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Review

Lipid-Based Niclosamide Delivery: Comparative Efficacy, Bioavailability, and Potential as a Cancer Drug

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Abstract: Niclosamide, an FDA-approved anti-parasitic drug, has demonstrated significant potential as a repurposed anti-cancer agent due to its ability to interfere with multiple oncogenic pathways. However, its clinical application has been hindered by poor solubility and bioavailability. Lipid-based nanocarrier systems such as liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), and lipid nanoemulsions (LNE), along with lipid prodrugs, have successfully been employed by researchers to overcome these limitations and improve niclosamide's pharmacokinetic profile. Lipids are the core organic compounds which serve as the foundation of these advanced drug delivery methods and in turn play a critical role in enhancing niclosamide's therapeutic efficacy through improving drug solubility and bioavailability. Lipid-based nanoparticles encapsulate niclosamide, protect it from degradation, facilitate drug delivery and release, and may facilitate targeted delivery in the future. While niclosamide holds significant potential as an anticancer agent due to its multi-pathway inhibitory effects, the challenges associated with its poor bioavailability and rapid clearance underscore the need for innovative delivery methods and chemical modifications to unlock its full therapeutic potential. This review aims to present the latest instances of lipid-based delivery of niclosamide and to compile successful strategies which may be employed when aiming to develop effective anticancer therapies.

Keywords: lipid-based drug delivery; liposome; niclosamide

Citation: Woo, J.; Wiggins, R.W.; Mito, S. Lipid-Based Niclosamide Delivery: Comparative Efficacy, Bioavailability, and Potential as a Cancer Drug. *Lipidology* **2024**, *1*, 134–149. https://doi.org/10.3390/lipidology1020010

Academic Editor: Nicola Ferri

Received: 30 October 2024
Revised: 24 November 2024
Accepted: 29 November 2024
Published: 1 December 2024

1. Introduction

Niclosamide, an oral anthelminthic drug, has gained attention for its potential use as an anti-cancer drug [1]. This compound has demonstrated an ability to target multiple oncogenic pathways critical for cancer cell proliferation, survival, and metastasis, including Wnt/β-catenin, mTOR, NOTCH, KRAS, and STAT3. These pathways are often implicated in tumorigenesis of a variety of different cancers [2]. Studies have shown niclosamide inhibits the Wnt/β-catenin pathway and disrupts the stabilization and nuclear translocation of β-catenin, ultimately reducing the expression of genes involved in cancer cell proliferation and metastasis [3]. Niclosamide also suppresses the mechanistic target of rapamycin (mTOR) pathway through activation of the tuberous sclerosis complex (TSC), inhibiting mTORC1 and subsequently reducing cellular growth and survival [3]. In addition, niclosamide also suppresses the mTOR pathway by decreasing Notch1 and Notch2 protein expression through decreased production of notch mRNA [4]. It has also been shown to degrade KRAS protein, which, in abundance, contributes to uncontrolled cellular proliferation and resistance to apoptosis [5]. Lastly, it is known to act on the STAT3 pathway by preventing its activation through inhibition of STAT phosphorylation, subsequently preventing nuclear translocation and, in turn, inhibiting cellular proliferation and increasing rates of apoptosis [6]. Outside of these pathways, niclosamide also demonstrates activity as a mitochondrial uncoupler and disrupter of tumor metabolism [7].

Despite its promising antitumor effects, niclosamide faces significant hurdles for clinical application. These challenges are primarily attributed to its poor solubility in

aqueous environments (0.23 μg/mL), designating it a class II compound according to the Biopharmaceutical Classification System. Along with poor solubility, it is subject to rapid metabolism in the liver and intestine [8–10]. The hydrophobic nature of niclosamide severely limits its absorption in the gastrointestinal tract, while its rapid clearance through cytochrome P450-mediated hydroxylation and glucuronidation further reduces its systemic availability [8,9]. These pharmacokinetic challenges result in subtherapeutic plasma concentrations, necessitating higher doses that are difficult to achieve and sustain clinically, as well as increasing the risk of side effects. This is evidenced by a recent clinical trial evaluating the effectiveness of oral niclosamide as an adjunctive treatment administered with enzalutamide in the setting of castration-resistant prostate cancer (DU145). This dose-escalation study failed to consistently reach plasma concentrations corresponding to what is believed to be the lower threshold of its therapeutic index (81.8 ng/mL; IC_{50}: 330 ng/mL) determined in vitro [11]. Additionally, the dose escalation study was aborted due to dose-limiting toxicities. The two patients receiving 1000 mg TID both experienced side effects, with one patient experiencing nausea, vomiting, and diarrhea > 72 h, and the other experiencing abdominal pain, diarrhea, and colitis [11].

Given these limitations, there is clearly a need for improved drug formulations and delivery systems. Strategies to improve bioavailability, such as the development of niclosamide derivatives via *O*-alkylamination and halogenation, have shown promise in enhancing solubility and bioavailability [12–14]. Other various approaches, including cyclodextrin complexes, polymeric nanoparticles, and nanohybrids, have also demonstrated potential to improve drug stability and delivery efficiency [15,16].

Among these, lipid-based nanocarrier systems are being explored as affordable alternatives which aim to improve niclosamide's pharmacokinetic profile while offering targeted delivery to tumor tissues [1]. These efforts aim to maintain niclosamide's potent anticancer activity while overcoming the pharmacological barriers that currently limit its clinical effectiveness.

In this review, we specifically focus on lipid-based delivery systems to highlight their unique potential in addressing the challenges of niclosamide bioavailability and pharmacokinetics. Lipid-based nanotechnologies are the largest subset of nanotechnology with FDA approval for clinical use. This is due to their relative ease of synthesis in conjunction with their favorable safety profiles attributed to the low immunogenicity and biocompatibility of naturally occurring lipids [17]. Lipid-based nanocarrier systems, such as liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid nanoemulsions (LNEs), and self-microemulsifying drug delivery systems (SMEDDS), along with lipid conjugated (LC) prodrugs, have been utilized to overcome the pharmacokinetic challenges of niclosamide and improve its solubility profile (Tables 1 and 2) [18–20].

Liposomes, for instance, form concentric lipid layers around the drug, enhancing solubility in aqueous environments and increasing drug circulation time (Figure 1d) [18,21]. These lipid nanotechnologies also allow for the incorporation of other compounds which contribute to more favorable pharmacokinetic and dynamic drug profiles. This allows for greater accumulation at tumor sites while reducing systemic toxicity. Additionally, certain formulations like solid lipid nanoparticles (SLNs) allow for controlled drug release, which sustains ideal plasma drug concentrations over time (Figure 1c) [18,21]. This is particularly beneficial for drugs like niclosamide, which are rapidly metabolized and cleared from circulation [8]. Nanostructured lipid carriers (NLCs), a modification of SLNs, offer the same delivery advantages gained when using SLNs but allow for even greater drug-loading content (DLC) due to the inclusion of both solid and liquid lipids (Figure 1b). The inclusion of liquid lipids in the vehicle design prevents drug expulsion during storage and further enhances therapeutic delivery in vivo [22]. These lipid-based strategies not only improve niclosamide's bioavailability but also enhance its accumulation in tumor tissues, enhancing its anti-cancer potential [18,21]. This review will discuss how these delivery methods, and more, have been applied to deliver niclosamide in both in vivo and in vitro models below (Section 2) in more detail.

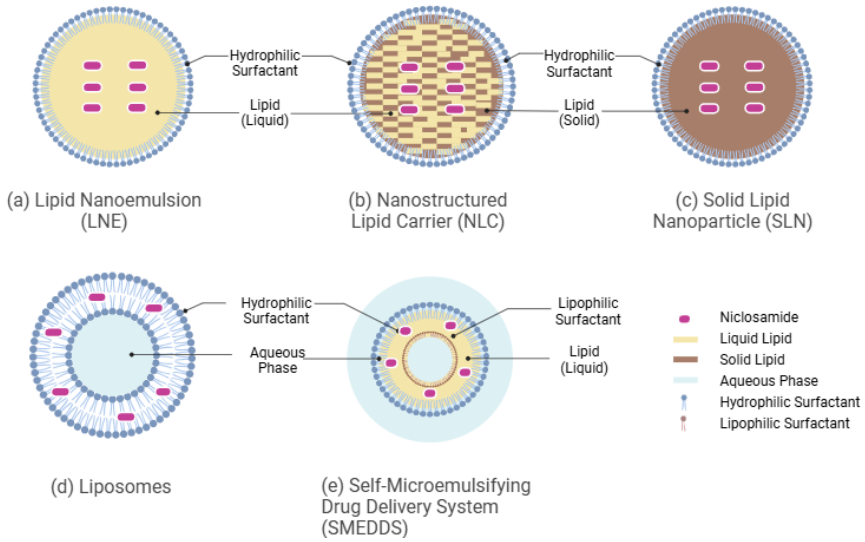

Figure 1. Structures which have been employed in lipid-based niclosamide delivery. Niclosamide represented as a payload in five different lipid-based drug delivery systems: (**a**) LNE (lipid nanoemulsions): niclosamide solubilized within lipid droplets in an aqueous phase, (**b**) NLC (nanostructured lipid carriers): niclosamide enclosed in a hybrid core of solid and liquid lipids, (**c**) SLN (solid lipid nanoparticles): niclosamide encapsulated in a solid lipid core with a lipid bilayer, (**d**) liposome: niclosamide encapsulated in a spherical vesicle with a lipid bilayer, and (**e**) SMEDDS (self-microemulsifying drug delivery system): niclosamide in a solid molecular dispersion of oil, surfactants, and cosurfactants [17–19,21]. Created in BioRender.

Table 1. The main lipid-based delivery systems and techniques.

Lipid Based Delivery	Composition
Solid lipid nanoparticles (SLNs)	Solid lipid core w/surfactant [17,18,21]
Nanostructured lipid carriers (NLCs)	Core of mixed solid and liquid lipid w/surfactant [17,18,21]
Lipid prodrug	Conjugated with a lipid moiety [20]
Lipid nanoemulsions (LNEs)	Oil droplets in water w/surfactants [17,18,21]
Self-microemulsifying drug delivery system (SMEDDS)	Hydrophilic surfactant, lipophilic cosurfactant, and oil phase [19]
Liposome	Lipid bilayer vesicle with an aqueous core [17,18,21]

Table 2. Comparison of pharmacokinetic characteristics of lipid-based niclosamide delivery systems.

Formulation Name	Delivery Type	Composition	Size (nm)	Zeta Potential (mV)	DLC (%)	EE (%)	Cancer Type	IC_{50} (μM)	Free NIC IC_{50} (μM)
NIC-loaded liposomal thermogel system [23]	Liposome	Egg lecithin Cholesterol	108.26–207.43	−13 ± 9.71	7.00–20.00	58.21–94.16	Melanoma (SK-MEL-28)	1.828	3.210
Ncl-Lips [24]	Liposome	DSPE-PEG2000 Cholesterol	136.97 ± 0.54	−13.5 ± 0.98	32.25 ± 0.49	89.49 ± 2.40	* Pulmonary Fibrosis	-	-
Niclosamide-loaded liposomes [25]	Liposome	-	~108	-	-	-	Colon Cancer (CT26)	4.4	2.5

Table 2. *Cont.*

Formulation Name	Delivery Type	Composition	Size (nm)	Zeta Potential (mV)	DLC (%)	EE (%)	Cancer Type	IC_{50} (μM)	Free NIC IC_{50} (μM)
Nic-loaded solid lipid nanoparticles (NIC-SLNs) [26]	SLN	Tween 80 Soya lecithin Glyceryl monostearate Stearyl amine	197.3 ± 18.08	+10 ± 3.43	8.16	75.64	** Phosphate buffer with dialysis membrane	-	-
NFM-3 [27]	SLN	Stearic acid PEG-400 Tween 80	204.2	−33.16	5.27	84.4	-	*** AUC 16.74 (μg h mL^{-1}) rabbit in vivo	*** AUC 1.51 (μg h mL^{-1}) rabbit in vivo
PBA-Niclo-SLNs [28]	SLN	Tween 80 Pluronic F-68 Stearylamine	112.18 ± 1.73	23.8 ± 2.7	8.3 ± 0.42	82.21 ± 0.62	TNBC	-	-
Chitosan-coated NLC [29]	NLC	Precirol ATO 5 Compritol 888 ATO, Oleic acid Tween 80 Diacetyl phosphate	189.6~334.5	-	-	98.8–99.7	Breast (solid Ehrlich)	-	-
NSPT [29]	Lipid prodrug	Stearate DSPC Cholesterol DSPE-PEG2000	30 ± 5	-	-	-	Osteosarcoma (143B, MG63, U2OS, and SaOS2)	0.2–2 μM	1.16 μM
NL-CSLE [30]	LNE	-	307.8	-	>9.0	-	-	-	-
NL-PSLE [30]	LNE	-	162.2	-	>9.0	-	-	-	-
Nano-NCL [31]	SLE	Miglyol® 812, Poloxamer 188	~200	-		~90	Colorectal (HCT-116)	1.259 (48 h)	5.460 (48 h) DMSO
Coarse-NCL [31]	SLE		>1 μm	-		~90	Colorectal (HCT-116)	4.504 (48 h)	5.460 (48 h) DMSO
Nic-SMEDDS [32]	SMEDDS	Labrasol ALF Plural oleique Labrafac lipophile WL1349	~150	−6.8	>75	-	HCC-PDX	-	-

Abbreviations: DLC, drug-loading content; EE, encapsulation efficiency; NIC/NCL/NL, niclosamide; SLN, solid lipid nanoparticle; DSPC, distearoylphosphatidylcholine; AUC, area under curve; PEG, polyethylene glycol; TNBC, triple-negative breast cancer; NLC, nanostructured lipid carrier; LNE, lipid nanoemulsion; SLE, submicron lipid emulsion; DMSO, dimethyl sulfoxide; SMEDDS, self-microemulsifying drug delivery system. Improved IC_{50} of niclosamide/lipid nanocarrier formulations are compared to free niclosamide when data available. *: Pulmonary fibrosis, though not a type of cancer, is included to ensure a comprehensive review. **: Included for the same purpose as noted above. ***: AUC (Area Under the Curve): Represents overall drug response; included as no IC50 data was available.

2. Lipid-Based Drug Delivery of Niclosamide

2.1. Liposomes

Liposomes are spherical, self-assembled vesicles made of one or more phospholipid layers. They are capable of encapsulating both hydrophilic drugs within their aqueous core and hydrophobic drugs within the lipid bilayer (Figure 1d) [33]. This dual encapsulation capability allows liposomes to improve the solubility and bioavailability of various types of drugs, including niclosamide [34]. A niclosamide-loaded liposome formulation utilizes phospholipid concentric layers to encapsulate the drug, resulting in significantly enhanced solubility and stability in aqueous environments compared to the unformulated variant [25,35,36]. These liposomal formulations not only increase drug solubility but may

also prolong the stability of both the drug and the delivery vehicle over extended periods of time through the addition of other compounds in the vehicle design [35,36].

Liposomes used as drug delivery systems offer several advantages: improved bioavailability, enhanced cellular permeability, prolonged circulation time, controlled drug release, immunoevasion (stealth properties), passive targeting, and improved pharmacokinetics [34]. Moreover, liposomal formulations can be functionalized with other molecules to optimize drug pharmacokinetics and pharmacodynamics. For example, functionalizing liposomes with monoclonal antibody fragments (Fab) enables active tumor targeting. One such experimental drug, MM-302, a HER2-targeted PEGylated immunoliposome doxorubicin conjugate, was administered in conjunction with trastuzumab in phase I clinical trials in HER2-positive metastatic breast cancer [37]. Unfortunately, the drug would fail to improve survival compared to the control protocol, chemotherapy with trastuzumab [38]. Although not as effective as current HER2-positive metastatic breast carcinoma treatment protocols, MM-302's favorable safety profile still offers a source of encouragement regarding the eventual development and utilization of immunoliposome formulations in clinical practice [37].

Challenges associated with liposome-based drug delivery include decreased long-term stability/shelf-life (something shared by most lipid-based delivery systems), problems surrounding sterilization (chemical and heat-based sterilization techniques disrupt liposome stability and integrity), and historically having lower encapsulation efficiencies, especially with hydrophobic drugs [39].

Recent Liposome Utilization

A drug repurposing study conducted by Shah et al. demonstrated that niclosamide-loaded liposomes (NIC-loaded liposomes) were able to achieve a 1.756-fold increase in cytotoxicity against melanoma cells (SK-MEL-28), with an IC_{50} of 1.828 μM compared to 3.210 μM for free niclosamide, highlighting the improved therapeutic potential of the liposomal formulation [23]. These liposomes were created using a modified ethanol injection method, producing liposomes with sizes ranging from 108.26 to 207.43 nm, aligning within the preferred range of 100–200 nm for efficient cellular uptake. Shah et al. achieved a zeta of -13 ± 9.71 mV, which suggests moderate stability, although a zeta potential greater than ±30 mV is generally considered ideal for preventing aggregation. They also recorded drug loading content (DLC) rates between 7 and 20%, more than the generally acceptable 1–10% for liposome encapsulation. They provided a range of their encapsulation efficiency (EE), the percentage of drug successfully encapsulated within the nanoparticle system, from 58.21 to 94.16%, also an acceptable range for liposome encapsulation [23,34]. They describe the fabrication process as the following: niclosamide, egg lecithin, and cholesterol were weighed in ratios equivalent to 10.5 mM and dissolved in an organic phase composed of a 1:1 mixture of acetone and ethanol. The organic phase was then added dropwise to the aqueous phase and stirred. The organic phase was then evaporated using a rotary evaporator.

Similarly, Yu et al. utilized liposomes to improve niclosamide bioavailability for application in the setting of pulmonary fibrosis [24]. They fabricated and investigated the efficacy of niclosamide-loaded lipid nanoparticles (Ncl-Lips). Niclosamide was introduced into a DMSO (dimethyl sulfoxide) solution and then lyophilized. DSPE-PEG2000 and cholesterol were then introduced and mixed in ethanol using ultrasonic dispersion. This produced an average liposome size of 136.97 ± 0.54 nm with a polydispersity index (PDI) of 0.14–0.17. They reported an EE of 89.49 ± 2.40%, with a DLC as high as s 32.25 ± 0.49% and a zeta potential of -13.5 ± 0.98 mV. After intravenous injection in a mouse model, their formulation was detected in serum. They were able to confirm the deposition of niclosamide in their target (lung tissue) via circulation [24].

Additionally, another study utilizing a thin-film method demonstrated that nanoliposome encapsulation significantly enhanced the anti-tumor activity of niclosamide in B16F10 melanoma, both in vitro and in vivo [36]. Liposomal encapsulation not only in-

creased the drug's anti-cancer efficacy but also improved stability, reduced systemic toxicity, and prolonged circulation time [36]. This was achieved through the protection provided by the phospholipid bilayer, which shielded niclosamide from enzymatic degradation, facilitated its accumulation at the tumor site, and enabled controlled drug release. In a separate study targeting CT26 colon cancer cells, niclosamide-loaded liposomes (NicLLs) exhibited greater tumor inhibition in vivo compared to free niclosamide, despite the free drug showing higher cytotoxicity in vitro (IC_{50}: 2.5 μM vs. 4.4 μM for NicLL) [25]. This underscores the importance of solubility and stability for translation to real clinical outcomes with niclosamide.

2.2. Solid Lipid Nanoparticles (SLNs)

Solid lipid nanoparticles (SLNs), developed by Muller in the 1990s, are colloidal carriers composed of a solid lipid core which remains solid at room and body temperature (Figure 1c) [40]. This core, made from biocompatible and biodegradable lipids such as glyceryl monostearate, stearic acid, or acetyl palmitate, encapsulates the drug [41]. The lipid core is then stabilized by an overlaying layer of surfactant or emulsifiers like Tween 80, lecithin, or poloxamers, which prevent particle aggregation and ensure the stability of the nanoparticles in suspension [41,42].

SLNs offer the advantages of improved physical stability and controlled drug release in comparison to earlier systems (lipid nanoemulsion (LNE) and liposomes) [43]. These advantageous characteristics are achieved via their solid lipid core [44]. The solid lipid matrix protects the encapsulated drugs from environmental degradation, including damage mediated by oxidation and photodegradation [45]. Another advantage of the SLN system is its ease of production. Compared to other lipid-based systems such as liposomes, SLNs exhibit greater stability, have a reduced risk of drug leakage, and are easier to scale up for production [46]. This makes them a cost-effective and efficient solution for a variety of therapeutic applications, including cancer treatment, infectious diseases, and chronic conditions [46]. The ability of SLNs to enhance bioavailability and stability makes them particularly promising for hydrophobic drugs like niclosamide.

SLNs offer advantages such as enhanced stability and sustained release; however, there are some limitations. For example, SLNs have lower drug-loading content (DLC) due to the rigid structure of their solid lipid core, which restricts the amount of drug that is able to be encapsulated [41]. This may pose a challenge in cancer therapies requiring higher drug concentrations, where other delivery systems like LNE or liposomes may prove more effective. Additionally, SLNs are prone to polymorphic transitions during storage leading to drug expulsion, further driving down the DLC and reducing drug bioavailability [41]. The initial burst release (see Pindiprolu et al. below) associated with SLNs may also result in the fluctuation of drug levels, complicating the maintenance of steady therapeutic concentrations necessary for optimal serum concentrations [47]. Additionally, SLNs may suffer from particle aggregation and inconsistent size distribution, making other systems more appropriate when considering drug design [48]. Finally, in vivo SLNs may exert off-target effects, accumulating in liver, spleen, and lung tissues when they are consumed by macrophages. In these instances, SLNs may lead to neutrophilia and macrophage activation [49]. These limitations highlight the need to carefully weigh the benefits and drawbacks of SLNs for delivery purposes, particularly when alternatives may offer superior drug loading, stability, and scalability.

Recent SLN Utilization

In a recent in vitro study, niclosamide-loaded solid lipid nanoparticles (NIC-SLNs) were formulated and their ability for sustained release characterized [26]. NIC-SLNs were formulated using glyceryl monostearate, stearyl amine, soya lecithin, and Tween 80 through an emulsion solvent evaporation method. The nanoparticles had a size of 197.3 ± 18.08 nm, which falls on the higher end of the preferred 100–200 nm range for efficient cellular uptake. The zeta potential of +10 ± 3.43 mV indicates moderate stability, though a zeta potential

above ±30 mV is typically ideal for preventing aggregation [50]. The entrapment efficiency of 75.64% is within a favorable range (typically >70%), while the DLC of 8.16% is acceptable but could be improved for formulations aiming for higher drug payloads [51]. Overall, these findings suggest a promising formulation but with room for optimization.

The sustained release profile of Nic-SLNs is critical for maintaining prolonged drug availability, particularly for drugs like niclosamide with a short half-life and poor bioavailability. The study conducted in vitro analysis and showed an increased drug release profile (cumulative release of 44% over 24 h), indicating NIC-SLNs could provide controlled, extended drug delivery, thus improving therapeutic efficacy, reducing dosing frequency, and minimizing fluctuations in drug concentration, translating to better clinical outcomes with fewer side effects [26]. However, additional in vivo studies have yet to be conducted with this promising formulation, which are necessary to validate its anticancer efficacy and improved pharmacokinetics.

While the previous study was limited to in vitro testing, Rehman et al. conducted a comparative in vivo evaluation of their own NIC-SLN formulations. Stearic acid, polyethylene glycol (PEG-400), and Tween 80 were used to formulate five different NIC-SLNs (NFM-1~5) and were tested on their release profiles, where the specific formulation (NFM-3) with a particle size of 204.2 nm and polydispersity index (PDI) of 0.328 indicated a moderately narrow size distribution [27]. Typically, a PDI below 0.3 is considered ideal for monodisperse systems, meaning the particles are uniform in size [28,51]. A PDI of 0.328, while slightly above 0.3, still reflects an acceptable distribution for many nanoparticle formulations, indicating controlled size variation within the sample [28,51]. In addition, a zeta potential of −33.16 mV was recorded, suggesting strong particle stability due to repulsion between similarly charged particles, preventing aggregation [27]. NFM-3's EE was 84.4%, indicating a high proportion of niclosamide is encapsulated compared to other SLN formulations, where EE values typically range between 70% and 90% [27,52]. DLC was found to be 5.27%. In comparison to other studies, this DLC is on the lower end, as the average DLC for SLN formulations generally falls between 5% and 15% depending on the drug and lipid components used [46,53]. While this DLC is sufficient for therapeutic purposes, there is potential for optimization to increase drug loading, particularly in instances where clinical application demands higher doses of niclosamide.

In vitro release studies of NFM-3 demonstrated that NIC-SLNs exhibited a sustained release profile, with 93.21% of the drug being released over 12 h, following zero-order kinetics (a constant drug release rate independent of drug concentration) [27]. The drug release mechanism followed Case-II transport, indicating a combination of diffusion and erosion processes from the solid lipid matrix [54]. In this system, the drug diffuses while the lipid matrix undergoes gradual erosion, facilitating sustained drug release [54,55]. This controlled dual process is particularly beneficial for maintaining therapeutic drug levels over extended periods, reducing the need for frequent dosing and providing a steady release of the drug throughout treatment [55]. NFM-3's in vivo pharmacokinetic testing revealed a 2.15-fold increase in peak plasma concentration compared to free niclosamide [27]. The in vivo results demonstrate promising potential, with a relative bioavailability of 11.08, further confirming the findings of previous in vitro studies that the SLN system is highly effective in improving the aqueous solubility, permeability, and bioavailability of niclosamide, making it a prospective strategy for enhancing oral drug delivery [27].

Pindiprolu et al. also developed SLNs for the targeted delivery of niclosamide in triple-negative breast cancer (TNBC) cells using an emulsification–solvent evaporation method [56]. Stearylamine was employed as the solid lipid, while Tween 80 and Pluronic F-68 (intestinal drug efflux inhibitors) were used as surfactants. The lipid and aqueous phases were combined, homogenized, sonicated, and freeze-dried to form NIC-SLNs. The formulation was optimized by the Box–Behnken design, which evaluates both linear and quadratic interactions between variables while minimizing the number of experiments [56,57]. Spherical particles with a size of 112.18 ± 1.73 nm were produced, falling within the optimal range (100–200 nm) for nanoparticle-based drug delivery, ensur-

ing good permeability and enhanced cellular uptake. The zeta potential was measured at 23.8 ± 2.7 mV, indicating moderate stability, though values above ±30 mV are typically preferred for optimal stability in colloidal systems.

The EE and DLC were determined to be 82.21 ± 0.62% and 8.3 ± 0.42%, respectively. The EE is relatively high, as values above 70% are generally considered good for SLN formulations, indicating efficient encapsulation of niclosamide within the nanoparticles [53,56]. The drug release profile of Pindiprolu et al.'s formulation was examined at physiological pH (7.4) and acidic pH (5.5) to mimic tumor microenvironment. At pH 5.5, an initial burst release was observed, with 90% of the drug released within the first hour, compared to 25% at pH 7.4. This higher rate of drug release in acidic conditions favors cancer cell targeting, as tumor microenvironments are generally characterized by a lower pH [58]. The authors attributed the increased release to the protonation of the amino group in stearylamine at lower pH levels, in turn enhancing the drug's release in the tumor environment [56].

Furthermore, in vitro studies on TNBC cells revealed that NIC-SLNs exhibited significantly better cytotoxicity than free niclosamide, likely due to enhanced cellular uptake and efficient intracellular delivery of the drug [56]. The SLN-treated TNBC cells showed accumulation in the G0/G1 phase of the cell cycle (77.06%), higher than the 69.50% observed with free niclosamide. Additionally, apoptosis was significantly increased with NIC-SLN treatment, with 70% of cells undergoing apoptosis compared to 50% in cells treated with free niclosamide, demonstrating enhanced pro-apoptotic activity as well. Pindiprolu et al.'s formulation provided an optimal particle size for effective drug delivery, moderate stability based on zeta potential, and high encapsulation efficiency (EE) [56]. The enhanced drug release in acidic conditions, combined with improved cellular uptake, cell cycle arrest, and apoptosis induction, makes their NIC-SLN formulation a promising approach for targeting TNBC [56].

2.3. Nanostructured Lipid Carriers (NLCs)

While SLNs enhance the bioavailability and solubility of hydrophobic drugs, they face limitations due to the solid and rigid lipid core matrix, which can expel drugs from the crystalline structure over time and compromise long-term stability [59]. To avoid unwanted drug expulsion, nanostructured lipid carriers (NLCs) utilize a different core composition, combining solid and liquid lipids, creating a more disordered lipid matrix that enhances drug entrapment (Figure 1b) [60]. However, although NLCs demonstrate improved stability and drug entrapment compared to SLNs, they remain vulnerable to gastrointestinal fluid, specifically by degradation via bile salts [61]. To improve stability and avoid degradation, Elkholy et al. introduced a chitosan coating in their NLC formulation, which enhanced bioavailability, ensured structural integrity, and increased adhesion to intestinal walls, contributing to increased drug absorption [62].

Chitosan-coated NLCs loaded with niclosamide were prepared using micro-emulsification with solid lipids (Precirol ATO 5, Compritol 888 ATO), liquid lipids (oleic acid, Tween 80), and diacetyl phosphate as a charge modifier. Uncoated NLC formulations (F1, F2, F3) had particle sizes ranging from 189.6 nm to 334.5 nm, while coated formulations (F4, F5) had mean sizes of 259.5 nm and 268 nm, respectively [29]. NLCs ranging between 100 nm and 300 nm may be an optimal balance for drug delivery. Nanoparticles in this size range circulate longer by avoiding rapid kidney clearance (particles < 100 nm more at risk) and excessive uptake by the liver and spleen [29]. Nanoparticles in this size range also have enhanced cellular uptake via clathrin-mediated endocytosis, often even more so in the setting of cancer due to the enhanced permeability and retention effect observed in tumors [42]. This leads to a greater accumulation of the drug payload in cancerous cells [42]. EEs were high, ranging from 98.8% to 99.7%, attributed to niclosamide's inherent lipophilicity and its localization within the lipid matrix. Drug release over 10 h was slow (3–5%), with no significant difference between coated and uncoated NLCs, once again attributed to niclosamide's affinity for the lipid matrix [62]. This slow release of niclosamide from the NLCs is not necessarily a disadvantage. In fact, it is beneficial

in certain applications, particularly for oral drug delivery where sustained, controlled release is desired, as slow release may help maintain therapeutic levels of the drug over an extended period.

In vivo, NLCs significantly reduced tumor volume in mice with solid Ehrlich carcinoma by 73–79%, outperforming a niclosamide aqueous suspension [62]. In addition, histopathological analysis showed increased tumor necrosis and reduced mitotic figures, indicating the superiority of NLCs over the free suspension. Chitosan-coated NLCs were slightly more effective than uncoated NLCs, likely due to chitosan's mucoadhesive properties enhancing tumor cell targeting [62].

2.4. Lipid Prodrug

A lipid prodrug is a drug chemically modified by attaching a lipid moiety, such as fatty acids (e.g., oleic acid), glycerides, phospholipids, or cholesterol, to improve its pharmacokinetics [20]. These lipid attachments enhance the drug's solubility, bioavailability, and ability to cross lipid membranes [20]. Once administered, the lipid is enzymatically cleaved, releasing the active drug at the target site [63]. This approach is particularly effective for poorly water-soluble drugs, optimizing absorption, circulation, and delivery.

By formulating niclosamide into Niclosamide Stearate Prodrug Therapeutic (NSPT), Reddy et al. aimed to create a stable, injectable prodrug that can overcome the challenges associated with free niclosamide and deliver therapeutically effective concentrations of the drug directly to tumor sites [64]. NSPTs were created using a rapid solvent injection method, resulting in nanoparticles with an average size of 30 $\pm$ 5 nm, as measured by dynamic light scattering, which assesses particle size distribution based on the scattering of light. The nanoparticles consist of a core of niclosamide stearate (an esterified form of niclosamide) and a stabilizing lipid monolayer composed of DSPC (distearoylphosphatidylcholine) and cholesterol. Additionally, DSPE-PEG2000 (a PEGylated lipid) was incorporated into the monolayer to reduce protein binding and enhance circulation time in plasma by avoiding detection and clearance by the immune system (stealth properties). This nanoparticle structure aimed to ensure that NSPTs were stable in different environments, including water, equiosmotic solutions, and isotonic buffers such as PBS (phosphate-buffered saline) [64].

Pharmacokinetic studies in mice demonstrated that after intravenous administration of NSPTs, both niclosamide stearate and its active form exhibited a half-life of approximately five hours, significantly extending their circulation time compared to free niclosamide. The area under the curve (AUC), which measures the total drug exposure over time, was substantially higher for niclosamide stearate (3560 hμg/mL) compared to free Nic (1.4 hμg/mL from previous studies). This dramatic increase in AUC indicates that NSPTs deliver higher amounts of niclosamide into circulation, ensuring that therapeutically relevant concentrations can be maintained for longer periods of time [64].

In vitro stability tests also confirmed that NSPTs are resistant to hydrolysis, with a half-life of 17 days in PBS and over 24 h in plasma. This extended stability ensures a controlled and sustained release, providing better therapeutic outcomes. Notably, NSPTs showed significant efficacy in reducing cell viability and proliferation in osteosarcoma models. In both human and canine osteosarcoma cell lines, NSPTs inhibited cell growth at IC_{50} values ranging from 0.2 to 2 μmol/L, effectively targeting cancer cells while sparing normal cells [64].

While NSPTs have demonstrated potential, several areas remain in need of improvement. Although NSPTs demonstrated efficacy at both low and high doses (0.59 mg/kg and 50 mg/kg), the large difference in dose between these trials suggests the need for more precise dose–response studies to ensure maximum efficacy with minimal dosing. In addition, even though early preclinical data in mice and canine models show promise, the translation of these results into human clinical trials is pending. Testing of NSPTs in larger randomized controlled trials in canine osteosarcoma models could help solidify NSPT's clinical feasibility, safety, and long-term efficacy [64].

2.5. Lipid Nanoemulsions

Lipid nanoemulsions (LNEs), also referred to as submicron lipid emulsions (SLEs), are colloidal dispersions consisting of oil droplets stabilized by surfactants within an aqueous phase, with droplet sizes typically ranging between 100 and 500 nm (Figure 1a) [65]. These emulsions are designed to improve the solubility, stability, and bioavailability of hydrophobic drugs in vivo [65,66]. LNEs provide several advantages over other drug delivery systems such as SLNs, NLCs, and liposomes. Unlike SLNs, which are prone to polymorphic transitions and potential drug expulsion, and NLCs, which offer limited DLC, LNEs can incorporate higher drug concentrations and provide a greater level of stability over time [66]. Additionally, PEGylating LNEs can assist in evasion of the immune system and prolong serum circulation time [67]. While PEGylation may prolong circulation time, they still generally have a shorter half-life compared to other formulations due to rapid clearance [65]. Still, this rapid clearance may offer benefit when attempting to maximize safety and avoid toxicity. These attributes make LNEs a viable platform for delivering low-solubility drugs.

In a comparative bioavailability study, Zhang et al. explored employing LNEs in conjunction with PEGylation to enhance niclosamide bioavailability [30]. The researchers developed two LNE formulations: conventional niclosamide-loaded/submicron lipid emulsion (NL-CSLE) and PEGylated niclosamide-loaded/submicron lipid emulsion (NL-PSLE), using melt dispersion and high-pressure homogenization techniques [30]. The particle sizes were 307.8 nm for NL-CSLE and 162.2 nm for NL-PSLE, both of which exhibited an acceptable drug loading (>9.0%). For comparison, conventional SLE loading is generally between 5% and 15% but as high as 20% [30,68,69]. When administered orally, both formulations showed significantly enhanced bioavailability, increasing 441.11% for NL-CSLE and 463.55% for NL-PSLE in comparison to free niclosamide [30]. PEGylation further reduced particle size and enhanced the stealth properties of NL-PSLE, increasing circulation time and avoiding immune system recognition [30]. These findings demonstrate that SLEs, especially PEGylated variants, can be highly effective in overcoming the solubility challenges of niclosamide, providing enhanced bioavailability and therapeutic potential.

Another study performed by Barbosa et al. characterized the effect of using a long- vs. medium-chain lipid base in a niclosamide nanoemulsion (Nano-NCL) [31]. Niclosamide incorporated into both Nano-NCL (particle size < 200 nm) and Coarse-NCL (particle size > 1 μm, PDI > 0.6) forms of the same formulation shifted the cell viability curve leftward compared to the free drug, suggesting that the lipid systems potentiated the cytotoxicity of niclosamide. The similar IC_{50} values of Nano-NCL (8.775 μM) and Coarse-NCL (9.936 μM) at 24 h indicated that particle size was less critical during the early stages of treatment. At 48 h, however, the IC_{50} of Nano-NCL (1.259 μM) was 3.6-fold lower than that of Coarse-NCL, indicating that the smaller particle size of the nanoemulsion enhanced drug potency over time. This highlights the importance of the nanoscale formulation in increasing the therapeutic effect of niclosamide during longer treatment periods [31]. The study also demonstrated that Nano-NCL significantly enhanced the cytotoxicity of niclosamide against colorectal cancer cells (CRCs) (HCT-116) compared to the free drug solution. For instance, at 24 h, the IC_{50} of Nano-NCL was 5.842 mg/mL, nearly three times lower than the blank nanoemulsion control (15.82 mg/mL), demonstrating cytotoxicity was primarily driven by niclosamide [31].

Barbosa et al. confirmed that medium-chain lipids provide better solubility for niclosamide compared to long-chain lipids, supporting trends observed in broader nanoemulsion research [70–72]. Earlier studies have shown that shorter-chain lipids generally enhance the solubility of poorly soluble drugs by increasing the molar concentration of ester groups, which form stronger hydrogen bonds with polar solutes. For example, studies by Caliph et al. suggested that medium-chain lipids promote better solubility due to increased hydrogen bonding between ester groups and polar groups (-OH, -NH) in the drug molecule [72]. In addition, other research has also demonstrated that medium-chain lipids are better at forming interactions with drugs because of their increased ester content

and reduced hydrophobicity compared to long-chain lipids [70,71] Consistent with this phenomenon, the niclosamide nanoemulsion with long-chain lipids consistently failed to solubilize niclosamide, confirming that chain length plays a crucial role [31]. Building on the finding about niclosamide LNEs and chain length, the researchers suggested that medium-chain LNEs transported via the lymphatic system could improve oral delivery of niclosamide, especially in conditions like colon cancer where lymphatic metastasis affects prognosis [31]. This aligns with existing evidence that medium- and short-chain lipids support lymphatic drug transport [71,72].

In summary, this study demonstrates that the Nano-NCL formulation significantly enhances the cytotoxicity of niclosamide compared to the coarse dispersion and free drug solution, particularly at later time points (48 h), where the nanoscale size becomes crucial for potentiating drug efficacy. The improved activity is likely due to better cell uptake, tumor penetration, and interaction with critical cancer-related pathways like Wnt/β-catenin.

2.6. Self-Microemulsifying Drug Delivery System (SMEDDS)

The self-microemulsifying drug delivery system (SMEDDS) enhances the bioavailability of poorly water-soluble drugs by spontaneously forming nanometer-sized droplets upon contact with gastrointestinal (GI) fluids [19]. SMEDDS are composed of oils, surfactants, and cosurfactants, which are smaller, amphiphilic molecules that help reduce surface tension and stabilize the microemulsion (Figure 1e) [19]. They improve the system's flexibility, allowing for better emulsification [19]. The SMEDDS improves solubility and absorption more efficiently than other lipid-based systems like solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), and lipid nanoemulsions (LNEs), particularly for rapid oral drug delivery, though other systems may excel in areas when a sustained release or higher drug loading is desirable [73]. In addition to its rapid drug release when compared to SLNs and NLCs, it proves more stable than LNEs, with the added benefit of being easier and cheaper to manufacture than liposomes, though liposomes provide targeted drug delivery [74]. SMEDDS characteristics derive from its liquid structure and the absence of a solid lipid matrix which limits its drug loading capacity [75]. It is mainly suited for oral delivery, whereas LNEs can be used in parenteral routes. SMEDDS also may face stability issues with its surfactants over time. Despite these drawbacks, SMEDDS remains effective for enhancing the solubility and bioavailability of hydrophobic drugs, especially in instances which demand oral drug administration [75].

In a recent study, Liu et al. fabricated an optimized niclosamide self-microemulsifying drug delivery system (Nic-SMEDDS) which exhibited ideal physicochemical properties for drug delivery (Figure 2) [76]. They prepared the formulation by introducing niclosamide to various oils, surfactants, and cosurfactants (Tween80® purchased from Sigma-Aldrich (St. Louis, MO, USA), cycloheximide and D-luciferin potassium salt from Thermo Fisher Scientific Inc. (Waltham, MA, USA), PEG-8 CapRrylic/Capric glycerides (Labrasol® ALF), Plurol isostearique, and Labrafac lipophile WL 1349 from Gattesfosse (Paramus, NJ, USA)) using the shaking flask method. Supernatants were then collected and filtered through a 0.45 μm pore-size membrane filter [76]. The filtrates were then dissolved in methanol. Twenty mixtures were prepared; each oil phase was blended with a surfactant/cosurfactant mixture and stirred in a shaking water bath. They selected a SMEDDS formulation based on 66.94% Labrasol ALF, 13.36% Plural oleique, and 14.09% Labrafac lipophile WL1349 for niclosamide loading [76].

They were able to achieve a 4.1-fold increase in bioavailability compared to free niclosamide in non-tumor-bearing mice. They reported an average particle size of ~150 nm, a zeta potential of −6.8 mV, DLC of 5.6%, and a viscosity of 133.3 cp (an ideal viscosity for SMEDDS typically falls between 100 and 300 cp), balancing ease of flow and mixing with GI fluids [32]. Viscosity that is too high can hinder the mixing process and slow absorption, while too low of a viscosity may compromise the controlled release of the drug. They recorded a mean particle size of ~150 nm, which falls within the recommended nano-size of <200 nm for drug delivery [77]. These smaller particles increase the surface area available

for dissolution, which in turn enhances drug solubility and absorption across the intestinal membrane [77]. Particles larger than 200 nm may encounter absorption limitations due to reduced solubility and slower diffusion across biological barriers [77]. The negative surface charge of −6.8 mV is also beneficial, as surface charges around −30 to +30 mV are ideal for maintaining particle stability [78]. A charge in this range helps prevent particle aggregation, ensuring the nano-sized particles remain dispersed in solution [78]. Aggregation can hinder drug absorption by reducing the available surface area for dissolution and uptake [79]. The formulation had a slightly acidic pH of 3.96, which is well within the preferred range of 3–4.5 for oral formulations [80]. This range is ideal as it minimizes drug degradation in the stomach's acidic environment while ensuring the drug remains stable through the GI tract [80]. A more basic or neutral pH might lead to premature drug breakdown or reduced absorption efficiency in the acidic stomach [80].

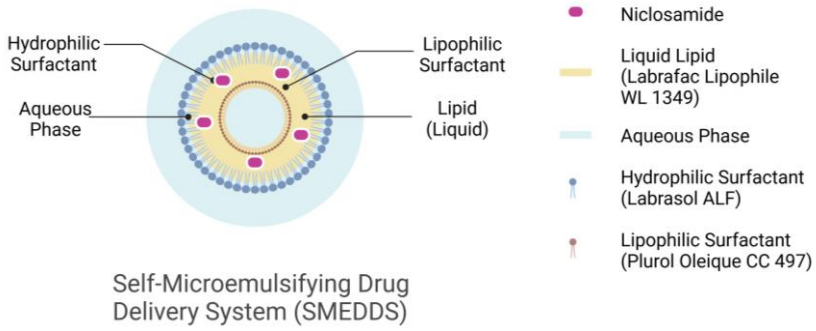

Figure 2. Schematic representation of a niclosamide loaded with SMEDDS formation, based on Liu et al. [76]. Created using BioRender.

They also conducted an in vivo anti-tumor efficacy study with their Nic-SMEDDS formulation. Pharmacokinetic analysis showed that doses of 60 mg/kg and 100 mg/kg, administered twice daily, achieved the highest niclosamide plasma concentrations and subsequent tumor growth inhibition compared to a saline-treated control group [76]. The 100 mg/kg bid dose led to the greatest suppression of tumor volume, outperforming niclosamide ethanolamine salt (NEN) at 200 mg/kg. The authors suggest that Nic-SMEDDS enhances the therapeutic efficacy of niclosamide by improving bioavailability and maintaining higher drug levels. They attribute the increase due to characteristics of the Nic-SMEDDS formulation, which allows for an enhanced interfacial area when dispersed in an aqueous solution, facilitating the easy partition of drugs from the oil phase into the aqueous phase. They also believe the intestinal efflux inhibiting surfactants included in the formulation contributed to favorable pharmacokinetics.

In addition, histological analysis revealed increased apoptosis and necrosis in tumor cells, as indicated by reduced Ki-67 staining (a marker of cell proliferation) and elevated caspase-3 levels (a marker of apoptosis). These findings, coupled with minimal body weight loss and no significant toxicity in major organs, suggest that Nic-SMEDDS is a potent and safe formulation for improving niclosamide's antitumor efficacy. Furthermore, Nic-SMEDDS demonstrated favorable effects on biochemical parameters, including reduced glucose levels and preserved liver function, reinforcing its potential as a safe and effective cancer treatment [76]. Despite the early clinical trial failures of free niclosamide, this novel formulation potentially offers an avenue for success through increasing bioavailability and reducing systemic toxicities [81,82]. Nic-SMEDDS also has potential for clinical translation as a treatment for other solid tumors, particularly where niclosamide's signaling inhibition mechanisms are relevant. Therefore, further preclinical and clinical studies are necessary to optimize the formulation for human use.

3. Conclusions

Lipid-based drug delivery systems act as accessible and realistic solutions for addressing the significant pharmacokinetic barriers associated with niclosamide. Niclosamide

formulations created from liposomes, SLNs, NLCs, and SMEDDS have repeatedly been shown to enhance niclosamide's bioavailability and therapeutic efficacy by improving solubility, altering drug release, and enabling the deposition of niclosamide in tissue. However, limitations such as low drug loading in SLNs, risk of drug expulsion, and storage instability in certain lipid systems suggest the optimal utilization of these methods is contingent on the system being properly attuned to achieve the desired delivery characteristics (quick or sustained release, GI-based delivery, etc.). While these lipid drug delivery systems have proven effective, combining lipid-based delivery with niclosamide derivatives offers another unexplored avenue. Derivatives like *O*-alkylamino-tethered niclosamide and halogenated niclosamide have also shown promise in improving solubility and potency in various tumors [12,13,83]. Combining derivatives with lipid delivery systems may result in a synergism which furthers the repurposing of niclosamide as an anticancer agent. In the future, it may be a worthwhile endeavor for researchers to test the feasibility of delivering niclosamide derivatives by way of lipid delivery systems when they are conducting pharmacokinetic studies aimed at improving aspects of the original compound for repurposing.

Author Contributions: Resources, data curation: R.W.W. and J.W.; writing—original draft preparation, J.W. and R.W.W.; writing—review and editing, R.W.W.; visualization, J.W.; conceptualization, validation, and supervision, S.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

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