et al. pH-sensitive polymeric micelles for targeted delivery to inflamed joints. *J Control Release*. 2017;246:133-141.

Introduction

Niosomes

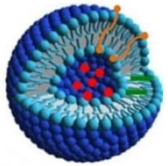
Niosomes are effective in the modulation of drug release properties; they can act as penetration enhancers through the skin. Their effectiveness is strongly dependent on their physical and chemical properties, such as formulation size, surface charge and lamellarity.



Marianecci C, Di Marzio L, Rinaldi F, et al. Niosomes from 80s to present: the state of the art. *Adv Colloid Interface Sci*. 2014;205:187-206.

Our work: pH-sensitive niosomes *in vivo*

pH-sensitive niosomes



Niosomes based on polysorbate-20 (**TW20**) or its pH-sensitive derivative, polysorbate-20 derivatized by glycine (**TW20GLY**) in comparison with:

- Veichle (**V**)
- Drugs in empty vesicles (**IBU** or **LID**)
- unstructured surfactant formulation composed of surfactant and cholesterol (**TG**).

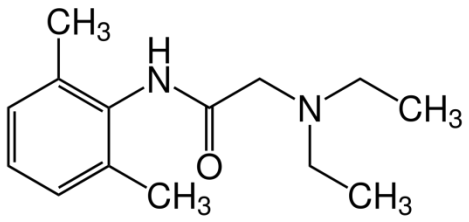
Rinaldi F, Del Favero E, Rondelli V, et al. pH-sensitive niosomes: Effects on cytotoxicity and on inflammation and pain in murine models. *J Enzyme Inhib Med Chem*. 2017;32(1):538-546.
Marzoli F, Marianecci C, Rinaldi F, et al. Long-Lasting, Antinociceptive Effects of pH-Sensitive Niosomes Loaded with Ibuprofen in Acute and Chronic Models of Pain. *Pharmaceutics*. 2019;11(2):62.

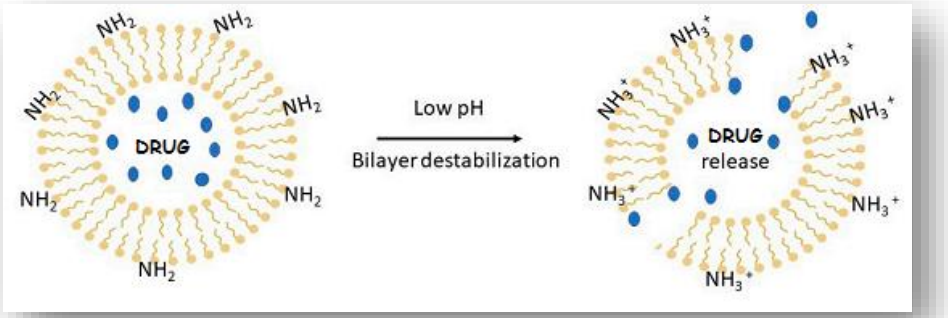
Our work: pH-sensitive niosomes *in vivo*

pH-sensitive niosomes

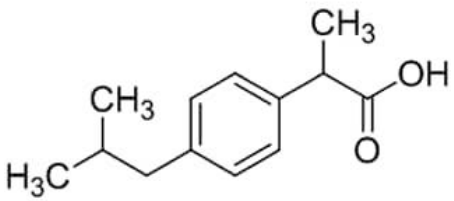
TW20 and TW20GLY were developed to deliver Ibuprofen (IBU) and Lidocaine (LID). We can see bilayer destabilization of the pH-sensitive niosomes at an acidic pH and consequent drugs release.

IBUPROFEN (IBU)





LIDOCAINE (LID)



Rinaldi F, Del Favero E, Rondelli V, et al. pH-sensitive niosomes: Effects on cytotoxicity and on inflammation and pain in murine models. *J Enzyme Inhib Med Chem*. 2017;32(1):538-546.
Marzoli F, Marianecci C, Rinaldi F, et al. Long-Lasting, Antinociceptive Effects of pH-Sensitive Niosomes Loaded with Ibuprofen in Acute and Chronic Models of Pain. *Pharmaceutics*. 2019;11(2):62.

Materials and Methods

Experiment to assess the *in vivo* efficacy of the IBU- and LID-loaded niosomes were carried out in murine models of nociception and inflammation:

- Formalin test
- Zymosan-Induced Paw Edema
- Writhing test
- Capsaicin test
- Zymosan-Induced Hyperalgesia
- Neuropathy-Induced Allodynia
- Neuropathy-Induced Hyperalgesia

Rinaldi F, Del Favero E, Rondelli V, et al. pH-sensitive niosomes: Effects on cytotoxicity and on inflammation and pain in murine models. *J Enzyme Inhib Med Chem*. 2017;32(1):538-546.
Marzoli F, Marianecci C, Rinaldi F, et al. Long-Lasting, Antinociceptive Effects of pH-Sensitive Niosomes Loaded with Ibuprofen in Acute and Chronic Models of Pain. *Pharmaceutics*. 2019;11(2):62.

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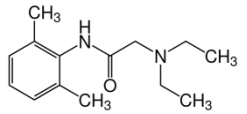
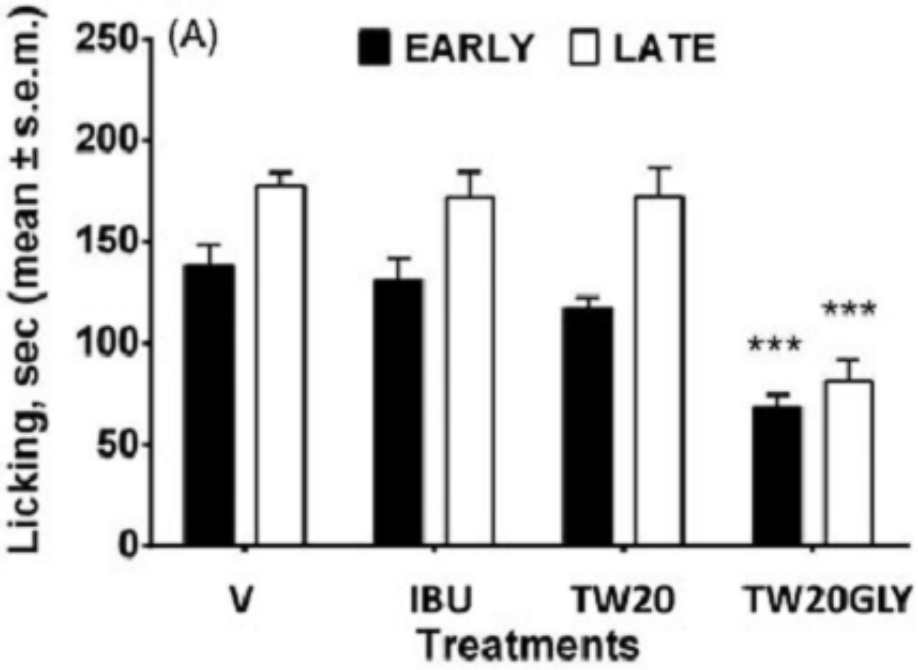
Results

IBU

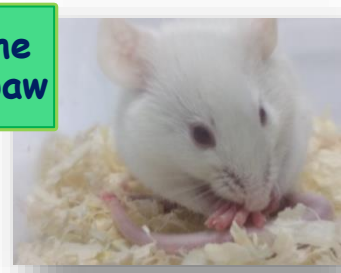


Formalin test



*** is for P<0,001 versus V. N=10.

Licking the injected paw



Subcutaneous injection of a dilute solution of formalin into the mice hind paw evokes nociception behavioral responses, which are considered indices of nociception. We reported the effects on the time spent licking the formalin-injected paw.
Samples were administered subcutaneously in the dorsal surface of mice paw 120 min before formalin in a volume of 40µL/paw.
When Ibuprofen was administered with TW20 empty vesicles or loaded in TW20 vesicles in the mice paw before formalin, we did not observe any differences in the paw licking induced by formalin. On the contrary, pH-TW20GLY niosome loaded with Ibu was able to strongly reduce licking activity induced by formalin in both test phases.

Rinaldi F, Del Favero E, Rondelli V, et al. pH-sensitive niosomes: Effects on cytotoxicity and on inflammation and pain in murine models. *J Enzyme Inhib Med Chem*. 2017;32(1):538-546.

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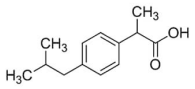
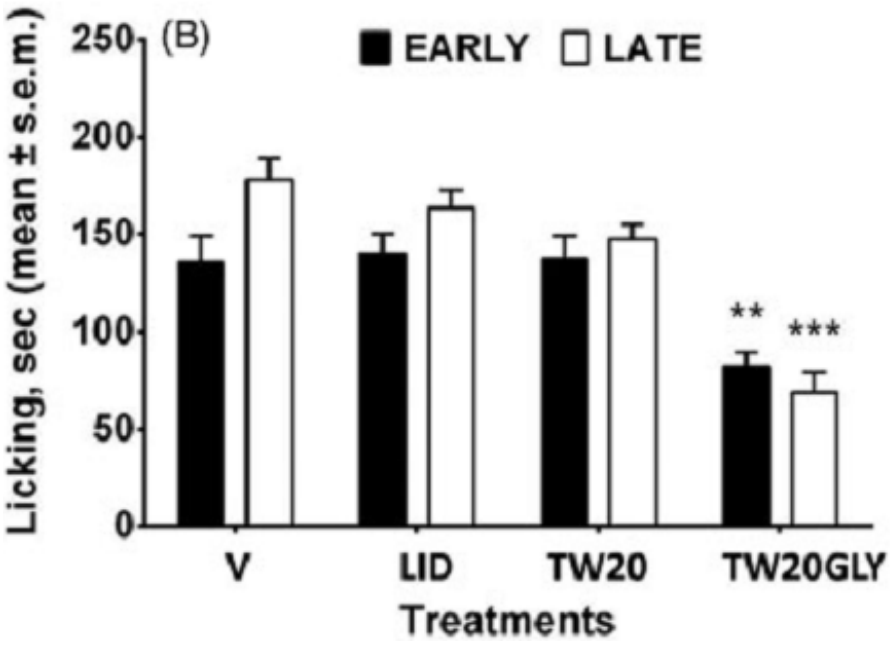
Results

LID

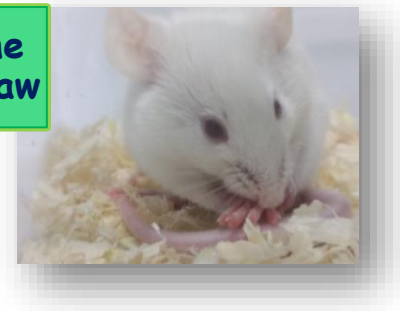


Formalin test



** is for P<0.01 and *** is for P<0,001 versus V. N=10.

Licking the injected paw



We reported the effects on the time spent licking the formalin-injected paw.
When Lidocaine was administered with TW20 empty vesicles or loaded in TW20 vesicles in the mice paw before formalin, we did not observe any differences in the paw licking induced by formalin. On the contrary, pH-TW20GLY niosome loaded with Lid was able to strongly reduce licking activity induced by formalin in both test phases.
Samples were administered subcutaneously in the dorsal surface of mice paw 120 min before formalin in a volume of 40µL/paw.

Rinaldi F, Del Favero E, Rondelli V, et al. pH-sensitive niosomes: Effects on cytotoxicity and on inflammation and pain in murine models. *J Enzyme Inhib Med Chem*. 2017;32(1):538-546.

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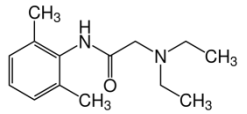
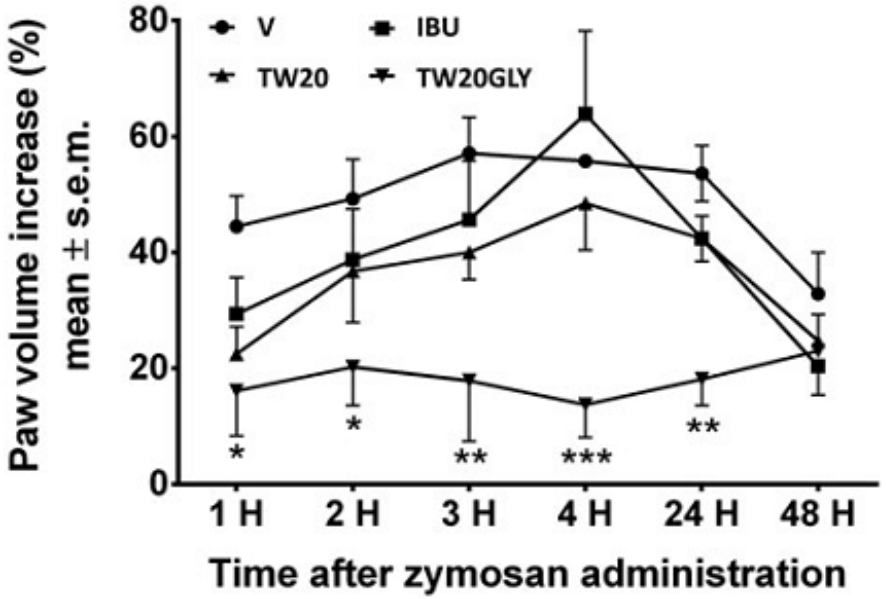
Results

IBU



Zymosan-Induced Paw Edema



*** is for P<0.05, ** is for P<0.01, and *** is for P<0,001 versus V. N=10.**

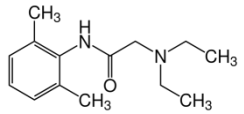
Paw volume

We evaluated the anti-inflammatory activities after a subcutaneous injection of zymosan into the dorsal surface of the paw. Paw volume was measured 3 times before the injections and at 1,2,3,4,24 and 48 hours thereafter, using a hydroplethysmometer. Samples were administered subcutaneously in the dorsal surface of mice paw 120 min before zymosan in a volume of 40µL/paw. The administration of Ibuprofen in empty vesicles or Ibuprofen in TW20 vesicles did not change paw edema increase.
When pH-TW20GLY niosome loaded with Ibu was administered before zymosan, a significant reduction in paw volume development was recorded. The reduction in paw volume increase started 1 hour after zymosan administration and was still present 24 hours thereafter.

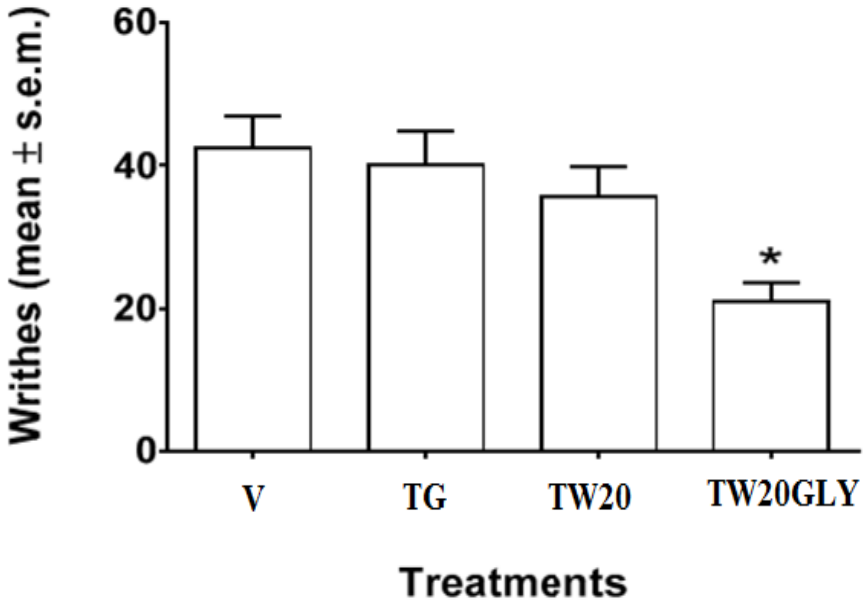
Rinaldi F, Del Favero E, Rondelli V, et al. pH-sensitive niosomes: Effects on cytotoxicity and on inflammation and pain in murine models. *J Enzyme Inhib Med Chem.* 2017;32(1):538-546.

Results

IBU



Writing test



*** is for P<0.05 versus TG. N = 8.**

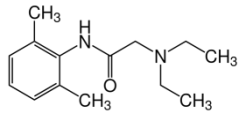
Writhes

Analgesic activity was determined by recording the decrease in the number of writhes in a 20 min period induced after intraperitoneal injection of acetic acid, used to induce peripheral pain.
Samples were subcutaneously injected 120 min before acetic acid injection. The administration of an unstructured surfactant formulation composed of surfactant and cholesterol (TG) or Ibuprofen loaded in TW20 vesicles did not change the response to acetic acid in mice.
Strong inhibition of the number of writhes was instead observed when pH-TW20GLY niosome loaded with Ibu was administered 120 min before the acetic acid.

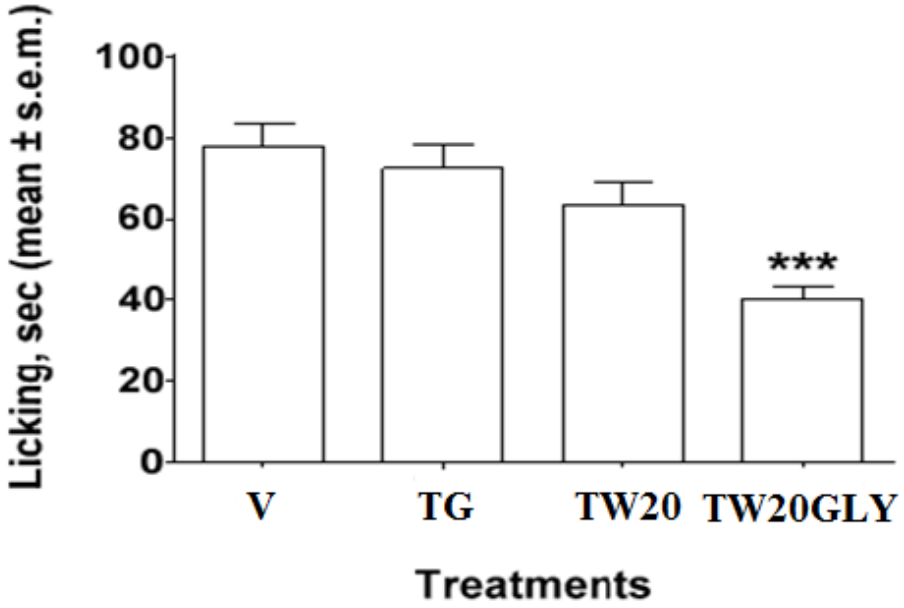
Marzoli F, Marianecci C, Rinaldi F, et al. Long-Lasting, Antinociceptive Effects of pH-Sensitive Niosomes Loaded with Ibuprofen in Acute and Chronic Models of Pain. *Pharmaceutics*. 2019;11(2):62.

Results

IBU

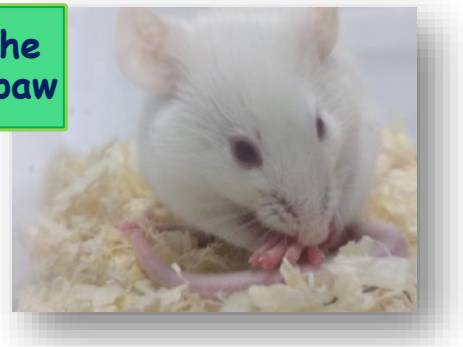


Capsaicin test



*** is for P<0,001 versus TG. N=10.

Licking the injected paw

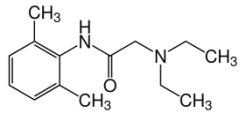


Antinociceptive activity was determined in the Capsaicin test. Samples were injected subcutaneously (40µL/paw) in the dorsal surface of the hind paw 120 min before capsaicin injection. The time the animal spent licking the injected paw, for a total period of 5 min, was registered and considered as indicative of pain. In this test, neither TG nor Ibuprofen loaded in TW20 vesicles reduced the duration of the licking response.
A statistically significant antinociceptive effect was shown for pH-TW20GLY niosome loaded with Ibu-treated mice.

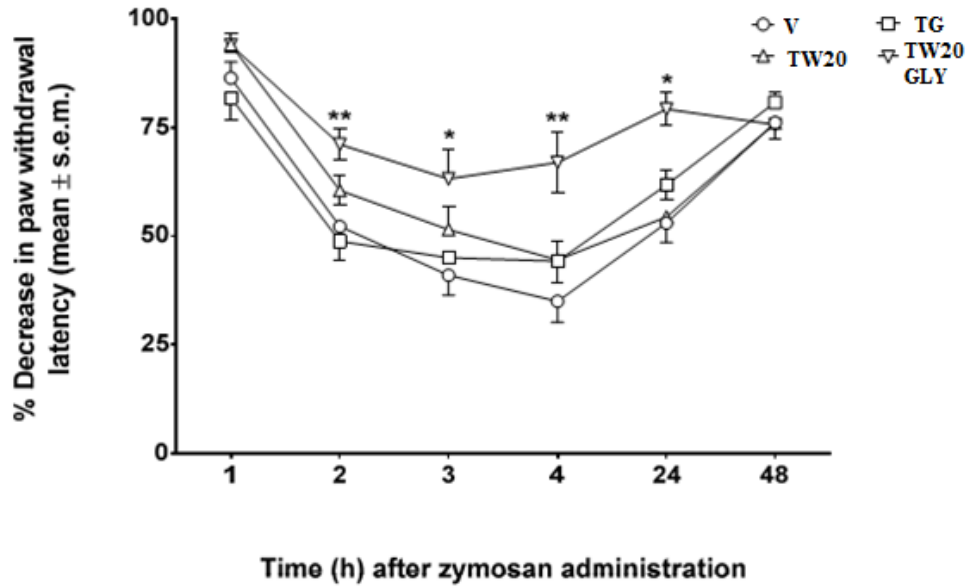
Marzoli F, Marianecci C, Rinaldi F, et al. Long-Lasting, Antinociceptive Effects of pH-Sensitive Niosomes Loaded with Ibuprofen in Acute and Chronic Models of Pain. *Pharmaceutics*. 2019;11(2):62.

Results

IBU



Zymosan-Induced thermal Hyperalgesia



*** is for P<0.05 and ** is for P<0.01 versus TG. N=10.**

Paw withdrawal latency (PWL)

A subcutaneously injection of zymosan into mice footpad induces persistent dose- and time-depend thermal hyperalgesia associated with inflammation up to twenty-four hours after treatment. Samples were subcutaneously injected (40µL/paw) in the dorsal surface of paw fifteen min before zymosan injection. Then, thermal thresholds were determined at 1,2,3,4,24 and 48 h thereafter using plantar test. The reduction in latency response to a thermal stimulus applied to a paw and induced by a zymosan was measured as a percentage.
The highest increase in pain threshold was observed in pH-TW20GLY niosome loaded with Ibu-treated mice.

Marzoli F, Marianecci C, Rinaldi F, et al. Long-Lasting, Antinociceptive Effects of pH-Sensitive Niosomes Loaded with Ibuprofen in Acute and Chronic Models of Pain. *Pharmaceutics*. 2019;11(2):62.

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Results

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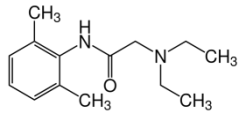
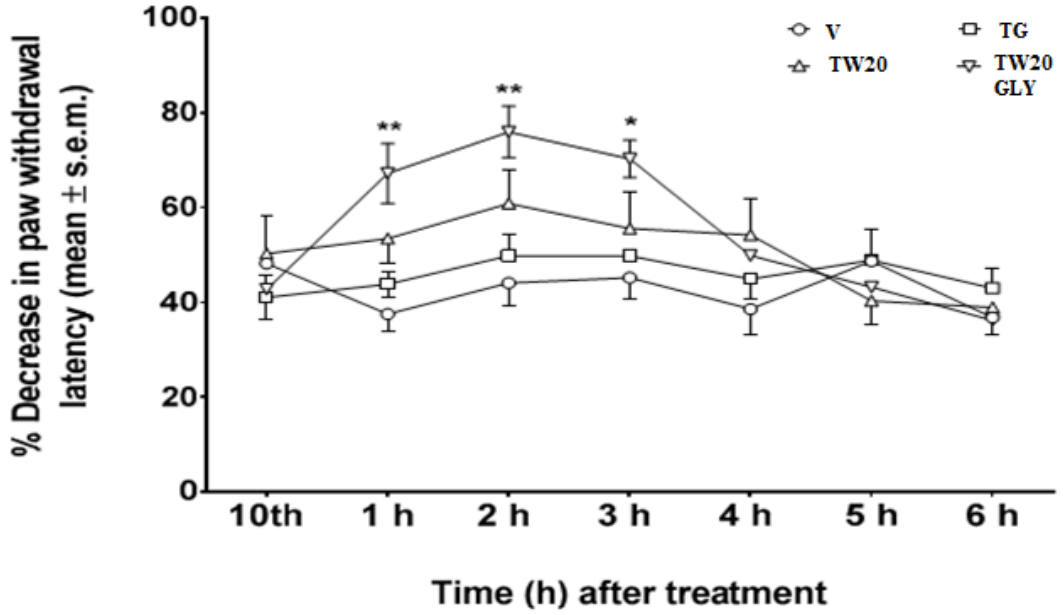
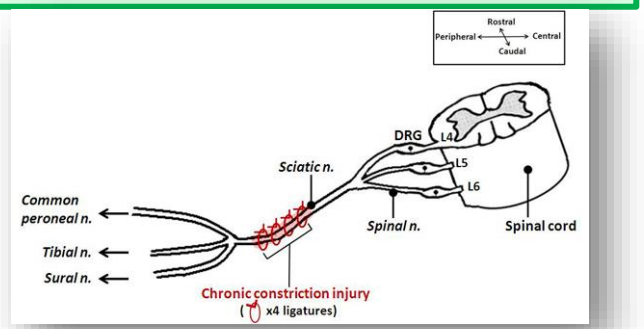


Neuropathy-Induced mechanical Allodynia



*** is for P<0.05 and ** is for P<0.01 versus TG. N = 8.**

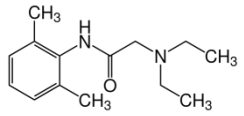


Effects on allodynia induced by a chronic constriction injury of the right sciatic nerve. Behavioral assessments of chronic constriction injury-induced mechanical allodynia and thermal hyperalgesia were carried out 10 days after nerve injury, and behavioral responses compared with the ones measured before surgery. Samples were injected subcutaneously (40µL/paw) in the dorsal surface of the hind paw.
When allodynia was measured, the results demonstrated that pH-TW20GLY niosome loaded with Ibu significantly increased paw withdrawal latency from 1 to 3 h after treatment. The thresholds for mechanical allodynia was assessed with the dynamic plantar aesthesiometer.

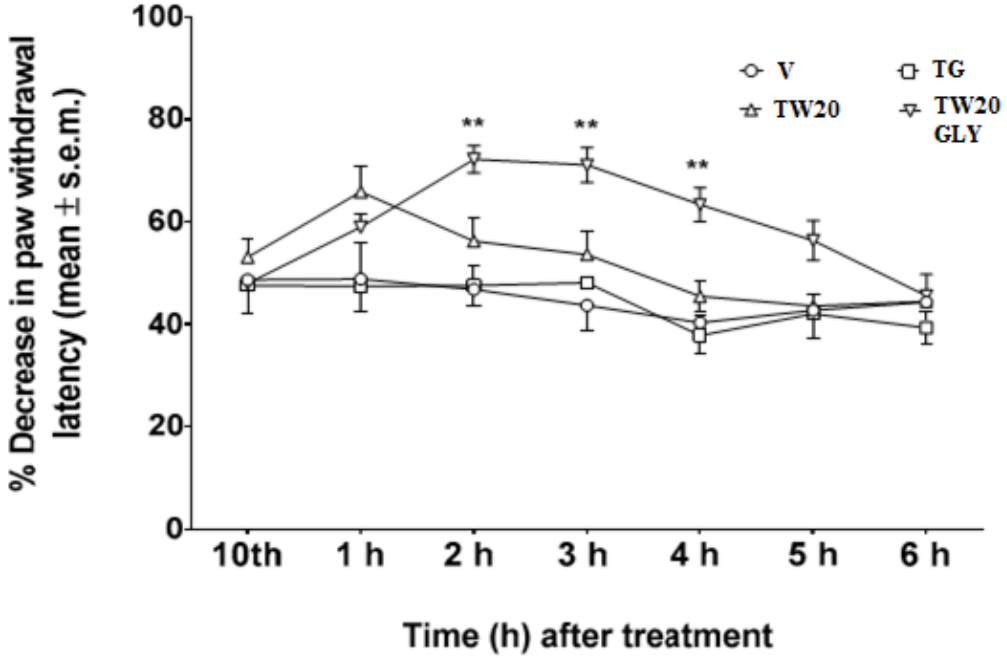
Marzoli F, Marianecci C, Rinaldi F, et al. Long-Lasting, Antinociceptive Effects of pH-Sensitive Niosomes Loaded with Ibuprofen in Acute and Chronic Models of Pain. *Pharmaceutics*. 2019;11(2):62.

Results

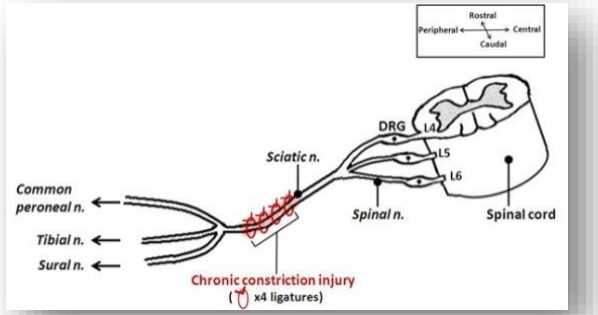
IBU



Neuropathy-Induced thermal Hyperalgesia



*** is for P<0.05 and ** is for P<0.01 versus TG. N = 8.**

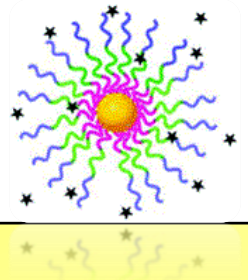


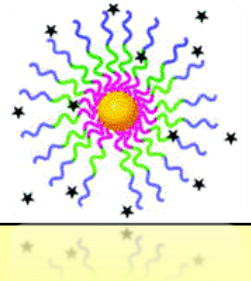
Effects on hyperalgesia induced by a chronic constriction injury of the right sciatic nerve. When hyperalgesia was measured with plantar test, pH-TW20GLY niosome loaded with Ibu increased the pain threshold from 2 h up to 4 h after treatment. Samples were injected subcutaneously (40µL/paw) in the dorsal surface of the hind paw.

Marzoli F, Marianecci C, Rinaldi F, et al. Long-Lasting, Antinociceptive Effects of pH-Sensitive Niosomes Loaded with Ibuprofen in Acute and Chronic Models of Pain. *Pharmaceutics*. 2019;11(2):62.

Conclusions

pH-sensitive liposomes and niosomes: efficient delivery systems for analgesic and anty-inflammatory drugs.





- **pH-sensitive liposomes:** efficient delivery systems for prednisolone in rheumatoid arthritis therapy.
- **pH-sensitive niosomes:** efficient delivery systems for Ibuprofen and Lidocaine in acute and chronic inflammation associated with pain.

Thanks for your attention!

Minosi P[1], Rinaldi F[2], Hanieh PN[2], Marianecci C[2], Marzoli F[1], Ciarlo L[1], Carafa M[2] and Pieretti S[1]

[1] National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, 00161, Rome, Italy.

[2] Department of Drug Chemistry and Technologies, Sapienza University, 00185 Rome, Italy.