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Review on : Micro- and Nanocarriers for pain alleviation

Authors : Alexandre da Silva, Sinda Lepetre-Mouelhi, Patrick Couvreur

Contact : alexandre.da-silva@universite-paris-saclay.fr ; sinda.lepetre@universite-paris-saclay.fr
; patrick.couvreur@universite-paris-saclay.fr

Institution : Université Paris-Saclay, CNRS, Institut Galien Paris-Saclay, 92296, Châtenay-Malabry, France.

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Abstract

Acute or chronic pain is a major source of impairment in quality of life and affects a substantial part of the population. To date, pain is alleviated by a limited range of treatments with significant toxicity, increased risk of misuse and inconsistent efficacy, owing, in part, to lack of specificity and/or unfavorable pharmacokinetic properties. Thanks to the unique properties of nanoscaled drug carriers, nanomedicine may enhance drug biodistribution and targeting, thus contributing to improved bioavailability and lower off-target toxicity. After a brief overview of the current situation and the main critical issues regarding pain alleviation, this review will examine the most advanced approaches using nanomedicine of each drug class, from the preclinical stage to approved nanomedicines.

Introduction

Pain is defined by the International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage [1]. This subjective experience relies on the integration of diverse nervous signals, or the lack thereof, emanating from the peripheral nervous system (PNS), and processed in the central nervous system (CNS) (Fig. 1). Pain is often classified by its etiology (nociceptive, neuropathic, central or psychogenic) and its duration (acute, chronic). Prevalence of pain is estimated at 25% of the general population and is higher among the elderly [2], [3]. When intense and/or chronic, pain can dramatically worsen patient's physical and mental health. Given its high prevalence and its tremendous impact on the quality of life, chronic pain can be considered as a disease *per se*, deserving dedicated management and treatment [4], [5].

To date, pain management includes pharmacological treatments, completed by physical (massage, exercise) or psychological (relaxation techniques) therapies. Drug-based strategies depend on the type and the intensity of the pain. Firstly, the well-known pain ladder, created by the World Health Organization (WHO) as a guideline for the use of classic analgesics, is composed of three steps. Step 1 includes acetaminophen and non-steroidal anti-inflammatory agents (NSAIDs) acting as cyclooxygenase (COX) inhibitors for mild pain ; step 2 comprises weak (codeine, tramadol) opioids for moderate pain ; and step 3 contains strong opioids (morphine, oxycodone, buprenorphine, fentanyl), used to treat severe pain. However, opioids, reintroduced in the 1980s, rapidly became the source of a major public health crisis, due to their severe sedating side effects. In the U.S., the authorization of increasingly powerful opioids, such as fentanyl, combined to their widespread misuse has led to a dramatic increase in addiction and fatal overdoses, with an annual death toll of nearly 50,000 in 2019 [6]–[8]. These serious drawbacks raised questions about the relevance of the current WHO pain ladder, leading some physicians to suggest a 4-step ladder [9], [10]. And above all, the lack of safer and effective alternatives to strong opioids remains an important medical concern. Moreover, some types of pain, mainly neuropathic pain, are not relieved by classic analgesics. Some antidepressant [11] and antiepileptic drugs [12] may relieve fibromyalgia, postherpetic neuralgia or other undetermined central or peripheral neuropathic or cancer pain [13], but only in a limited number of patients [14]. The same applies for recently authorized cannabinoid drugs (CBD, THC and synthetic analogues), whose efficacy on severe cancer pain, central pain and muscle spasticity pain remains uneven [15]–[19]. Lastly, local anesthetic drugs, which block nociceptive and part of non-nociceptive signaling, are an integral part of surgical and short-term post-surgical pain management [20].

The limited number of available treatments to alleviate pain and their lack of efficacy is indicative of the urgent need to develop safer and more effective analgesics. In this respect, research has been actively focusing on several targets involved in nociception and pain right after the identification of important pain signaling pathways. For instance, the discovery of opioid receptors and their endogenous ligands in 1974 [21]–[23] gave rise to the development of strategies aimed at potentializing the enkephalin-induced analgesia. In 1991, the identification of COX2 has initiated the development of COX2-specific inhibitors in order to decrease NSAIDs gastrointestinal side effects. Another example is the discovery of sodium channels in the 1960s and 1970s [24] and the subsequent identification of the mechanism of

action of already available drugs interfering with sodium channel blockers (SCBs). In that respect, local anesthetics or drugs primarily developed for non-analgesic purposes, represented the starting point of many clinical trials assessing their efficacy in neuropathic pain alleviation [25]. However, none of these drugs has revolutionized the treatment of severe or chronic pain.

Used as a treatment in an increasing number of diseases, nanomedicine can address some of the critical issues met with the aforementioned drugs. Firstly, thanks to their ability to transport the active pharmaceutical ingredient (API) either on their surface or into their core, nanocarriers allow improved biodistribution of many drug molecules or grant a reinforced protection against metabolization of fragile molecules, such as peptides. In addition, nanocarriers, whose size ranges between a few to several hundreds of nanometers, with spherical, rod, polygonal or sheet shapes, display different interactions with cells and tissues compared to free molecules. On one hand, their larger dimensions than single molecules prevent them from crossing some biological barriers. This property is exploited when nanocarriers are injected intravenously: vascular endothelium, mostly formed by a continuous wall, can only be crossed when it is fenestrated or when its basement membrane is incomplete, which is the case in some organs (liver, spleen, kidneys...) but also in neovascularized solid tumors. Called EPR (enhanced permeability and retention effect), this effect, although controversial [26], is exploited to accumulate nanoparticles carrying the transported API at the desired sites. A similar effect exists around inflamed tissues, due to endothelial tight junctions loosening. On the other hand, the surface of nanoparticles (NPs), when properly functionalized, allows an increased and/or a more specific binding to a tissue or a cellular population. Both characteristics contribute to a better targeting and limit the off-target release of the API, thus reducing systemic toxicity. Finally, NPs are capable of controlling the release of the API over the time. Depending on their composition, nanocarriers can undergo endocytosis, either by macrophages or by cells including those expressing high contents of LDL receptors (especially for lipidic nanoparticles). NPs can be eroded by enzymes or by a change of physical or chemical conditions (pH, temperature). These properties turn nanoparticles into tunable on-demand drug delivery devices, allowing more controlled and/or sustained delivery of drugs, which can be useful for acute or chronic pain relief. This review will examine the contributions of nanomedicine in the improvement of the treatment of pain, from the preclinical level to late-stage clinical trials and approved nanomedicines (Fig. 2 and 3). As pharmacokinetic (PK) and toxicity concerns are rather specific to each drug class, contributions will be listed by pharmacotherapeutic category.

Abbreviations

ADME-Tox : absorption – distribution – metabolism – excretion and toxicity

API : active pharmaceutical ingredient

AUC : area under the curve

cDNA : complementary desoxyribonucleic acid

CNS : central nervous system

COX : cyclooxygenase

DMPK : drug metabolism pharmacokinetics

MOP-R, DOP-R, KOP-R, NOP-R : μ , δ , κ , and nociceptin receptors

mRNA : messenger ribonucleic acid

NP : nanoparticle

NSAID : nonsteroidal anti-inflammatory drug

PCL : polycaprolactone

PEG : polyethylene glycol

PLGA : poly(lactic-co-glycolid acid)

PNS : peripheral nervous system

PK : pharmacokinetics

ROS : reactive oxygen species

SCB : sodium channel blocker

SNRI : serotonin and noradrenalin reuptake inhibitor

TCA : tricyclic antidepressant

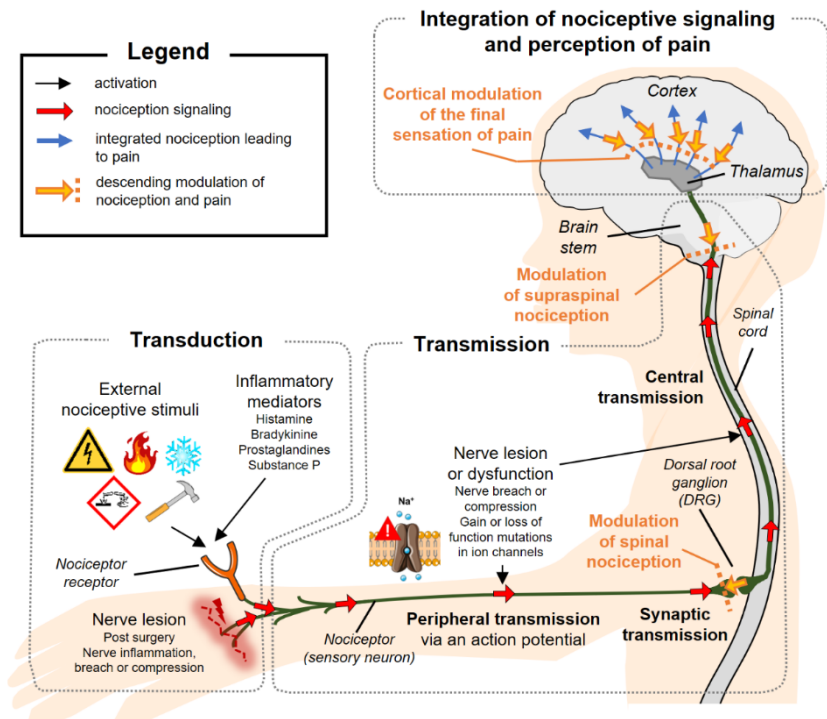


Figure 1 – Overview of ascending pathways of pain perception.

Pain is the result of the integration of nociceptive signaling (in red) and its modulation at several steps (in orange), mainly at the spinal (dorsal root ganglion) and supraspinal (mesencephalic) level. Nociceptive pain can arise from the activation of nociceptors in the skin, mucous membranes tissues and some internal organs by external stimuli (thermal, mechanical, chemical, electrical noxious stimuli), or by internal stimuli (inflammation mediators), both relying on a healthy nervous system. Other nociceptive signaling can emanate from a lesion (inflammation, compression, breach) or a conduction disorder within the sensory nerve, which results in neuropathic pain. Some pain occurrences, such as cancer pain, fall in both categories, hence the qualification of mixed pain. Lastly, nociplastic (or central) pain originates from a dysfunctional integration of nociception and/or descending modulation of nociception or pain in the central nervous system (brain and spinal cord); their underlying pathophysiology remains poorly understood.

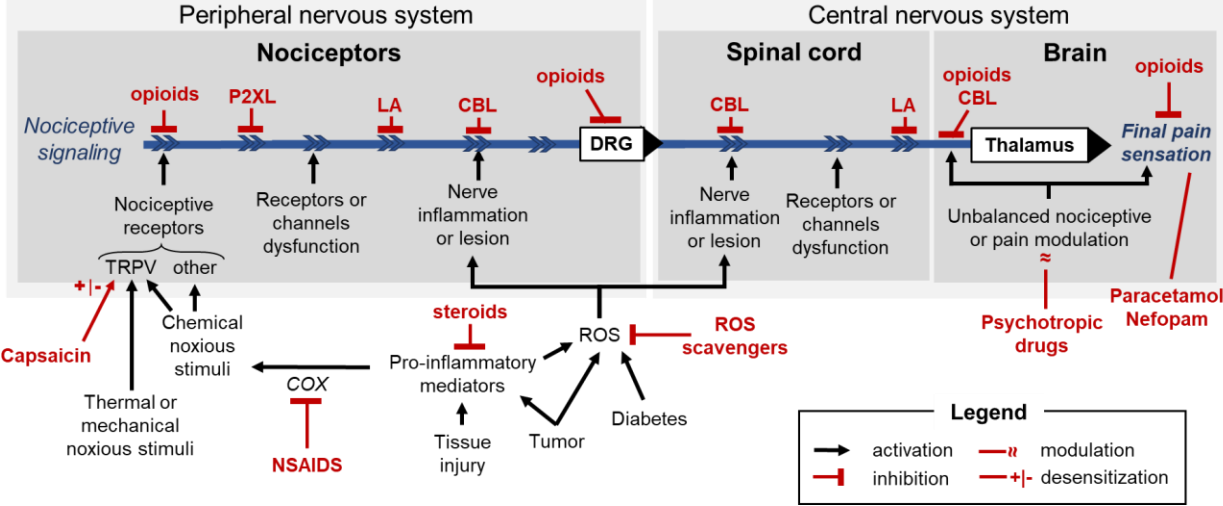


Figure 2 – Signaling pathways exploited by nanomedicine for pain relief

From the inhibition of the upstream noxious inflammation to the final blockage of the sensation of pain after the integration of the nociceptive signaling in the thalamus (in blue), several strategies have been investigated to alleviate the pain. Depending on the location of the target protein, channel or receptor, the final efficacy and side effects can greatly vary. P2XL: purinergic ligands; LA: local anesthetics; ROS: reactive oxygen species; CBL: cannabinoid ligands; COX: cyclooxygenase; DRG: dorsal root ganglion; TRPV: transient receptor potential vanilloid

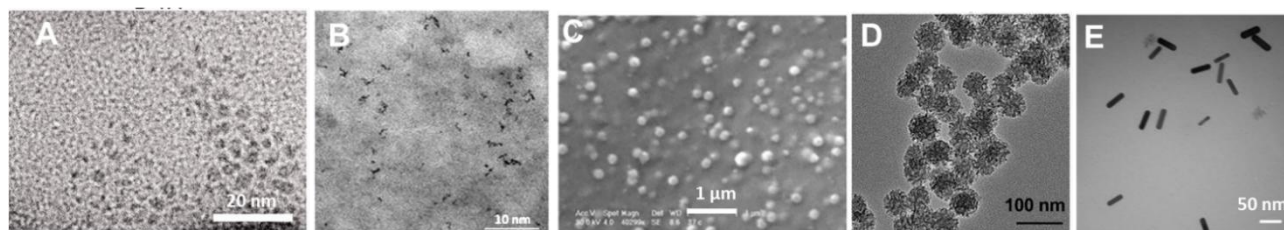


Figure 3 – Examples of nanoparticles used in pain alleviation. Lipidic, polymeric and metallic nanoparticles were all represented in the nanomedicine-based pain alleviation strategies. The pictures above are transmission electron microscopy (TEM) observations. (A) Triamcinolone-conjugated polyamidoamide dendrimers [28]. (B) Pluronic® F127-reduced graphene oxide carrying buprenorphin, using strong π - π interactions between the drug and the graphene oxide layers [29]. (C) PLGA-PEG polymeric nanoparticles, exploited to deliver CB13 [30]. (D) Hybrid liposome-mesoporous silica nanoparticles containing DADLE [31]. (E) Gold nanorods attached to liposomes carrying tetrodoxin. The gold nanorods convert near infrared light into heat, enabling a phase transition of the liposomes' lipid bilayers, ultimately leading to a faster drug release [32].

1 - Opioids

Opioids are ligands of the opioid receptors (OP-R), which belong to the family of 7-transmembrane spanning G-protein coupled receptors. Opioid receptors exist in 4 subtypes : μ (MOP-R), δ (DOP-R), κ (KOP-R) and nociceptin (NOP-R) [33]. They are mainly distributed in nervous tissues (central and peripheral nervous system) as well as in some immune cells (dendritic cells, monocytes, macrophages, ...), glands (adrenal glands, pancreas), organs (lungs, heart, liver, small intestine) and some skeletal muscles [33]–[37]. Their activation generates specific pharmacological effects (see Tab.1, [38]). Most of the opioids used in medicine, both opiates (derived from the opium, including morphine, codeine, buprenorphine or oxycodone) and synthetic opioids (fentanyl, tramadol, methadone) are agonists or antagonists that preferentially bind to MOP-R. This binding not only generates the desired intense analgesia but may also induce addiction, tolerance, sedation and respiratory depression (Tab. 1). The occurrence of these severe side effects is favored by two main factors. Firstly, the opiates are able to easily cross the blood-brain barrier and reach the central nervous system (CNS) and local opioid receptors involved in the reward system or in the autonomous control of ventilation. Secondly, the frequent misuse of these drugs leads to a rather rapid dose escalation, either due to increased tolerance or to recreational use when taken as a replacement of other psychoactive drugs.

Table 1- Main pharmacological effects of MOP-R, DOP-R and KOP-R agonists.

The intensity of the referred effects is expressed as a quantitative scale :

+ : minor, ++ :
[38].

mild, +++ : intense.

Effect or site of action	MOP-R (μ)	DOP-R (δ)	KOP-R (κ)
Analgesia	Supraspinal Spinal	Supraspinal Spinal	Spinal
Myosis	++	++	
Respiratory depression	+++	++	+
Diuresis	Antidiuretic		++
Constipation	++	++	
Smooth muscles	Spasm	Spasm	
Behavior	Euphoria Sedation ++	Euphoria Sedation ++	Dysphoria Sedation +
Physical dependence	++	++	+

1.1 - Opiates and synthetic opioids MOP-R opioid agonists

Several attempts have been made to limit the occurrence of opioid overdoses, all of them relying on sustained-release formulations that allow more stable plasmatic concentrations compared to classic forms. In that respect, we can find oral morphine sulfate sustained-release or extended-release tablets or capsules (e.g. Avinza, Kadian, Skenan LP [39], [40], and their approved generics), and controlled-release tablets with oxycodone (OxyContin), made with polymer-coated drug-containing beads or with hydrophilic polymer matrix [41]–[43]. Even longer deliveries, associated with a greater comfort and safety of use, are made possible using transdermal patches with buprenorphine or fentanyl [44].

Nanomedicine is also taking part in this endeavor. Controlled or sustained release were performed using very diverse nanocarriers (polymer, lipid, inorganic NPs) and their drug-release properties assessed both *in vitro* and *in vivo*. For example, morphine, the reference opioid, once encapsulated in liposomes, was able to relieve sciatic nerve ligation induced pain in Sprague-Dawley (SD) rats up to 7 days after a single subcutaneous injection near the ligation [45]. In a similar study, Planas *et al.* compared morphine encapsulated in liposomes to its free form when injected subcutaneously at a distant site, thus highlighting prolonged antinociception (8 h versus 4 h) and anti-inflammatory effect [46], which could act as a synergy in pain relief [47]. Another example of a vectorized form of morphine is its complexation with dendrimers. Ward *et al.* synthesized esterase-sensitive morphine prodrugs with aliphatic or aromatic ester linkers, which were then complexed with generation 5 poly(amidoamine) dendrimers. Such NPs displayed very disparate release profiles and nociception in rats and guinea pigs depending on prodrugs linker. Parenteral administration of the prodrug with aliphatic ester linker led to 6h of sustained analgesic effect [48]. Finally, despite their dimensions exceeding the nanometer scale, with an average diameter of 20 μm , patented DepoFoam liposomes containing morphine sulfate (DepoDur) were authorized in the U.S. The epidural administration of these liposomes were indicated for the treatment of lower limb post-surgery or cesarean section pain [49] but were discontinued without any declared reason [50].

Among other commonly used opioids, buprenorphine was also formulated into NPs for nociception experiments. In that respect, Wang *et al.* used the intravenous route to inject oil/fatty ester nanostructured lipid carriers, with buprenorphine and buprenorphine propionate, leading to both increased and prolonged analgesic effect in rats [51]. In another study, Zhang *et al.* incorporated buprenorphine into reduced graphene oxide nanosheets stabilized by pluronic F127, to further

formulate them as an improved hydrogel transdermal patch ; strong π - π interactions between the graphene oxide and the buprenorphine ultimately slowed down the drug release by extending analgesia to 15 days, compared to the 10-day efficacy without nanosheets in cold ethanol tail flick model [29]. Furthermore, bioerodable lipid hybrid NPs, designed by Zhu *et al.*, and composed of poly(carboxyphenyl propane co-sebacic acid):poly(lactic-co-glycolic) acid in 50:50 proportion, were chosen to encapsulate ketamine with a drug loading rate of 33%. These NPs enabled an impressive 28-day sustained release *in vitro*, and resulted in an anti-allodynic effect lasting up to 3 days on sciatic nerve-constricted mice after a single intrathecal injection of the NP suspension [52].

Nanomedicine has also been used to increase drug bioavailability or to speed up drug diffusion. For instance, Touitou *et al.* used the intranasal route in rats to deliver butorphanol, tramadol from soft nanovesicles. The peak concentration of these drugs in the plasma and in the brain was 2 to 5 times higher than with non-encapsulated forms only 10 min after the drug intake [53]. On the other hand, Chen *et al.* demonstrated that loperamide, an antidiarrheic opioid devoid of analgesic effect when taken orally, was able to significantly improve relief of formalin-induced inflammatory pain in mice after IV administration, when encapsulated in polysorbate 188-coated PLGA-PEG-PLGA NPs [54]. Both authors pointed out the advantages of having fast and increased peak plasma drug concentrations or increased drug concentrations in the brain. In fact, these NPs can be very useful in the case of acute and intense pain which require fast-acting analgesics, or can also be used as adjuvant of chronic analgesic treatment. However, as this efficacy essentially relies on the opioid receptors located in the CNS, side effects such as respiratory depression or later tolerance and addiction caused by high concentrations of MOP-R agonists, are likely to occur.

Given the serious concerns related to centrally induced analgesia, the idea of generating analgesia restricted to the peripheral opioid receptors has gained interest. Along with opioid analogues which are effluxed out of the CNS by p-glycoproteins or ionized analogues at physiological pH that are unable to cross the blood-brain-barrier (BBB) [34], [55], nanocarriers could also be used to hinder the BBB passage of opioids. Only a few studies have really demonstrated preferential or exclusive peripheral targeting of nanoparticles carrying MOP-R agonists. But in a noteworthy paper, González-Rodríguez *et al.* have developed dendrimer-like nanocarriers, using hyperbranched polyglycerol, covalently bound to morphine. Intraplantar or intravenous administration of these nanocarriers in a complete Freund's adjuvant (CFA)-induced paw inflammation pain in rats was found to display similar or better analgesic activity than morphine but without sedation or constipation. Nevertheless, these experiments would have deserved to last longer than 60 min in order to better compare the duration of action. This peripheral-only activity was confirmed by microdialysates showing negligible concentrations of morphine in the blood and the brain compared to concentrations found in the inflamed paw, when nanocarriers were injected using both routes of administration. These results suggest that this nanomedicine accumulated in inflamed tissues owing to an EPR-like effect [56]. Another use of targeted delivery capabilities of nanocarriers in the same pain model has been exploited by Hua *et al.* using "immunoliposomes". These are conventional liposomes grafted with anti-ICAM1 (intercellular adhesion molecule-1) monoclonal antibody, an adhesion factor contributing to the migration and interaction of opioid-containing immune cells towards inflamed tissue and sensory neurons [57]. When loaded with loperamide hydrochloride, these immunoliposomes exerted a prolonged antinociceptive effect (more than 50h), compared to a total absence of effect both from non-ICAM1-grafted loperamide-loaded liposomes and free loperamide. Moreover, these immunoliposomes allowed significantly higher concentrations of loperamide in the paw and lower in other organs, including the brain, in comparison with those found with conventional liposomes. Besides, their antinociceptive effect was abolished after beforehand administration of peripheral-only opioid antagonist, the naloxone methiodide, validating the exclusively peripheral activity of the treatment [58]. To the best of our knowledge, this encouraging results still have not resulted in any clinical study.

1.2 - Endogenous opioids and analogues

Human endogenous opioids are made up of 3 main families of peptides: β -endorphin, derived from the proopiomelanocortin, enkephalins (Leu- and Met-enkephalins), which precursor is the preproenkephalin, and dynorphins (dynorphin A, B and neo-dynorphins), the final products of the prodynorphin. These neuropeptides preferentially bind respectively to the MOP-R, DOP-R and KOP-R. DOP-R peptide agonists (enkephalins) are of particular interest by virtue of their lower undesired side effects, i.e. less gastrointestinal disorders [59], lower drug abuse potential [60], reduced respiratory depression [61] and cognitive impairment [62]. Furthermore, enkephalins, such as other drug peptides, possess high specificity and efficacy, with low off-target toxicity [63]–[65]. However, they are prone to fast metabolization, leading to an extremely short half-life (not exceeding 3 min in plasma [66], [67]), which greatly reduces its drugability in its original form.

Several strategies have thus been elaborated to exploit pharmacological properties of enkephalins. In the 1970s and 1980s, many enkephalin analogues have been synthesized, either by modifying the peptide sequence, by adding non-peptide moieties [21], [68]–[71] or by cyclizing their primary structure [72]–[74]. Unfortunately, these methods only resulted in one clinical trial, aborted due to poor tolerance [68]. Another strategy was to potentialize the endogenous enkephalin signaling using enkephalinase inhibitors [75]–[82]. As regards this category, racecadotril was authorized, but

only as an antidiarrheic drug, and three other candidates (PL37, PL265 and STR-324) are currently undergoing clinical trials [83]–[85]. Of note, opioidin, an endogenous peptide present in human saliva, and which originated the STR-324, were loaded in PEGylated liposomes by Mennini *et al.* Compared to the peptide free, this vectorization attempt resulted in an increased bioavailability (up to 80% of increase in AUC) and a prolonged analgesic activity (+50%) in rats using IV route of administration [86].

Therewith, increased protection of enkephalins or its analogues against circulating enzymes can be performed through vectorization, achieving both enhanced distribution and efficacy. Given the high lability of these peptides, some researchers focused on entrapment efficiency (EE) and controlled release. Increased EE was achieved with polymeric NPs, such as methoxyPEG-PLGA NPs, which showed to be able to encapsulate dalargin with EE up to 91% [87]. Controlled release was attempted with Met-enkephalin in RGD grafted PEG-poly(methylmethacrylate) (PEG-PMMA) thermoresponsive NPs with a phase transition at 38 to 40°C, allowing selective NP degradation and drug delivery to tumors, with higher temperature than normal [88]. As regards liposomes, the use of different surface charges alone or in combination with iontophoresis enabled an enhancement of entrapped Leu-enkephalin delivery *ex vivo* through human skin [89], [90]. However, none of these strategies have been translated to *in vivo* pain alleviation applications.

Besides *in vitro* assays, bioavailability, distribution and efficacy of entrapped enkephalins were investigated on nociceptive pain. For example, Schroeder *et al.* demonstrated that dalargin-loaded poly(cyanobutylacrylate) NPs were able to cross the BBB. This passage was enhanced by polysorbate 80 coating that allowed a 3-fold increase in dalargin concentration in the brain, and enabled an antinociceptive effect after a single intravenous injection in mice [91]. More recently, we administered Leu-enkephalin, as squalene-coupled prodrug-based NPs, allowing much higher drug loading (55%) than polymeric NPs (usually under 5%). A unique intravenous injection of these squalene-based NPs on paw-inflamed rats enabled a longer analgesia than morphine. The abolition of the pain alleviation in the presence of naloxone methiodide, as well as the accumulation of these NPs in the inflamed paw without reaching the brain, highlighted a peripheral-only induced analgesia (Fig. 4) [92]. Furthermore, the underlying signaling pathways triggered by DOP-R agonist binding were confirmed by Jimenez-Vargas *et al.* In a mice model using DADLE ([D-Ala2, D-Leu5]-enkephalin loaded in liposomes with a silica mesoporous shell. The intravenous administration of these NPs near the dorsal root ganglion innervating the intestine led to the inhibition of the nociceptive signaling emanating from intestinal colitis. This effect was associated with a preferential endocytosis of DADLE-containing liposomes by the DOP-R expressing cells. Moreover, the endosomal signaling by protein kinase C (PKC), among others, was significantly reduced [31].

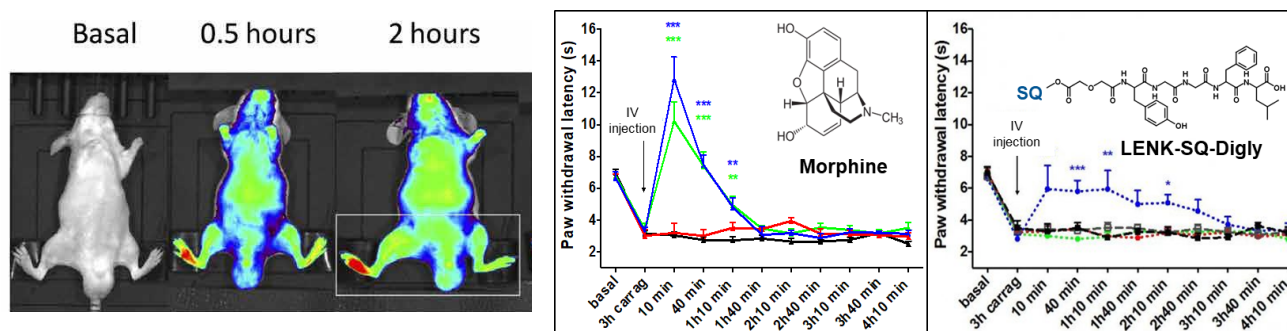


Figure 4 – An example of targeted delivery of opioids for inflammation pain treatment: the accumulation of Leu-enkephalin-squalene nanoparticles in a carrageenan-induced paw edema.

Injected intravenously, these NPs displayed a peripherally restricted anti-hyperalgesic effect and were able to deliver the Leu-enkephalin preferentially in the inflamed tissue (DiD dye labeled NPs, left). Their efficacy was measured using the Hargreaves test (thermal plantar test) in a carrageenan-induced hyperalgesia model. Compared to morphine, this nanomedicine carrying enkephalin was able to relieve pain for longer durations, by restoring the normal levels of pain sensitivity (right, paw withdrawal latencies, morphine vs. Leu-enkephalin-squalene NPs) [92].

Whether pain relief is central or peripheral, most of studied cases rely on an invasive intravenous or local injection, raising some concerns about the occurrence of infections or the drug compliance by the patient, especially in the context of chronic pain. In this respect, the most convenient route of administration of analgesic drugs should be safe, minimally invasive, versatile and easy-to-use. Transdermal and intranasal routes cited above meet these criteria. However, skin, and particularly the stratum corneum, remains hard to cross, while nasal route, although richly vascularized, suffers from the small exchange surface and constant mucociliary clearance. The intranasal route was explored by Godfrey *et al.* taking advantage of the short distance between the nasal epithelium and the brain. In that respect, a single series of Leu-enkephalin-loaded chitosan NPs instillations resulted in a 4h central analgesia on CFA-induced paw inflammatory pain [93]. Regarding the oral route, it is the most used and the most obvious administration route. However, the harsh conditions of the gastrointestinal tract still represent an unresolved challenge for the administration of peptides [94]. Yet,

Schroeder *et al.* reused dalargin loaded poly(butylcyanoacrylate) by oral route and obtained analgesia lasting over 2 h, with similar pharmacodynamic profiles than dalargin NPs injected intravenously. Even though authors claimed that BBB passage was enhanced, this assertion was not confirmed without biodistribution or opioid antagonist assays. [95]. The most unexpected results are from Lalatsa *et al.*, who managed to reach measurable and prolonged nociceptive pain relief (8 h) with quaternary ammonium palmitoyl glycol chitosan NPs carrying Leu-enkephalin or Tyr-palmitoyl-enkephalin after a single oral intake in Balb/C mice. Pharmacokinetic studies in rats showed an increase of Leu-enkephalin bioavailability by 67% [96]. Given the very short half-lives of both peptides and the lack of any efficacy when taken in their free form, these surprising outcomes are hardly conceivable without an intact passage of NPs through the gastrointestinal wall and could represent potential breakthroughs. However, since 2012, no further studies have been published and those results were not confirmed so far. Overall, endogenous opioids and opiates-based nanomedicines are still currently blocked at the experimental stage but certainly deserves to be encouraged.

Table 2 – Opioid micro- and nanomedicines displaying pain relief.

Abbreviations : d_{HD} : hydrodynamic diameter ; ZP : zeta potential ; EE : encapsulation efficiency ; DL : drug loading ; CFA : Complete Freund's adjuvant ; CCI : chronic constriction injury ; SNL : spinal nerve ligation ; adm.: administration ; dev. : development ; PEG : polyethylene glycol ; PLGA : poly(lactic-co-glycolid acid) ; CPP-SA : poly-carboxyphenoxy propane co-sebacic acid ; SC subcutaneous

Active molecule	NP composition or type	d_{HD} (nm)	ZP (mV)	EE (%)	DL (%)	Route of adm.	Targeted pain (pain model) / Pain test	Furthest stage of dev.	Main benefits	Limitations	Ref.
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; IV : intravenous ; TD : transdermal ; IT : intrathecal ; IN : intranasal ; PO : per os

Morphine (Depodur®)	Liposomes (Depofoam®)	17-23x10 ⁵	-	-	-	SC, epidural	Nociceptive pain	Phase 4 - discontinued	Sustained release and pain alleviation	Micrometer size	[97]
Morphine (prodrug)	Polyamidoamide dendrimers complex	-	-	-	-	IV	Nociceptive pain / Paw pressure test	<i>In vivo</i>	Sustained release and pain alleviation	Lack of NP characterization	[48]
Morphine	Hyperbranched polyglycerol prodrug complex	-	-	-	-	IV, SC	Inflammatory pain (CFA-induced inflammation) / Paw pressure test	<i>In vivo</i>	Targeted release Peripherally restricted analgesia ; no central sedative effect	Lack of NP characterization	[56]
Morphine Oxymorphone	Liposomes	-	-	-	-	SC	Neuropathic pain (SNL) / Hargreaves test	<i>In vivo</i>	Sustained release and pain alleviation	No biodistribution assay	[45]
Buprenorphine	Solid lipid (oil/fatty ester)	180-200	-31 to -21	-	-	IV	Nociceptive pain / Cold tail-flick test	<i>In vivo</i>	Sustained release and pain alleviation Improved bioavailability	No biodistribution assay	[51]
Loperamide	Antibody-conjugated liposomes (immunoliposomes)	286	-	99	-	IV	Inflammatory pain (CFA-induced inflammation) / Paw pressure test	<i>In vivo</i>	Targeted release Improved analgesic and anti-inflammatory effects (vs non-conjugated liposomes) Exclusively peripheral effect	-	[58]
Loperamide	Polysorbate coated PLGA/PEG copolymer	148	1.5-3	72-74	-	IV	Inflammatory pain (Formalin-induced inflammation) / Hot-plate and licking tests	<i>In vivo</i>	Improved bioavailability (brain)	No biodistribution assay ; risk of central side effects	[54]
Buprenorphine	Pluronic F127-reduced graphene oxide hydrogel as nanosheets	158	-32	-	-	TD	Nociceptive pain / Tail flick test	<i>In vivo</i>	Sustained pain alleviation	No biodistribution assay	[29]
Hydromorphone Ketamine	Lipid-polymer (hybrid) CPP-SA/PLGA 50:50	120-500	-	66	33	IT	Neuropathic pain (CCI) / Von Frey test	<i>In vivo</i>	Sustained pain alleviation	IT route ; no biodistribution assay	[52]
Nalbuphine	Solid lipid (phospholipid)	170	-22	93	26	IN	Inflammatory pain / Hot plate test	<i>In vivo</i>	Sustained release and pain alleviation	-	[98]
Butorphanol Tramadol	Phospholipid nanovesicle	99-508	-	-	-	IN	Nociceptive pain (Acetic acid-induced pain) / Writhing test	<i>In vivo</i>	Improved bioavailability	-	[53]
Leu-enkephalin (Squalene-conjugated prodrug)	Squalene-conjugated prodrug (lipid)	75-120	-30 or 30	-	53-59	IV	Inflammatory pain (Carrageenan-induced paw edema) / Hargreaves test	<i>In vivo</i>	Sustained pain alleviation and targeted release Peripherally induced antinociception, intensity similar to that of morphine	-	[92]
Leu-enkephalin (Drug or palmitoyl-conjugated prodrug)	Chitosan polymer	44	-	-	-	IV, PO	Nociceptive pain / Tail flick test	<i>In vivo</i>	Improved bioavailability	Central analgesia ; missing NP surface properties	[96]
Dalargin	Polysorbate coated poly(butylcyano acrylate)	288	-	-	-	IV, PO	Nociceptive pain / Hot plate test	<i>In vivo</i>	Improved bioavailability	-	[95], [99]
DADLE ([D-Ala ² , D-Leu ⁵]-Enkephalin)	Silica mesoporous shell coated liposomes	176	32	73-5	-	Local instillation on DRG	Inflammatory pain (Dextrane sulfate sodium induced colitis) / Patch clamp of DRG assay	<i>In vivo</i>	Inhibition of the nociceptive signaling	No pain relief assay	[31]
Endomorphin-1	Poly(butylcyano acrylate)	27	-	-	-	IV	Nociceptive pain / Tail-flick test	<i>In vivo</i>	Sustained release and pain alleviation Improved bioavailability	No biodistribution assay	[100]

2 - Steroidal and non-steroidal anti-inflammatory drugs

Inflammation is one of the main causes of pain. Inflammatory pain is a physiological symptom acting as a signal alerting about the occurrence of an ongoing tissue injury and of the subsequent innate immune response, involving pro-inflammatory mediators (leukotrienes, histamine, serotonin, prostaglandin, bradykinin, substance P...) and cytokines (IL-1, IL-6, TNFα...). However, it becomes prejudicial when too intense and/or persistent [101]. The use of anti-inflammatory drugs, deeply rooted in medicine since the use of salicylate-rich willows in ancient Egypt and the derived aspirin, is aimed at blocking the prostaglandin pathway, by inhibiting the cyclooxygenase (COX), which converts the arachidonic acid into prostaglandins.

2.1 - Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAID) are currently the most used anti-inflammatory drugs, and, by extent, pain relieving drugs, aside from paracetamol. However, they are far from being devoid of undesired effects : aspirin inhibits platelet aggregation, and safer NSAIDs, when taken on the long run, still cause severe gastrointestinal damage, such as ulceration or hemorrhage [102] as well as renal dysfunction [103]. Furthermore, some poorly vascularized and painful

inflammation sites, particularly in the case of joint inflammation (e.g., arthritis), requires local injections. Parenteral administration of NSAIDs, such as intravenous or intramuscular injections, or topical administrations could be performed to better alleviate pain with less toxicity [104]–[107], but they require frequent administration due to a low residence time.

In that respect, nanomedicine offers a very promising alternative to address some of the limitations related to the use of NSAIDs. Many studies have focused on improving the bioavailability of these molecules through noninvasive routes to display systemic anti-inflammatory activity and remote pain control. For instance, transdermal administration of

Active molecule	NP composition or type	d _{HD} (nm)	ZP (mV)	EE (%)	DL (%)	Route of adm.	Targeted pain (pain model) / Pain or inflammation test	Furthest stage of dev.	Main benefits	Limitations	Ref.
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naproxen incorporated in chitosan/carrageenan NPs (NAP-loaded CS/CRG NPs) dispersed in carbopol gel, or of indomethacin in polyamidoamine (PAMAM) NPs, allowed significantly improved skin permeation (+30 to +150%) [108], [109] and lowered inflammation [109] compared to their free form counterparts. Yiyun *et al.* also used polyamidoamide NPs to load diflunisal and ketoprofen for transdermal administration to mice. Not only have they achieved a 2.5-fold increase of transdermal permeation of both APIs, but they also obtained a significant reduction of pain levels in a model of intraperitoneally-injected acetic acid induced abdominal pain [110]. As regards the intranasal route, the use of anti-inflammatory drugs is mainly directed to the treatment of asthma ; little evidence of pain alleviation by such drugs is available, except the aforementioned use of NPs with ketoprofen and opioids [53].

Targeted, controlled, delayed and sustained delivery applications of nanocarriers remains much less explored for NSAIDs. One of the most noteworthy examples was issued by Kim *et al.*, who highlighted a notable decrease in inflammation of surgically-sutured tissues after a local injection of mannose-PEG-coated PLGA NPs containing diclofenac, compared to mannose-coated PLGA NPs and free diclofenac in solution. This improved effect relied on a better targeting of macrophages [111].

Other research focused on the enhanced delivery of COX-2 inhibitors. Celecoxib is a selective COX-2 inhibitor developed to decrease the occurrence of gastrointestinal side effects caused by COX-1 inhibition. However, this molecule has limited bioavailability. To improve bioavailability and efficacy, Mishra *et al.* entrapped celecoxib in Eudragit S100-coated nanostructured lipid carriers. After oral administration to mice, lower mastocyte infiltration and a decreased level of pro-inflammatory factors in colon wall were observed, comparatively to free celecoxib [112]. Kalangi *et al.* used the intravenous route for the administration of the same molecule, coupled with mercaptopropionic acid-capped CdTe quantum dots (QDs), leading to a localization of the latter in a carrageenan-induced paw edema after 24h [113]. However, surprisingly, neither inflammation, nor antinociceptive effect were measured.

2.2 - Steroidal anti-inflammatory drugs

Steroidal anti-inflammatory drugs or other purposed drugs with anti-inflammatory properties, with broader range of actions and higher toxicity were also incorporated in NPs for sustained delivery, although pain alleviation was not their only indication. A longer-lasting inflammation inhibition could be noticed after intraperitoneal or intrathecal injection of respectively dexamethasone-loaded PLA [114] and triamcinolone acetonide-loaded PAMAM [28] NPs. In addition, the latter were able to decrease neuropathic pain better and longer than did the free drug. Notwithstanding the high relevance and the benefits that a targeted delivery of corticoids would bring, no treatment or even any clinical trial has been released so far for pain alleviation.

Table 3 – Nonsteroidal anti-inflammatory drug-based nanomedicines used for pain relief

Pain relief assessment was not tested on all the mentioned nanomedications ; however, as inflammation levels is highly correlated to pain intensity, publications in which the inflammation intensity was measured were relevant enough to be included in the following table. Abbreviations : d_{HD} : hydrodynamic diameter ; ZP : zeta potential ; EE : encapsulation efficiency ; DL : drug loading ; SNL : spinal nerve ligation ; adm.: administration ; dev. : development ; PEG : polyethylene glycol ; PLGA : poly(lactic-co-glycolid acid) ; TNFα : tumor necrosis factor α ; TD : transdermal ; IV : intravenous ; PO : per os ; IT : intrathecal ; IP : intraperitoneal

Naproxen	Chitosan/ carrageenan NPs dispersed in carbopol gel	150- 350	25-35	93-97	-	TD	Inflammatory pain (Formalin induced edema) / Measurement of the volume of the inflamed paw	<i>In vivo</i>	Sustained release Better transdermal passage Reduced inflammation	No pain relief evaluation	[115]
Indomethacin	Polyamidoamide (PAMAM) dendrimers	-	-	-	-	TD	Inflammatory pain (Carrageenan-induced edema) / Measurement of the volume of the inflamed paw	<i>In vivo</i>	Improved bioavailability Lower paw inflammation	No pain relief evaluation No NP characterization	[109]
Diflunisal Ketoprofen	Polyamidoamide (PAMAM) dendrimers	-	-	-	-	TD	Inflammatory pain (Acetic acid-induced writting pain) / Measurement of writting frequency	<i>In vivo</i>	Improved bioavailability and pain alleviation Better transdermal passage	No NP characterization	[110]
Ibuprofen	Lipid nanocapsules	47-56	0.46- 0.91	94-98	-	IV, PO	Nociceptive pain / Tail flick test	<i>In vivo</i>	Sustained release and pain alleviation Prolonged antinociception only using oral route	-	[116]
Diclofenac	Mannose coated PEG/PLGA copolymer	89- 327	-44 - -28	-	1.5	Local instillation in surgical suture	Nociceptive pain (Surgical wound) / Histopathological assessment of inflammation and expression of inflammatory markers	<i>In vivo</i>	Improved bioavailability Stronger inhibition of prostaglandin E2 Lower inflammation	No pain relief evaluation	[111]
Triamcinolone acetoneide	Polyamidoamide dendrimers	3-10	-2	-	-	IT	Neuropathic pain (SNL) / Von Frey test	<i>In vivo</i>	Improved bioavailability Sustained release and pain alleviation Reduction of transcription of pro-inflammatory factors	IT route	[28]
Dexamethasone Diclofenac	PLA polymer	210- 230	-29	-	66	IP	Inflammatory pain -	<i>In vivo</i>	Sustained release Improved bioavailability Decrease of TNF α levels	No pain relief evaluation	[114]
Celecoxib	Nanostructured lipid carriers	250	-25	60	-	PO	Inflammatory pain (Dextrane sulfate sodium-induced colitis) / Histopathological assessment of inflammation and quantification of pro- inflammatory factors	<i>In vivo</i>	Sustained release Improved bioavailability Reduction of infiltration of mastocytes in the colon wall Reduced levels of pro- inflammatory factors	No pain relief evaluation	[112]

3 - Local anesthetics

Local anesthetics (LAs) are small, lipophilic molecules used for short and superficial to regional anesthesia, owing to their short half-life (< 3 h) and a low bioavailability. They inhibit the entry of Na⁺ ions into sensory neurons through voltage-gated sodium (Na_v) channels, thus stopping the generation and propagation of the nociceptive signaling along sensory neurons. Unlike nociceptive pain, neuropathic pain originates from a functional impairment of the nervous system itself, such as nerve lesions or dysfunction of ion channels [25], [117], [118]. The ability of LAs, and by extension, of sodium channel blockers (SCBs) to modulate the action potential makes them the obvious choice for the treatment of neuropathic, and to a certain extent, of central or nociplastic pain.

Na_v channels are transmembrane proteins formed by three subunits: one α subunit composing the pore, and two β subunits, outside the pore, which play a role in the modulation of the channel activity. Most of the SCBs bind to the α subunit of Na_v channels. Both α and β subunit types exist in different isoforms and have definite locations in the body (Tab. 4). However, unlike the location of these channels, SCB drugs binding is poorly specific, which blurs the borders

between local anesthetics, antiepileptics, antidepressants, and even antiarrhythmic drugs, and favors off-target toxicity [117], [119].

LAs are a well-known drug class. Lidocaine, the LA reference, was authorized for the first time in 1947. LAs are mainly

Na _v isoform	Tissue expression	Role in pain	Other therapeutic relevance
Na _v 1.1	CNS, PNS		Epilepsy
Na _v 1.2	CNS, embryonic PNS		Epilepsy, autism
Na _v 1.3	CNS, embryonic PNS	Neuropathic, inflammatory	Epilepsy
Na _v 1.4	Skeletal muscle		Myotonia
Na _v 1.5	Cardiac muscle		Cardiac rhythm disorders

administered by TD or SC routes or even instilled during surgeries, while there is no consensus about their use by IV route, because of their binding to cardiac Na_v 1.5 and the subsequent disturbance of cardiac rhythm such binding would cause [120]. The contribution of nanomedicine in literature shows to be very dependent on the targeted tissues, and essentially consisted in improving the pharmacological potency thanks to a higher bioavailability, and an enhanced passage through the BBB or the blood-nerve barrier (BNB). It successfully gave rise to the US-marketed drug Exparel[®], made of bupivacaine-loaded liposomes. Exparel[®] relies on the same DepoFoam technology as the morphine containing DepoDur (withdrawn, see above). Indicated for local analgesia by infiltration for field block and interscalene brachial plexus nerve block [121], [122], it displays better analgesia compared to bupivacaine free, and enables a decrease of post-surgery opioid intake [123], [124]. Moreover, recent results obtained from a phase 3 clinical trial are in favor of the extension of their indications [125]. Other formulations made of polymer, lipid or hybrid NPs feature interesting *in vitro* and *in vivo* properties [126]. For instance, Ulery *et al.* designed polyanhydride NPs with tunable release that allowed a 3-fold longer half-life of ropivacaine, up to 41h [127]. Same longer-release improvements have been found with articaine-containing PEG-polycaprolactone (PEG-PCL) NPs displaying lower toxicity as compared to the free drug [128]. Subcutaneous injection in rats of multilamellar vesicles releasing ropivacaine led to an impressive 10-fold release time increase, correlated to higher levels of anesthesia in guinea pigs as opposed to the same quantities of free LA [129]. Other experimental studies focused on tetrodotoxin (TTX), a powerful neurotoxin produced by some fishes (e.g. fugu) or by some bacteria [130]. Although it is not currently used as a LA, TTX is a promising SCB for cancer pain treatment [131]. For instance, Liu *et al.* demonstrated that when instilled locally in rats, TTX-loaded hollow silica NPs could penetrate the sciatic nerve and exert nerve blockade at lower doses compared to free TTX form [132]. In a similar approach, Zhan *et al.* used liposomes carrying both TTX and dexmedetomidine to trigger the release of those drugs thanks to thermoresponsive gold nanorods attached to the liposomes. These nanorods have the ability to convert near-infrared (NIR) light into heat, thus triggering phase transition of liposomes' lipid bilayers resulting in an on-demand drug release [32]. Even so, these promising achievements must not make forget that TTX is even less specific than the approved LAs (Tab. 5), which might strictly limit the drugability of this toxin.

As regard to topical analgesia, several lipid NPs loaded with lidocaine, benzocaine or tetracaine showed either higher potency or longer-lasting effect compared to the reference EMLA cream [133]–[137]. Lastly, NPs might broaden the scope of LAs, as suggested by Song *et al.*, who performed the administration of lidocaine and thalidomide co-loaded PEG-functionalized graphene oxide (LDC–THD–GO) nanosheets. Of note, LDC and THD were loaded onto PEGylated GO nanosheets through π – π stacking. This treatment was performed in a streptozocin-induced diabetic mouse model of neuropathic pain, ultimately leading to a pain intensity decrease up to 4 weeks [138]. However, the individual effect of lidocaine remains not so clear, and despite lactate dehydrogenase toxicity assays showing no additional cytotoxicity due to NPs, their long-term toxicity could be redhibitory [139].

Table 4 voltage- sodium	Na _v 1.6	CNS, PNS, glia	Ataxia, motor neuron disease	– Human gated (Na_v) channels α
	Na _v 1.7	PNS (sensory)	Neuropathic, inflammatory, hereditary	
	Na _v 1.8	PNS (sensory)	Neuropathic, inflammatory	
	Na _v 1.9	PNS (sensory)	Inflammatory	

subtypes.

9 isoforms of α subunit were identified identified, with different tissue distribution and roles in nociception and pain. These channels can be blocked by sodium channel blockers (SCB). Some of them are exploited for therapeutic applications. [117], [140].

Abbreviations : CNS : central nervous system ; PNS : peripheral nervous system

Table 5 – LA-based micro- and nanomedicines used for pain relief.

Abbreviations : d_{HD} : hydrodynamic diameter ; ZP : zeta potential ; EE : encapsulation efficiency ; DL : drug loading ; adm.: administration ; dev. : development ; PEG : polyethylene glycol ; PLGA : poly(lactic-co-glycolid acid) ; PCL : polycaprolactone ; PLA : polylactic acid ; IT : intrathecal ; IV : intravenous

4 - Psychotropic drugs

In neuropathic pain management, the first-line therapy is composed of tricyclic antidepressants (TCA) (e.g. amitriptyline, imipramine...), serotonin and noradrenalin reuptake inhibitors (SNRI) (duloxetine, venlafaxine), and gabapentinoids antiepileptic drugs (gabapentin, pregabalin) [11], [12], [145]. As the drug class name of these molecules suggests, they were not primarily designed for pain alleviation. However, their binding to many ionic channels (including sodium channels explained above) and adrenergic receptors might explain their relative efficacy on neuropathic and central pain. The major drawbacks of these treatments are their incomplete efficacy together with substantial side effects. Indeed, only a reduced fraction (often under 40%) of patients affected by fibromyalgia, VIH- or chemotherapy-induced pain or diabetic neuropathic pain, feel a significant relief, and some of the psychoactive and somatic effects (lethargy, anticholinergic effects, dizziness, ataxia, increased suicide risk, QT prolongation...) are not always well tolerated [13], [14], [146], [147].

Among this rather limited range of molecules, lamotrigine was formulated in PLGA NPs to improve its poor pharmacokinetics. When decorated with transferrin and lactoferrin ligands and injected intravenously in rats with sciatic nerve injury, these nanoparticles led to a greater accumulation of the drug into the brain and a significantly better and

Active molecule	NP composition or type	d _{HD} (nm)	ZP (mV)	EE (%)	DL (%)	Route of adm.	Targeted pain (Pain model) / Test	Furthest stage of development	Main benefits	Limitations	Ref.
Bupivacaine (Exparel®)	Liposomes (DepoFoam®)	24-31x10 ³	-	-	-	SC/local infiltration	Nociceptive pain Post-surgery pain	Phase 4 for local analgesia by infiltration for field block and interscalene brachial plexus nerve block Phase 3 for sciatic and adductor canal nerve block	Improved bioavailability Better analgesia, lower post-surgery opioid intake	Micrometer size	[121], [123], [124], [141]
Bupivacaine	PLGA polymer	150	-	-	24	IT, local injection	Nociceptive pain (Compressed dorsal root) / Von Frey test	<i>In vivo</i>	Sustained pain alleviation Accumulation of NPs in the dorsal root ganglion, Prolonged antinociception	IT route	[142]
Lidocaine	Pegylated DSPE nanolipid carrier functionalized with cell-penetrating peptides	157	25.2	81.8	1.6	Topical	Nociceptive pain / Hot plate test	<i>In vivo</i>	Sustained and reinforced analgesic effect	-	[133]
Lidocaine	Liposomes	-	-	-	-	Topical	Nociceptive pain Acute mechanical pain / Pinprick test	Phase 1	Sustained analgesic effect	Lack of NP characterization	[135]
Lidocaine	PEG-PCL copolymers	202	-	98.5	39.4	Topical + ultrasound	Nociceptive pain / Tail flick test	<i>In vivo</i>	Faster and more efficient analgesia	-	[143]
Lidocaine Thalidomide	Pegylated graphene oxide nanosheets	-	-	-	50-70	IV	Neuropathic pain (Diabetic mouse model of neuropathic pain) / Mechanical allodynia and hot plate tests	<i>In vivo</i>	Sustained pain relief	Lack of NP characterization No assessment of the individual effect of lidocaine	[138]
Benzocaine	Solid polyoxyethylene sorbitan ester lipid	189-337	-30 - -18	7-32	-	Topical	Nociceptive pain / Tail flick test	<i>In vivo</i>	Sustained and more potent anesthesia	-	[134]
Tetracaine	Liposomes	-	-	-	-	Topical	Nociceptive pain / Pinprick superficial and deep pressure Visual anagocic pain scale	Phase 1	Better superficial analgesia but no difference in deeper analgesia	No NP characterization	[136], [137]
Ropivacaine	Multilamellar vesicles	400-20 000	-	-	-	Intracutaneous injection	Nociceptive pain / Pinprick superficial pressure	<i>In vivo</i>	Sustained superficial analgesia	-	[129]
Ropivacaine	PEG/PLA copolymer	-	-	-	-	Local instillation (surgical suture)	Nociceptive pain (Post paw incision pain) / Hargreaves and von Frey tests	<i>In vivo</i>	Sustained analgesic effect	No NP characterization	[144]

longer (up to 48 h) antinociceptive response, compared to both ungrafted NPs and the free drug [148]. Likewise, duloxetine, amitriptyline, doxepin and imipramine when encapsulated in PLGA NPs and injected by intrathecal route,

displayed the same trends [149], [150]. Nonetheless, the low specificity of these drugs remains a major limitation of this strategy and, beyond these specific applications, raises the same problems as found with MOP-R opioids. Moreover, owing to the complexity of neuropathic pain, the animal models used in the studies reviewed here are not ideal to mimic all the types of neuropathic pain [151], [152]. Nociceptive pain, without any identified cause, is even more difficult to simulate [153], which explains the lack of available treatment and nanomedicine applications.

Table 6 – Psychotropic drug-based nanomedicines used for pain relief.

Abbreviations: d_{HD} : hydrodynamic diameter ; ZP : zeta potential ; EE : encapsulation efficiency ; DL : drug loading ; adm.: administration ; dev. : development ; CCI : chronic constriction injury ; SNL : spinal nerve ligation ; PLGA : poly(lactic-co-glycolid acid) ; IV intravenous ; IT intrathecal.

5 - Cannabinoids

Cannabinoids (CBs) are a group of molecules interacting with cannabinoid receptors (CBR). Their name derives from cannabis, from which cannabidiol (CBD) and δ -9-tetrahydrocannabinol (THC) were the first identified compounds. However, this category also englobes synthetic cannabinoids and endocannabinoids, such as arachidonic acid-derived anandamide and 2-arachidonoylglycerol. CBR are G-protein coupled receptors that exist in 2 isoforms (CBR-1 and CBR-2). CBR-1 is expressed in the CNS and the PNS, while CBR-2 is more localized in the microglia and in the cells and tissues of the immune system [154]. CBR agonists (mainly CBR-1) are psychoactive, affecting many nervous functions such as memory, mood and reflexes, hence its illegal status in many countries. However, they also modify pain perception. Recently, a series of drugs containing CBD, THC, and some derivatives were authorized in the U.S. (Epidolex[®], Marinol[®], Syndros[®], Cesamet[®]) [155] and in some European countries (Epidolex[®], Sativex[®]) [156]–[159]. Their use is restricted to the treatment of very specific pain, such as multiple sclerosis spasticity (Sativex[®]), although their real-life efficacy is still widely discussed [15]–[19], [158].

CBs possess high permeability and low water solubility properties (BCS (Biopharmaceutical Classification System) class

Active molecule	NP composition or type	d_{HD} (nm)	ZP (mV)	EE (%)	DL (%)	Route of adm.	Targeted pain (Pain model) / Test	Furthest stage of development	Main benefits	Limitations	Ref.
Lamotrigine	Transferrin and lactoferrin functionalized PLGA polymer	133-151	-12	-	78	IV	Neuropathic pain (SNL) / Hargreaves test	<i>In vivo</i>	Increased bioavailability in the brain Better and longer-lasting antinociception	IV route	[148]
Duloxetine	PLGA polymer	379	-39	31	-	IT	Neuropathic pain (SNL) / Von Frey test	<i>In vivo</i>	Sustained mechanical allodynia alleviation, anti-inflammatory effect	IT route	[149]
Amitriptylin Doxepin Imipramin	PLGA polymer	373-480	-	44-56	31-40	IT	Nociceptive/ neuropathic pain (CCI) / Hargreaves and mobility tests	<i>In vivo</i>	Stronger and sustained anti-allodynia effect	IT route	[150]

II). To increase their poor oral bioavailability (4-12%) [160], equimolar THC and CBD were included into proprietary nanomicelles (Nanocelle[®]), branded as NanaBis[®]. Intended to be used in a buccal spray, it is currently under phase 3 clinical trial for bone pain issued from metastatic cancers [161], [162]. The same CBs incorporated in lipid nanospheres functionalized with piperine as an absorption enhancer also underwent a phase 1 clinical trial. Following oromucosal administration, this drug enabled a nearly 2-fold increase of bioavailability and a 3- to 4-fold increase of maximal plasmatic concentration of the active molecules compared the non-vectorized molecules [163]. However, these encouraging results have not been pursued. Regarding preclinical data, another CB, CB13, was formulated by Berrocoso *et al.* into PEG-PLGA NPs. Once given orally to rats with constricted sciatic nerve, these NPs were able to reduce the intensity of pain for up to 11 days versus 9h after administration of CB13 free, which was in line with the sustained release found *in vitro* [30].

Following the same rationale as for opioids, targeting peripheral CBR-1 with CB-loaded nanoparticles could theoretically tackle the pain without harmful central side effects. Although, at preclinical stage, there is some evidence of peripherally restricted analgesic activity of CBs nanomedicines [164], [165], their activity on acute pain has not yet been achieved in humans [166]–[168]. Of note, WIN 55,212-2, a dual CBR-1 and CBR-2 agonist, was designed by Linsell *et al.* to exploit EPR-like effect near injured nervous or non-nervous peripheral tissue without crossing the BBB. In that respect, drug-loaded styrene maleic acid micelles attenuated allodynia for longer and slightly decreased motor ataxia during 1,5 h compared to the free drug. Here, peripheral restriction hypothesized by the authors would have deserved a convincing biodistribution imagery or pharmacokinetic analyses. An opposite strategy was adopted by Xie *et al.*, who achieved a 28-day long centrally restricted pain alleviation in mice using mesoporous silica NPs with THC and ARA290 (an erythropoietin-derived polypeptide) ; a sharp decrease in pro-inflammatory levels was observed, confirming the significant anti-inflammatory effect of THC already noticed in other publications (Fig. 5) [169].

Table 7 – Cannabinoid drug-based nanomedicines used for pain relief.

Abbreviations: d_{HD} : hydrodynamic diameter ; ZP : zeta potential ; EE : encapsulation efficiency ; DL : drug loading ; adm.: administration ; dev. : development ; CCI : chronic constriction injury ; PLGA : poly(lactic-co-glycolid acid) ; $\Delta 9$ -THC : delta-9-tetrahydrocannabinol ; CBD : cannabidiol ; PO : per os ; IV intravenous ; IP intraperitoneal.

Figure 5 – Example of a sustained drug release enabled by nanocarriers: THC and ARA290 containing mesoporous silica nanoparticles. Once injected intraperitoneally in mice, these NPs enable a sustained delivery of tetrahydrocannabinol (THC) and cibinetide (ARA290), leading to a sustained antihyperalgesic ((a) thermal hyperalgesia assay) and antiallodynic effect ((b) mechanical allodynia assay), compared to THC or ARA260 in solution [169].

6 - Nucleic acid therapy

Nucleic-based therapy to treat diseases consists in introducing a new gene, in replacing a disease-causing gene with a healthy copy or in inactivating a disease-causing gene (silencing) that is not functioning properly. To be efficient, gene therapy requires (i) the transport of the genetic material (i.e. RNA, DNA as plasmids, circular or hairpins of variable length) from the site of administration to the site of action without any degradation, (ii) the entry of the genetic material inside the cell and (iii) the delivery of the genetic material into the relevant subcellular compartment (ie., cell cytoplasm or cell nucleus) [172], [173]. Inspired by the intrinsic ability of viruses to infect cells, the use of nanomedicines for the delivery of DNA or RNA has generated much interest and hope for the treatment of severe diseases and the

Active molecule	NP composition or type	EE	DL	Route of administration	Targeted pain	Further stage	Main benefits	Limitations	Ref.
$\Delta 9$ -THC CBD equimolar	Nanocellulose patented nanomicel	-	-	IP	Neuropathic pain (CCI) / Hargreaves and allodynia mechanical aesthesiometer tests	<i>In vivo</i>	Decreased concentrations of pro-inflammatory mediators Sustained pain alleviation	-	[161], [170]
$\Delta 9$ -THC CBD	Lipid nanosphere (triglycerid phospholipid)	-	-	IP				-	[163]
CB-13	PEG-PLG copolymer	-	-	IP				elief	[30]
WIN 55,212-2	Styrene maleic acid micell	-	-	IP				ation	[171]
$\Delta 9$ -THC ARA290	Mesoporous silica	80	-	3-6 IP				ation	[169]

development of vaccines, some of them being already approved by EMA and/or FDA [174], [175].

Genetic material-based therapy studies for pain treatment purposes rely on RNA and DNA sequences coding for a large panel of active molecules. Carriers which are commonly used for the delivery of nucleic acids are either modified viruses or synthetic nanodevices.

6.1 - Viral carriers

Endogenous opioid pathways has been potentialized using preproenkephalin (PPE) circular DNA (cDNA) coding sequences into recombinant herpes simplex virus carriers (HSV1) [176]–[182]. The natural neurotropism of HSV [183] may have contributed to significant antinociception and anti-inflammatory activity in inflammatory and neuropathic pain models in both rats and mice. This fruitful piece of work, carried out by Fink *et al.*, has already reached clinical trials using intradermal injections of HSV-mediated PPE cDNA, encoding for Leu and Met-enkephalins. Despite a good tolerance and a dose-dependent analgesia lasting more than 2 weeks, clinical trials assessing the efficacy of this approach on cancer pain were discontinued after phase II [176], [184], [185]. Adenovirus is another frequently used viral vector. It enables high yield levels of transgene expression, and is one of the most investigated virus for gene therapy [186]. Although still limited to preclinical studies, several genes coding for anti-inflammatory cytokines and endorphins transported by adenovirus have meaningfully lowered levels of pain [187], [188]. An atypical application is the transfection of shRNA in adeno-associated virus, which was able to knockdown $Na_v1.3$ activity in diabetic neuropathic pain, 3 weeks after intrathecal administration [189]. Such strategy could be of great interest to tame neuropathic pain resulting from activation disorders in mutated Na_v like erythromelalgia [190].

6.2 - Non-viral nanocarriers

Non-viral vectors also accounted for preclinical achievements on pain treatment. For instance, p38 kinase siRNA-loaded in PLGA NPs were able to silence, in microglial rat cells, the expression of p38, a kinase protein playing a role in neuropathic pain through the production of pro-inflammatory IL-1 β and TNF α . In addition, Shin *et al.* showed a prolonged allodynia compared to free siRNA [191]. Likewise, plasmids or transgenes coding for the anti-inflammatory IL-

Nucleic acid type	Precursor and active molecule	Type of NP or vector	d _{HD} (nm)	ZP (mV)	EE (%)	Route of adm.	Targeted pain (Pain model) / Test	Furthest stage of dev.	Improvements	Limitations	Ref.
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10 cytokine, either in PLGA microparticles or in hybrid liposome-mesoporous silica NPs, have proved efficient on nociceptive and neuropathic pain [192], [193]. Regardless of their micrometer dimensions, PLGA carriers, injected intrathecally, accumulated in meningeal tissues and allowed a 75-day long neuropathic pain relief, although no effect of associated nitric oxide gas production was detected [192]. Nanoparticles composed of the same polymer were also used to deliver Foxp3 (Forkhead transcription factor3) plasmid in microglia of rat brain, allowing an enhanced and sustained inhibition of microglial activity associated with neuropathic pain [194]. Long-lasting effect on neuropathic pain was also demonstrated using liposomes as nanocarriers. As an illustration, Tsuchihara *et al.* transfected human hepatocyte growth factor gene in rats near a constricted sciatic nerve thus restoring blood flow and myelination. In addition, it allowed a decrease of several markers of nerve injury, which resulted in a sustained reduction of both allodynia and hyperalgesia [195]. Noteworthy, beyond pain alleviation, this method could be also relevant for nerve regeneration after traumatic injuries [196]. In the same context of gene therapy, gold NPs were also used as “gene guns” by Chuang *et al.*, with PPE and proopiomelanocortin cDNA to treat interstitial cystitis pain in rats. Local bladder wall injection or infusion of these nanomedicines decreased the frequency of bladder contraction for one week [197], [198].

Overall, the absence of clinical trials of these non-viral gene vectors, compared to the viral ones, is usually explained by a lower efficiency [199]. However, the recent approval of mRNA-based lipid nanoparticles for SARS-CoV-2 vaccination represents a real scientific and technological revolution [196], [197], [198] showing that such non-viral nanocarriers can combine safety and efficacy. This indisputable breakthrough could pave the way to new applications in vaccine and drug therapy [203]. Even if making the connection between the success of mRNA vaccines and some preclinical evidence related to the efficacy of non-viral vectors for pain alleviation is highly tempting, it should be noted that the underlying mechanisms radically differs. In addition to the aforesaid lack of specificity from some drugs classes both in terms of receptor targeting and tissue distribution, pain can be highly multifactorial, as opposed to viral infections in which the target protein for vaccination is clearly defined (ie., S protein). Additionally, vaccines can act at the injection point, triggering a general immune response, while nociception and pain treatment, which rely on a fixed nervous system, are more demanding as regards to biodistribution. Evident improvement might be the use of surface-functionalized nanocarriers for enhancing tissue targeting and *in situ* expression of the active molecule.

Table 8 – Nucleic acid-based micro- and nanomedicines used for pain relief.

Abbreviations: dHD : hydrodynamic diameter ; ZP : zeta potential ; EE : encapsulation efficiency ; DL : drug loading ; adm.: administration ; dev. : development ; CCI : chronic constriction injury ; PLGA : poly(lactic-co-glycolid acid) ; SC : subcutaneous ; IT : intrathecal ; cDNA : complementary DNA ; pCMV : cytomegalovirus plasmid ; PPE : preproenkephalin ; ENK : enkephalin ; HSV herpes simplex virus ; siRNA : small interfering RNA ; shRNA : short hairpin RNA ; TNF : tumor necrosis factor ; DMSO : dimethylsulfoxide.

cDNA	Endomorphin-2 + glutamic acid decarboxylase	Replication defective herpes simplex virus (HSV)	-	-	-	SC	Inflammatory pain (CFA-induced pain) / Von Frey test	Phase 2	Sustained pain relief	-	[177], [178], [184]
cDNA into pCMV plasmid	Pro-opiomelanocortin β -endorphin	Gold NPs (gene gun)	1500	2000	-	Injection in the bladder wall	Inflammatory pain (Acetic acid induced interstitial cystitis)	<i>In vivo</i>	Reduction of ladder intercontraction time, reversed by naloxone	Route of administration	[198]
cDNA	Preproenkephalin (PPE) Enkephalin (ENK)	Gold NPs (gene gun)	1500	2000	-	Bladder infusion	Inflammatory pain (Capsaicin induced pain)	<i>In vivo</i>	Reduction of ladder intercontraction time, reversed by naloxone	Route of administration	[197]
cDNA	PPE ENK	HSV	-	-	-	Topical after skin abrasion	Nociceptive pain (CFA-induced rheumatoid arthritis) / Hargreaves and horizontal mobility tests	<i>In vivo</i>	Increased mobility and reduced pain intensity	-	[181]
cDNA	PPE-A ENK	HSV	-	-	-	Topical after skin abrasion	Nociceptive pain (Capsaicin or DMSO induced pain)	<i>In vivo</i>	Abolition of sensitization to noxious stimuli	-	[180]
cDNA	PPE-A ENK	HSV	-	-	-	Local application (pancreas)	Inflammation pain (Dibutyl dichloride induced pancreatitis) / Spontaneous exploratory behavioral activity measurement and pancreatic tissue histology	<i>In vivo</i>	Reduction of inflammation and sequelae Improvement of exploratory behavior	Route of administration	[182]
P38 siRNA	-	PLGA polymer	153	25	-	IT	Neuropathic pain (SNL) / Von Frey test	<i>In vivo</i>	Prolonged anti-allodynia effect compared to free siRNA Reduced production of pro-inflammatory mediators	IT route	[191]
TRPV-1 siRNA	-	Solid lipid	50-100	-34-23	77-98	Topical, intraplantar	Neuropathic pain (Capsaicin-induced hyperalgesia) / Eddy's analgesiometer test	<i>In vivo</i>	Significant relief of thermal pain	-	[204]
TNF siRNA	-	Gold nanorod complex	45	-2.4	-	Local intracerebral injection	Nociceptive pain (CCI) / Frey and Hargreaves tests	<i>In vivo</i>	Decrease of TNF levels in hippocampal tissue Decrease in thermal hyperalgesia, transient decrease in mechanical allodynia	Route of administration	[205]
Plasmid DNA	Hepatocyte Growth Factor (HGF)	Liposomes	-	-	-	SC	Neuropathic pain (CCI) / Von Frey and Hargreaves tests	<i>In vivo</i>	Prolonged anti-allodynic and anti-hyperalgesic effect	No NP characterization	[195]
Plasmid DNA	IL-10	PLGA polymer	4,670	-28	-	IT	Neuropathic pain (CCI) + Von Frey test	<i>In vivo</i>	Accumulation in meningeal tissues and sustained pain relief (75 days)	IT route Micrometer size	[192]
Plasmid DNA	Foxp3	PLGA polymer	224	-18	-	IT	Neuropathic pain (SNL) + Von Frey test	<i>In vivo</i>	-	IT route	[194]
Transgene DNA	IL-10	Hybrid: amorphous mesoporous silica coated with phospholipid bilayers	230	24	-	IT	Nociceptive pain (Sciatic nerve constriction) + Von Frey test	<i>In vivo</i>	Significant pain relief	IT route	[193]
shRNA-Na _v 1.3	-	Adeno-associated virus	-	-	-	IT	Neuropathic pain (Streptozocin-induced diabetic neuropathic pain) / Von Frey test	<i>In vivo</i>	Reduced excitability of dorsal root ganglion Reduced tactile allodynia	IT route	[189]

7 - Other categories

7.1 - TRPV ligands: capsaicin

TRPV (transient receptor potential vanilloid 1) receptors are ionotropic receptors activated either by thermal stimuli (over 43°C), protons, or molecules such as capsaicin or allyl isothiocyanate [206]. Stimulation of TRPV1 is interpreted as a burning sensation, often felt after oral consumption of capsaicin-containing hot chili pepper. Immediate burning pain is followed by a desensitization of sensory neurons, owing to the temporary loss of membrane potential, and, when applied on the skin, a reversible retraction of epidermal and dermal nerve fiber terminals occurs [207]. Desensitization finally results in an analgesic effect. Capsaicin-induced analgesia is already exploited by E.U.- and U.S.-approved capsaicin topical and transdermal patches (Qutenza™ with 8% of capsaicin) [207] and other drug candidates are currently under clinical trials [208]. As regards nanomedicine applications, capsaicin has been incorporated into inorganic or lipid NPs [209], [210], both injected in mice hind paws. Baskaran *et al.* showed a prolonged release of capsaicin and a faster Ca²⁺ influx in TRPV1 expressing HEK293 cells in presence of PLGA-coated ferromagnetic NPs, compared to non-coated NPs and the free drug on a mice model of inflammatory pain [209]. However, the claimed reduction of the paw edema resulting from capsaicin-loaded NPs in relation to the free drug is not significant. In another study, Puglia *et al.* found that capsaicin-containing lipid NPs improved immediate nociceptive pain alleviation, whereas no desensitization

occurred 7 days after the administration, contrarily to what was observed with capsaicin free [210]. The eventual prejudicial effects resulting from long-term exposure (long-term blockade, down-regulation of receptors, nerve destruction, tumorigenesis [188]) were investigated but only after a single-injection which was not representative of long term toxicity, and not an evidence of definitive desensitization.

7.2 - Paracetamol and nefopam

Paracetamol is the most widely used drug in the world and is the standard treatment together with NSAIDs in the step 1 of the pain ladder. Although its mechanism of action is complex and has not yet been fully elucidated [212], paracetamol is long known for its hepatic toxicity [213], both after acute or chronic intake [214]. The few contributions of nanomedicine in this respect were essentially focused on the mitigation of hepatic and renal side effects at the preclinical level, using nanosized crystalline paracetamol [215], or diverse types of NPs loaded with antioxidants and hepatoprotective compounds [216]–[219].

Nefopam, a more rarely used centrally acting drug, used to treat nociceptive pain and with possible neuropathic pain applications [220], [221], was also encapsulated in NPs. The related preclinical experiments, relying on various polymer NPs and micrometer particles, mainly resulted in a sustained release and analgesic activity of this drug [222], [223].

7.3 - Bisphosphonates

Bisphosphonates are structural analogues of pyrophosphates, a class of molecules released by osteoclasts involved in bone resorption. Bisphosphonates possess the ability to inhibit diverse enzymatic functions required for bone demineralization [224]. This property led some of these molecules to be used in the treatment of osteoporosis. Besides this indication, bisphosphonates have been found to exhibit pain relieving effect, although the mechanism of action remains unknown [225]. When encapsulated into liposomes, two bisphosphonates drugs, clodronate and zoledronic acid, were described to display an anti-nociceptive activity on neuropathic pain after IV administration in rats [226] and mice [227]. Concerning the mechanism of action, clodronate was shown here to deplete the dorsal horn microglia, which ultimately reduced the initiation of neuropathic pain. However, the pain relief was of short duration. On the contrary, zoledronic acid, a drug indicated in the treatment of osteoporosis, was able to alleviate pain for 7 days.

7.4 - Purinergic ligands

Purinergic receptors play an important role in pain and nociceptive transduction in musculoskeletal system, skin and viscera [228]. Purinergic receptor antagonists are currently being developed for pain alleviation and non-pain related purposes [229]. One of them, the A-317491, was administered as chitosan oligosaccharide-g-stearic acid polymeric micelles and tested on a mice model of endometriosis, a still poorly understood disease which pain is greatly incapacitating in affected women [230], [231]. It was shown in this study that these nanoparticles accumulated in the ectopic endometrium tissues and led to a delayed but sustained effect on thermal and mechanical pain [232] (Fig. 6).

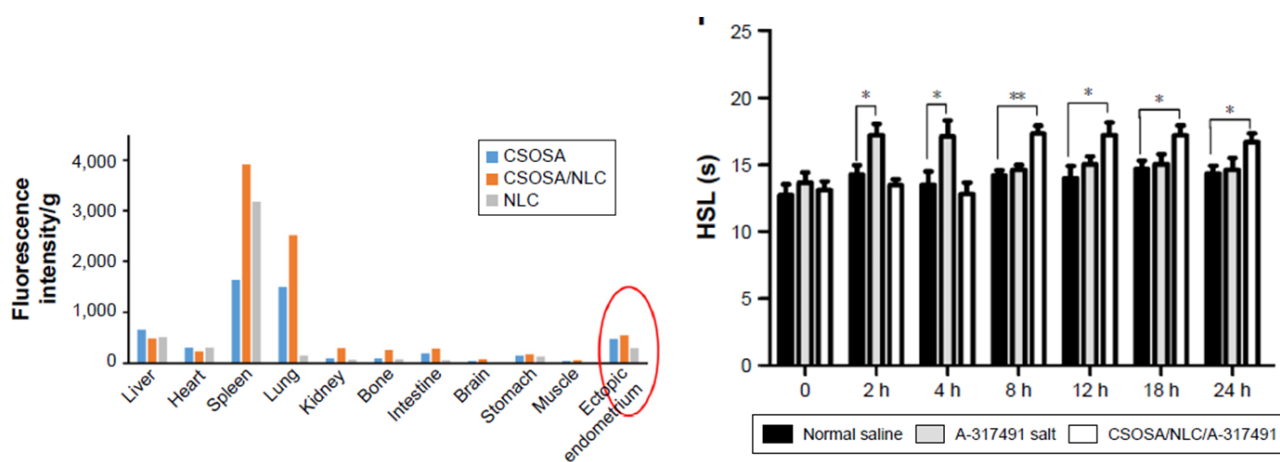


Figure 6 – Example of tissue-specific active targeting using nanoparticles: nanostructured lipid carriers (NLCs) carrying A-317491 (P2X₃ agonist) coated with chitosan oligosaccharide-g-stearic acid (CSOSA) polymer micelles

Accumulation of CSOSA/NLCs in the target tissue (ectopic endometrium) is higher than that of non-coated NLCs (fluorescence intensity of DiR-labeled micelles and nanocarriers, left). The targeting enhancement enabled by CSOSA in drug-loaded CSOSA/NLC NPs resulted in a delayed but prolonged pain relief compared to the drug free (thermal latency in seconds for thermal pain assessment, right) [232].

7.5 - Enzymes and other ligands

Some experimental drugs possess atypical or indirect mode of action in pain. One of them is carbon monoxide releasing molecule-2 (CORM-2), a CO-transporting ruthenium complex. Aside from its high affinity for hemoglobin that explains the toxicity of this molecule regarding dioxygen supply to cells, CO has many other functions, such as being involved in anti-inflammatory, anti-proliferative or cytoprotective mechanisms. Interestingly, CORM-2 and CO strongly inhibits neuropathic pain, by blocking the inducible nitric oxide synthase (iNOS) pathway thanks to their ability to dismutate superoxide radicals. However, its use is hindered by a short half-life and poor solubility properties [233]. In that respect, Joshi *et al.* demonstrated that intraperitoneal administration of CORM-2 loaded solid lipid NPs in a rat model of neuropathic pain led to a substantial 50-fold increase in CORM-2 half-life, a reduced concentration of iNOS-induced compound and a decrease of pain intensity compared to CORM-2 in solution [234]. Inflammatory pain might also be contained, as suggested by Lee *et al.*, who performed topical application of CORM-2 loaded liposomes in a mouse model of ear edema. Although pain itself was not evaluated, mRNA expression levels of pro-inflammatory cytokines were significantly lower and ear thickness were notably reduced [235]. However, the cytotoxicity of ruthenium-based compound [236] could be a limiting factor for further development.

Other metalloproteins, such as superoxide dismutase (SOD), displayed similar pain alleviation trends. In that respect, Kartha *et al.* incorporated this enzyme in polymersomes and achieved prolonged neuropathic pain alleviation in rats by intrathecal administration [237]. Contrary to CORM-2, SOD is an endogenous enzyme, which is not limited to the dismutation of superoxide radicals. Furthermore, some metallic NPs were found to display by their own some painkiller activity, owing to intrinsic antioxidant properties exploited in the mitigation of oxidative stress generated by free radicals. Manganese oxide NPs were successfully used by Kuthati *et al.* on this purpose, in the same conditions as for SOD polymersomes [238]. Sustained release of metallic or metalloprotein NPs with antioxidant properties could also be of great interest for other neurodegenerative diseases, some of them being at the origin of neuropathic pain [239]–[241].

Table 9 – Other experimental micro- and nanomedicines for pain relief improvement.

Abbreviations: d_{HD} : hydrodynamic diameter; ZP: zeta potential; EE: encapsulation efficiency; DL: drug loading; adm.: administration; dev.: development; CCI: chronic constriction injury; SNL: spinal nerve ligation; ALP: alkaline phosphatase; AST: aspartate transaminase; ALT: alanine transaminase; PLGA: poly(lactic-co-glycolid acid); SC: subcutaneous; IV: intravenous; PO: per os; IT: intrathecal; PMA: polymethacrylate acid; PHB: poly(hydroxybutarate); PCL: polycaprolactone; PBD: polybutadiene; PPO: polypropylene oxide; CSOSA: chitosan oligosaccharide-g-stearic acid; CORM-2: carbon monoxide releasing molecule-2; TPA: tetradecanoylphorbol acetate; DRG: dorsal ganglion route)

Active molecule	NP composition or type	d_{HD} (nm)	ZP (mV)	EE (%)	DL (%)	Route of adm.	Targeted pain (Pain model) / Test	Furthest stage of dev.	Main benefits	Limitations	Ref.
Capsaicin	PLGA-coated magnetic Fe NPs	10-20	-	89	9	SC	Inflammatory pain (Carrageenan-induced paw edema) / Measurement of the paw edema thickness	<i>In vivo</i>	Sustained release, faster and more potent pharmacological effect	No statistically significant reduction of inflammation No pain relief evaluation	[209]
Capsaicin	Softisan 100 – tween 80 lipid nanocarriers	287	-	-	-	SC	Nociceptive pain / Measurement of nociceptive behavior (paw licking, lifting, and shaking)	<i>In vivo</i>	Increased and sustained release, reduction of paw edema and faster antinociceptive action	-	[210]
Paracetamol	Drug-only Nanocrystals	24	-	-	100	IV	Nociceptive pain / ALP, AST and ALT quantification	<i>In vivo</i>	Decrease liver toxicity	No pain relief evaluation	[215]
Nefopam HCl	Eugradit RL/RS 100 and span 80 nanospheres	328	4	84	21	PO	Neuropathic pain (CCI) / Cold allodynia (acetone) test	<i>In vivo</i>	Sustained release and pain relief	-	[222]
Nefopam HCl	PHB-PCL microspheres	189,000	-17	-	-	PO	Nociceptive and post-operative pain / Tail flick, thermal and mechanical allodynia tests	<i>In vivo</i>	Sustained release and pain relief	Micrometer size	[223]
Clodronate	Liposome	-	-	-	-	IT	Neuropathic pain (SNL) / Von Frey test	<i>In vivo</i>	Specific depletion of spinal microglia Attenuation of induction but not maintenance of neuropathic pain	No NP characterization IT route	[226]
Zoledronic acid	Pegylated liposomes	241	-	-	70	IV	Neuropathic pain (SNL) / Von Frey test	<i>In vivo</i>	Reduction of allodynia	No NP characterization	[227]
A-317491	CSOSA-coated nanostructured lipid carriers	48	28	64	2	IV	Endometriosis pain (endometrial tissue implantation) / Von Frey and thermal withdrawal tests	<i>In vivo</i>	Targeted delivery to endometrial tissues, delayed but sustained pain alleviation	-	[232]
CORM-2	Solid lipid	120	-4	94	-	IP	Neuropathic pain (CCI) / Von Frey and cold allodynia tests	<i>In vivo</i>	Decreased concentration of inflammatory mediators and oxidative stress metabolites Better and sustained pain alleviation	IP route	[234]
CORM-2	Phosphatidyl choline liposomes	100	43	32	-	Topical	Inflammatory pain (TPA-induced skin inflammation) / -	<i>In vivo</i>	Decreased concentration of inflammatory mediators and edema volume	No pain relief evaluation	[235]
Superoxide dismutase	PEG-PPO-PEG/PEG-PBD polymersomes	110	-	-	-	Local instillation (near DRG)	Neuropathic pain (nerve root compression injury) / Paw withdrawal latency test	<i>In vivo</i>	Sustained pain relief	-	[237]
NP per se	Manganese oxide	100-300	-	-	-	IT	Neuropathic pain (partial sciatic nerve transection) / Thermal and mechanical paw withdrawal tests	<i>In vivo</i>	Reduced induction of inflammatory factors, sustained pain relief	IT route	[238]

General discussion

Unfavourable pharmacokinetics or biodistribution have always represented a considerable hurdle in the development of new drugs. The implementation of computational modeling methods and DMPK/ADME-Tox studies in the early stages of drug development significantly reduced the share of PK and bioavailability issues in the attrition rate among drug candidates in the last 20 years [242]–[244]. However, bioavailability and PK of many authorized drugs are still far from optimal, with insufficient efficacy, leading to massive, frequent or invasive administrations along with high toxicity. The active molecules listed in this review are no exception to this assessment. The abundance of research data obtained with nanovectorized painkillers and the promising results on pain relief discussed in this review demonstrates how rationally designed drug delivery nanosystems can be a game-changer in pharmacotherapy. Most of them were employed for sustained delivery or improved bioavailability, in order to increase respectively the duration of action of the drug and its distribution to tissues, while fewer of them were designed for targeted delivery in order to limit off-target toxicity. Although the versatility of NPs, in terms of composition, structure and physicochemical properties, enables the controlled delivery of a large range of drugs, the overall effectiveness and the relevance of nanomedicine for pain alleviation still largely depend on the route of administration, the nature of the encapsulated active molecule and the pharmacological target.

In nanomedicine, intravenous injection is generally the first-line systemic route of administration, owing to the facile access it can provide to virtually all the body tissues. This route was also used in many painkiller nanomedicine applications. The encapsulation of fragile molecules, such as neuropeptides or nucleic acids, allowed them protection from fast metabolism in the blood circulation and prevented the first pass effect, thus enabling a sustained-release. Moreover, as intravenous administration is the most used route for injection of nanomedicines in experimental proofs of concept, their fate is well known and more predictable. Indeed, there is a consensus on the nanoparticles' fate with a tendency to EPR or EPR-like effect – of particular interest for respectively cancer or inflammatory pain alleviation nanomedicines - along with opsonization phenomenon, hepatic accumulation and elimination. To mitigate opsonization process, polymer-coated NPs, commonly defined as second-generation NPs, are increasingly being used, enabling a slower hepatic accumulation and an increased duration of circulation of the drug in the bloodstream. Furthermore, although the absorption, pharmacokinetics and/or biodistribution of nanomedicines can depend on the nanocarriers size, surface charge and shape, none of the articles related to nanocarriers for pain alleviation have investigated this influence. However, intravenous route has major downsides. It is quite invasive, and even though it is more commonly used than the intrathecal route chosen in some of the listed experimental studies above, it can greatly alter the patient's compliance in case of frequent or prolonged use occurring in chronic pain treatment. In addition, pain has often multifactorial origins of which psychosomatic, with various pharmacological targets, making it more difficult to find the appropriate therapeutic approach. Given the versatility of nanocarriers and because the intravenous route is systemic, the properties of nanocarriers must be properly modulated to efficiently tackle a specific type of pain, in a safe and reproducible manner. For instance, improved bioavailability is hardly beneficial for opioids that are already able to cross the BBB and cause considerable side effects in the CNS. Surprisingly, the targeted delivery approach using EPR or EPR-like effect, theoretically exploitable for inflammatory and some cancer pain, is rarely sought in the literature. Indeed, while the majority of the research mentioned here describe painkiller nanomedicines improving the passage of the drug through the BBB, only three papers explicitly take advantage of their ability to restrict the delivery of opioids to the peripheral nervous system in order to prevent opioid central side effects [58], [56], [92]. In return, targeted delivery is not always possible, which is the case when the target is unknown or when the targeted organ or tissue is not reachable with enough specificity. Some types of neuropathic or central pain, with no identified organic causes, fall within this category. However, in the future, a third generation of nanocarriers could allow active targeting for more precise delivery, thanks to the grafting of ligands on their surface. These ligands can be either antibodies specific to proteins that are overexpressed by cancer cells (e.g., HER-2, epidermal growth factor), or organ-specific markers. Another important issue in the design of nanocarriers is the use of safe materials. In that respect, the use of non biodegradable nanomaterials, such as graphene oxide nanosheets encapsulating lidocaine and thalidomide [138] remains questionable because of potential bioaccumulation. It is important to highlight that lipid NPs are the most represented class in this paper. These nanocarriers constituted of endogenous and/or biodegradable lipids and surfactants, were already approved in many clinical trials, mainly in oncology, and might tip the scale in favor of this type of NPs for pain alleviation.

Oral route, for its part, is the other major systemic route of administration, especially relevant when repeated and long-term administration of analgesic drugs are needed. It is non invasive, ensures patient comfort, provides convenient access to drug administration thus allowing frequent and prolonged treatments compatible with chronic diseases. Moreover, the protective role and enhanced bioadhesion properties of some NPs in the gastrointestinal tract could trigger specific interest to improve poor gastrointestinal absorption of many drugs. However, this contribution for pain alleviation remains modest. The few examples of pain alleviation nanomedicine through oral route essentially, aimed at improving the bioavailability of ibuprofen [116] and nefopam [222], [223]. The main issues are related with the intestine wall. Indeed, NP-based strategies aiming at targeting the intestine (e.g. for the treatment of inflammatory bowel diseases) often failed due to a poor retention time and a lack of sufficient tissue bioadhesion to allow the specific release of the molecule [245]. Furthermore, the translocation of NPs through the intestinal wall is severely limited by the presence of tight junctions between the intestine epithelial cells. Even If the drug influx to the blood circulation is significant, the remaining drug still has to undergo the intense first pass effect, inherent to oral route, which might be redhibitory for fragile molecules. In the light of these factors, the only evidence of efficient delivery of Leu-enkephalin to the brain using chitosan NPs administered by oral route, proposed by Lalatsa *et al.* is hardly conceivable, and the mechanism rather difficult to understand [96].

Skin and mucosa are minimally invasive systemic routes of administration. The low drug residence time and the relative impermeability of these barriers, allow the passage of only small lipophilic molecules. Nonetheless, these routes offer the benefit of being minimally invasive and allow drug molecule to circumvent the first pass effect. In the same way as for the intestinal wall, nanoparticles are not expected to cross the skin or the mucosa, but some of them were found to be able to act as a permeation enhancer of molecules that can already intrinsically cross these barriers. In that respect, the transdermal passage was hence improved for some anti-inflammatory drugs [109], [110], [115] or buprenorphin [29], while the intranasal route took advantage of NPs to increase the passage of neuropeptides to the blood and the brain [93]. In addition, the transoromucosal passage of cannabinoids was greatly augmented when entrapped into nanomicelles. This approach, currently under clinical trials [163], represents a solid alternative to the oral route for this class of molecules.

As opposed to systemic delivery, the use of nanoparticles for local delivery, which includes topical administration, and intradermal or intratissular injections, essentially aims at releasing the drug in the immediate surrounding tissues from the site of application or injection, without the requirement of crossing biological barriers. Since this type of administration already represents a targeted delivery *per se*, sustained or on-demand release nanocarriers are the most relevant for this application, particularly when the administration is invasive (e.g. local infiltration). By combining high concentrations of local anesthetics or opioids, for longer durations, and in a delimited area (which contributes to limit off-target toxicity), this strategy is currently one of the most efficient and the most developed among pain-relieving micro- and nanodrugs, as demonstrated by the authorization of Exparel®. However, the injection or the implantation of such NPs, even for long durations (weeks or months) is limited to superficial tissues, which limits the range of applications.

Conclusion

Pain is a hypercomplex and multiple phenomenon, which involves not only one or several physiological mechanisms but also emotional, social, cognitive and cultural components, which are intimate. Moreover, there is no specific biomarker for pain. Therefore, pain alleviation, which still relies on treatments with high toxicity (opioids) and low efficacy (neuropathic pain drugs), represents a very difficult challenge because ligands and molecular targets involved in nociceptive signaling often have poor specificity both in terms of pharmacological activity and biodistribution. For these reasons, since decades, the discovery of new safe and efficient drugs is dramatically lacking and most of the drug on the market are still old. On the other hand, because of the fluctuating nature of the pain sensation, it is necessary to deliver the pain-relieving compound at the right dose at the right time and at the right location of the body. This is the main objective of the nanomedicines allowing to control the drug release kinetic and biodistribution. With a size of a few dozen to a few hundred nanometers, nanocarriers have made it possible to control the pharmacokinetics and biodistribution of encapsulated active ingredients. Many nanomedicines have been approved by the EMA in Europe and/or by the FDA in the USA, but mainly in the field of oncology. Unfortunately, the research concerning the application of nanotechnologies for pain alleviation remains very limited and, as shown in this review, only very few painkiller nanomedicines have reached the market. However, some innovative research paths would deserve to be encouraged for further development, like the better delivery of endogenous neuropeptides, such as endorphins or enkephalins, which have the capacity to naturally reduce the pain threshold, without inducing addictive effects as with opiates. The use of nucleic acids either RNA or DNA for activating some endogenous opioid pathways or for triggering the silencing of pro-inflammatory cytokines is another nanomedicine challenge. But, improving the administration and biodistribution of old drugs, like morphine derivatives or local anesthetics for a safer analgesic effect, still remains another important confront for nanomedicines.

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Authors contribution

ADS wrote the review, SLM and PC reviewed and completed it.

Conflict of interest

The authors declare no conflict of interest.

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