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Research Article

Pharmacokinetic Study of Intravenous Lomustine and Intranasal Lomustine-Loaded Solusome Nanovesicle in Rats for Brain Targeting

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Abstract

Background: Glioblastoma multiforme is a Grade IV, very aggressive malignancy and the most frequent primary cancer of the central nervous system. Despite its favorable alkylating agent property and use in the treatment of GBM, the low aqueous solubility and high dose-related systemic toxicity restrict the clinical use of lomustine. ***Objectives***: The aim of the study was to design and optimize a high-performance liquid chromatography assay to measure lomustine and to assess the pharmacokinetic properties of a novel nano-vesicular delivery system, lomustine-loaded solusomes, to estimate nose-to-brain targeting. ***Methods***: Solosomes of lomustine were developed by the thin-film hydration method. Their ability to target the brain was tested in vivo with 84 Wistar rats. The pharmacokinetic parameters were determined after intranasal administration of solusomal formulation and compared with intravenous administration of a lomustine solution for 72 hours. ***Results***: When delivered intranasally, lomustine-loaded solusomes achieved greatly improved brain targeting with a 2.3-fold greater maximum brain concentration (Cmax) than with intravenous delivery and a shorter time to peak concentration (Tmax). The formulation showed high targeting efficiency, with drug targeting efficiency (DTE% 486.22) and direct transport percentage (DTP% 64.8). Histopathological analysis was used to establish the preservation of the integrity of nasal mucosa, and the formulation was found to be safe and non-irritating. ***Conclusions***: Solusomes administered through the nasal route are effective in bypassing the blood-brain barrier through olfactory and trigeminal routes. The method has proven to be an effective way of increasing concentrations of therapeutic agents in the brain without worsening the safety profile.

Keywords: Brain targeting; Glioblastoma multiforme; HPLC validation; Lomustine; Nose-to-brain delivery; Solusomes.

دراسة حركية دوائية للوموستين المُعطى وريديًا والحويصلات النانوية المحملة باللوموستين عبر الأنف في الجرذان لاستهداف الدماغ

الخلاصة

الخلفية: الورم الأرومي الدبقي متعدد الأشكال ورم خبيث شديد العدوانية من الدرجة الرابعة، وهو أكثر أنواع السرطانات الأولية شيوعًا في الجهاز العصبي المركزي. على الرغم من خصائص لوموستين القاتلة للخلايا واستخدامه في علاج الورم الأرومي الدبقي متعدد الأشكال، إلا أن قلة ذوبانه في الماء وارتفاع سميته الجهازية المرتبطة بالجرعة يحدّان من استخدامه السريري. **الأهداف**: تصميم وتحسين اختبار كروماتوغرافي سائل عالي الأداء لقياس تركيز اللوموستين، وتقييم الخصائص الحركية الدوائية لنظام توصيل الحويصلات النانوية الجديدة المحملة باللوموستين، لتقدير استهداف الدماغ من الأنف. **الطرائق**: تم تطوير حويصلات اللوموستين النانوية باستخدام طريقة ترطيب الغشاء الرقيق. تم اختبار قدرتها على استهداف الدماغ في الجسم الحي باستخدام 84 جرذا من سلالة ويستار. تم تحديد المعايير الحركية الدوائية بعد إعطاء تركيبة سولوسومية عن طريق الأنف، ومقارنتها بإعطاء محلول لوموستين عن طريق الوريد لمدة 72 ساعة. **النتائج**: عند إعطاء الجسيمات النانوية المحملة باللوموستين عن طريق الأنف، تم تحقيق استهداف دماغي مُحسَّن بشكل كبير، حيث بلغ تركيز الدواء الأقصى في الدماغ 2.3 ضعفًا مقارنةً بالإعطاء الوريدي، مع تقليل الوقت اللازم للوصول إلى ذروة التركيز. أظهرت التركيبة كفاءة استهداف عالية، حيث بلغت كفاءة استهداف الدواء 486.22% ونسبة النقل المباشر 64.8%. استُخدم التحليل النسيجي المرضي للتأكد من سلامة الغشاء المخاطي للأنف، ووُجد أن التركيبة آمنة وغير مُهيّجة. **الاستنتاجات**: تُعد السولوسومات المُعطاة عن طريق الأنف فعالة في تجاوز الحاجز الدموي الدماغي عبر المسارين الشمي والعصبي الثلاثي التوائم. وقد أثبتت هذه الطريقة فعاليتها في زيادة تركيز العوامل العلاجية في الدماغ دون التأثير سلبًا على مستوى السلامة.

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INTRODUCTION

The WHO classifies glioblastoma multiforme (GBM) as a Grade IV brain tumor, the most aggressive and malignant cancer that starts in the central nervous system [1]. In the U.S., GBM is the most common primary malignancy with an incidence of about 10,000 per year. [2]. This type of tumor accounts for almost 80% of all malignant primary brain tumors [3]. GBM is characterized by strong tumor heterogeneity and genetic instabilities, which promote its aggressively invasive phenotype, long-term angiogenesis, and resistance to therapy [4]. Lomustine (LOM) is an alkylating agent that is a member of the nitrosourea category of drugs. This compound, chemically known as cyclohexyl nitrosourea, is used to treat various malignancies in chemotherapy, although it has been shown to be especially effective in treating brain tumors [5]. LOM is an alkylating agent, which has its cytotoxic effects through forming cross-links within the DNA, which inhibits cancer cell division and results in programmed cell death (apoptosis) [6]. Despite its therapeutic benefits, the clinical use of LOM is often limited by significant dose-dependent adverse effects, most notably bone marrow suppression

(hematologic toxicity) and respiratory complications (pulmonary toxicity) [7]. Furthermore, the hydrophobic nature of LOM complicates its intravenous delivery, frequently leading to severe complications such as vascular embolization and respiratory failure. These risks often arise from the poor solubility of the drug in aqueous environments, which can result in the formation of precipitates within the bloodstream [8]. Consequently, it's a critical requisite to pioneer a novel delivery approach that enhances both the therapeutic effectiveness and the safety profile of LOM for clinical use [5]. Encapsulating drugs in nanoparticles, liposomes, polymeric nano-vesicles, and other nano-carriers offers numerous benefits, including solubilization of poorly soluble drugs, stability, avoidance of precipitate upon dilution, and defense against destabilizing chemicals [9]. Solusomes in pharmaceutical drug delivery: Solusomes are a new type of nano-vesicular carriers combining the structural advantage of the conventional liposomes with the solubilizing effect of the amphiphilic polymer Soluplus to increase the maximum efficiency of the drug [10]. The effectiveness of nose-to-brain delivery of drugs via the nasal route is based on the anatomical structure of the nasal cavity and its intertwined neural pathways. These specialized structural mechanisms enable a circumvention of the blood-brain barrier (BBB), a vital route of delivery of therapeutic agents to the central nervous system [11]. The intranasal route has received a lot of research attention as a strategic methodology of overcoming the BBB [12]. Intranasal to central nervous system delivery has been reported to be safe, bypass hepatic first-pass metabolism, easier to administer, and patient-adherent [13]. Since delivery via olfactory conduits is non-invasive and is a way of circumventing the BBB and delivering therapeutics directly to the brain, intranasal delivery has received considerable attention [14]. This phenomenon is because the nasal cavity is lined with a thickly vascularized mucous membrane that facilitates quick absorption of therapeutic substances [15]. The major central nervous pathways, the olfactory and trigeminal nerves, play crucial roles in mediating direct intranasal-to-brain pharmacological transfer. [16]. Although the pharmacokinetics evaluation is promising from a clinical perspective, it has not been documented at all [17]. Prior inquiries regarding lomustine quantification predominantly focused upon the characterization of lomustine following oral delivery [18]. Nevertheless, extensive literature addresses the pharmacodynamics, toxicological, and pharmacokinetic attributes of lomustine; consequently, comprehensive and systematic methodological development and validation remain unclear [19]. Consequently, the objective of our research is to develop and validate a distinct, robust, and cost-effective HPLC technique for lomustine quantification in rat plasma and brain and study the pharmacokinetics of the lomustine solution and lomustine-loaded solusomes after intravenous and intranasal route administration to Wistar rats, respectively.

METHODS

Materials

Soya lecithin phosphatidylcholine 98% (SLPC) and Lomustine (LOM) were provided by Baoji Guokang Bio-Technology Co., Ltd., China. Cholesterol and propyl paraben (internal standard, IS) were purchased by Kathy Chemical Company, Iraq, from Wuhan Senwayer Century Chemical Co., Ltd., China. Absolute ethanol, HPLC-grade methanol, and acetonitrile were donated from the National Center for Drug Control and Research (Baghdad/Iraq). Soluplus® was purchased from BASF Pharmaceutical Co., Ltd., Germany.

Compositions of Lomustine-loaded solusomes (LOMLS)

The formulation of LOMLS via the application of the thin-film hydration method [20]. It involves organic phase preparation by using specific ratios of the drug (LOM), soya lecithin phosphatidylcholine (SLPC), cholesterol (for membrane stability), and the polymer Soluplus®, as shown in Table 1.

Table 1: Compositions of LOMLS Nanovesicle

Ingredient	Amount (mg)
Lomustine	10
SLPC	50
Soluplus®	25
Cholesterol	10

Chromatographic equipment and circumstances

The HPLC system (Shimadzu, Japan) was coupled with an LC-20AD solvent delivery pump, an SIL-20A HT autosampler, a column oven, a DGU-20A 5R degassing unit, and a PDA detector. The Pharmaceutical Research Department, National Centre of Drug Control and Research (Baghdad, Iraq), carried out data acquisition and processing. Separations were done by chromatography with a reversed-phase C18 column. The mobile phase was acetonitrile and water (50:50, v/v), which was pumped at a fixed rate of 1.0 mL/min. A column oven was used to keep the column temperature at 30°C, and UV detection was performed at 254 nm with an injection of 20 µL [21].

In vivo study design

The study involved 84 Wistar rats aged 3 months with an average weight of 250±30 g in two sets of rats, including six rats in each time interval (0, 0.1, 0.2, 0.25, 0.5, 0.75, 1, 3, 6, 12, 24, 36, 48, and 72 hours). Both food and water were provided to rats with unrestricted access. after receiving 0.3 ml of ketamine as an intramuscular injection to induce anesthesia [22]. For 72 hours, a 0.5 mg/kg animal dose per weight was the LOM dosage. To enable the animals in group I (intranasal set) to take the entire formulation, a micropipette was utilized to gradually infuse a volume of the optimized IN LOMLS (50 µL) into each nostril. In contrast, the animals in group II (intravenous set) received an intravenous injection of freshly prepared LOM solution in their tail vein. After each group

received LOM, in each group, six animals were sacrificed at each time interval, and 3-4 ml of carotid artery blood samples had been taken, and their brains were extracted. Plasma and brain samples were isolated and frozen at -20ºC for the following preparation and investigation [23]. Blood samples were collected in an ethylenediaminetetraacetic acid (EDTA) tube, a powerful anticoagulant that binds calcium to prevent blood from clotting [24]. The protein-precipitating agent, methanol, was then added (4 mL of methanol/1 mL of blood), and it was vortex mixed after 1 minute. The solution was then centrifuged at 4000 rpm/30 min [25]. The supernatant was filtered and then transferred into glass vials, leaving the rest of the methanol to evaporate at 45°C. The residue was reconstituted with the internal standard (IS); 100 µL of plasma was mixed with 10 µL of IS and then diluted with 60 µL of mobile phase. A brief vortex and centrifugation of the mixture at 10,000 rpm in 5 minutes were performed. Lastly, 100 µL of the supernatant aliquots were taken to be analyzed using HPLC. [26]. Protecting the light of the samples was carried out in the course of the experiment. The concentration of drugs in the brain was analyzed by homogenizing the brain tissue with normal saline in a 1:9 (w/w) ratio using a tissue homogenizer to prepare brain homogenates [27]. Methanol (400 µL), which is a protein-precipitating reagent, was added to 100 µL of the sample, along with 10 µL of internal standard. After blending the materials for 15 minutes in a vortex, then using the centrifuge device for 10 minutes at 4000 rpm [28]. After that, the supernatant was collected and filtered to prepare it to inject 100 µL into the HPLC device for LOM detection.

Outcome measurements

The direct transport percentage (DTP%) and drug targeting efficiency (DTE%) for intranasal (IN) LOMLS have been calculated according to the AUC data for the plasma and brain. Also, the outcomes were contrasted with those from intravenous administration; equations 1, 2, and 3 were used to calculate these parameters [30]:

$$DTE\% = \frac{(AUCbrain)IN/(AUCplasma)IN}{(AUCbrain)IV/(AUCplasma)IV} \times 100 \ldots (1)$$

$$DTP\% = \frac{[(AUCplasma)IN - Bx]}{(AUCplasma)IN} \times 100 \ldots (2)$$

Equation 3 determines Bx, or the amount of LOM that entered the brain through systemic distribution after intranasal administration:

$$Bx = \frac{(AUCplasma)IN \times (AUCbrain)IV}{(AUCplasma)IV} \ldots (3)$$

Where (AUCbrain)IN, (AUCplasma)IN, (AUCbrain)IV, and (AUCplasma)IV are used to calculate the AUC_{0-72} of LOM in brain and plasma samples after intranasal and intravenous injection, respectively.

Histopathological evaluation

Isolated fragments of the nasal mucosa were preserved using a 10% formalin solution [31]. Prior to analysis, specimens were debrided of all extraneous connective tissue. Individual sections underwent staining with hematoxylin and eosin. Ultimately, the prepared slices were examined via light microscopy to evaluate for evidence of necrosis or structural degradation [32].

Ethical considerations

Animal experiments were carried out in vivo with the approval of the Research Ethics Committee of Experimental Investigations at the College of Pharmacy, University of Baghdad, Iraq, in accordance with protocol number REC03243A.

Data analysis

Results are presented in terms of mean values in terms of standard deviation (±SD) with the sample size of n = 3. Statistical significance was determined based on the threshold P-value of < 0.05. A non-compartmental pharmacokinetic model to determine the LOM concentration versus time relationship for brain and plasma samples. We quickly determined the Cmax and Tmax for intravenous and intranasal delivery based on the concentration-time profile. Using the linear trapezoidal rule, it was possible to find the area under the curve (AUC0-72) [29]. The pharmacokinetic results have been compared for the two groups, and their statistical significance has been examined using a two-way ANOVA (Analysis of Variance) in the GraphPad Prism (version 8) software program.

RESULTS

The spiked plasma chromatogram demonstrated a clear distinction between LOM and internal standards, with LOM having a retention time of 18.13 minutes in spiked plasma and 18.15 minutes in spiked brain. The IS exhibited a signal at 7.78 minutes in spiked plasma and 7.83 minutes in spiked brain, indicating that there is no interference between LOM and IS, as depicted in Figure 1.

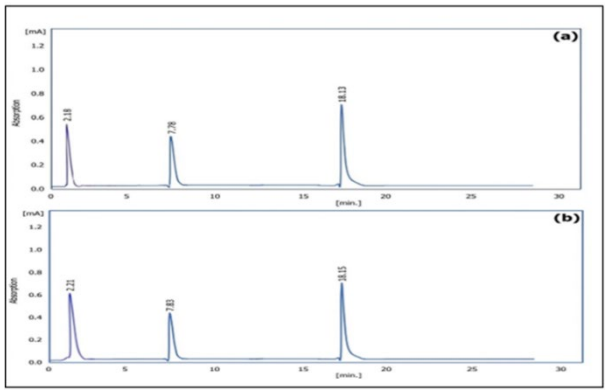

Figure 1: HPLC graph of LOM and IS in (**a**) spiked plasma sample and (**b**) spiked brain sample.

The following method was precise, specific, and sensitive for determining LOM in the spiked plasma and spiked brain samples. Each validation parameter was within the permitted limits. The application of HPLC-validated parameters satisfactorily identified LOM, with a retention time of approximately 18 minutes. We determined the comparative bioavailability of LOM in the intranasal (IN) LOMLS versus the intravenous (IV) LOM solution, as seen in Figure 2.

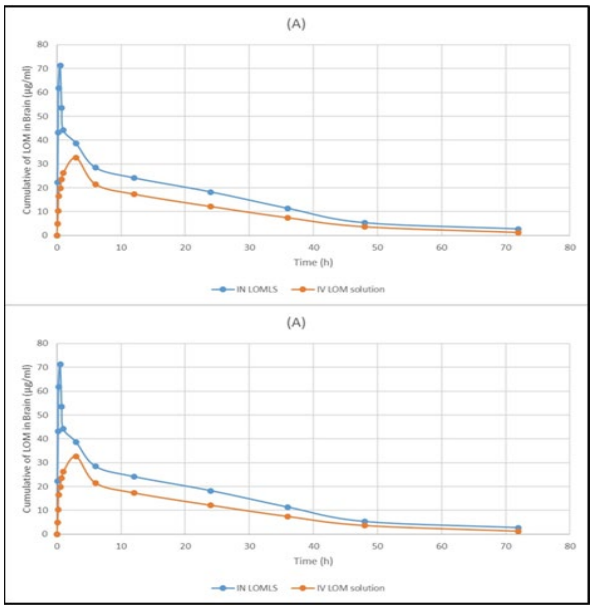

Figure 2: Illustrating of **(A)** the cumulative concentration of the LOM versus time curve for the IN LOMLS and IV LOM solution infusion of the brain; **(B)** the cumulative concentration of the LOM versus time curve for the IN LOMLS and IV LOM solution infusion of plasma.

The average plasma and brain drug concentration time curves after intranasal and intravenous administration of LOM formulations are shown in Figure 2. Intranasal delivery of the LOMLS formulation showed a higher brain concentration (2.3-fold) than intravenous administration of LOM solution, according to these studies. The pharmacokinetic results in Table 2 show that the IN LOMLS formulation achieved the higher concentration of LOM in the brain and the lower concentration in plasma. The intravenously administered LOM demonstrated the highest concentration of LOM in plasma and the lowest concentration in the brain. Additionally, the difference is even more significant within the first half hour, indicating that the IN LOMLS formulation delivers LOM to the brain 4.3 times more effectively than the IV LOM solution. Intranasal delivery of the LOMLS formulation resulted in a significantly faster and higher brain concentration of LOM ($p< 0.05$) than those in rats administered intravenous LOM (Table 2). After one week, the nasal membrane from the intranasal LOMLS formulation and the intranasal LOM solution administered to rats were isolated to assess for histopathological toxicity. As seen in Figure 3, after intranasal LOMLS, image (a) section showed normal preserved nasal mucosal lining epithelium with goblet cells and also the submucosal glands (H & E, X400), and image (b) section showed mucus secretion with the presence of secretory acini (H & E, X400). While for the intranasal LOM solution, the image (c) section showed the hypertrophy of nasal cartilage with some minor inflammatory cells (H & E, X100), and the image (d) section showed damage of the nasal lining epithelium with loss of cilia (H & E, X400).

Table 2: Pharmacokinetics parameters of IN LOMLS and IV LOM solution infusion

Formula	Brain			Plasma			Brain targeting parameters	
	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-72} (h.µg/ml)	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-72} (h.µg/ml)	%DTE	%DTP
IV LOM solution	32.65	3	435.521	67.45	0.1	924.115		
IN LOMLS	71.21	0.5	1043.302	21.18	3	484.831	486.22	64.8

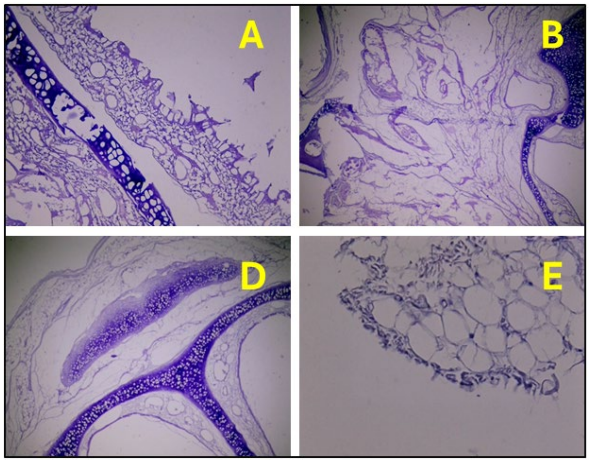

Figure 3: Histopathological examinations of nasal mucosal sections for intranasal LOMLS (A and B) and intranasal LOM solutions (C and D).

DISCUSSION

In previous studies, standard lipid matrices were employed to formulate conventional bilayers [37], and thermoreversible systems were investigated to improve the retention in the nasal cavity [38]. The carrier design was improved in this study by using a solusome produced by the thin-film hydration method. The solution combines the structural integrity of Soya Lecithin Phosphatidylcholine (SLPC) with cholesterol as a membrane stabilizer and the unique solubilizing properties of the amphiphilic polymer Soluplus®. The structural synergy gives a new strategy to deliver the strong hydrophobicity of lomustine, leading to better drug stabilization and avoidance of precipitation upon dilution. Also recognized as a unique mechanistic advantage over traditional liposomal and nanoemulsion formulations. Pharmacokinetic experiments revealed the role of the trigeminal and olfactory routes in the direct nose-to-brain delivery of LOM when the intranasal LOMLS formulation was used. Further, the direct transport efficiency (DTE%) was used as a measure of the amount of direct drug delivery between the nose and the brain [33]. In this experiment, the intranasal LOMLS formulation exhibited the highest DTE% values, as indicated in

Table 2. The value of DTE% (486.22), which is between 0 and infinity, and a DTP% of 64.8 (greater than 0) mean that the drug is being successfully directed to the brain through the olfactory and trigeminal routes, while a DTE% value of more than 100 shows that the medicine is more effective when administered intranasally than intravenously. Also, a high value of DTE% and DTP% obtained with the intranasal LOMLS formulation indicates that a higher percentage of drug was targeted after intranasal administration, while a minimum fraction was distributed by blood circulation [34,35]. This study looks at the pharmacodynamics of lomustine alone, rather than previous liposomal studies that looked at its use with synergistic agents [37]. This allows for a pure, unconfounded pharmacokinetic profile of the drug in a solosomal system and fulfills the necessary requirement for monotherapy baseline data for nose-to-brain targeting. This study goes beyond the simple in vitro release profile analysis [39]. A robust and highly specific HPLC method was developed and validated to detect lomustine in rat plasma and brain tissue. The study has provided strong quantitative evidence that the SLPC-based solusome bypasses systemic circulation directly via olfactory and trigeminal pathways, as shown by the precise calculations of DTP% of 64.8 and DTE% of 486.22. The Fisusi *et al*. (2016) trial and our study have the same goal of improving the delivery and safety profile of lomustine for the treatment of brain cancer, but the nanocarrier designs and administration methods to achieve these goals are different. For instance, Fisusi *et al*. injected mice with Molecular Envelope Technology (MET) nanoparticles composed of a modified chitosan polymer to achieve high-dose systemic distribution without dose-limiting bone marrow suppression and liver damage [17]. On the other hand, the actual solusome research employs intranasally administered nanovesicles composed of soya lecithin phosphatidylcholine and the amphiphilic polymer Soluplus® to bypass the blood-brain barrier directly in Wistar rats. The MET nanoparticles are designed to specifically target the brain endothelium, modulating systemic biodistribution and drug release, without crossing the blood–brain barrier. While the intranasal solusome formulation was formulated to deliver directly to the brain by nose-to-brain delivery through olfactory and trigeminal neural pathways, resulting in an astonishingly high direct brain targeting efficiency (DTE% of 486.22) with very limited systemic exposure. According to the pharmacokinetic analyses conducted on the rats in this investigation, the brain Cmax of IN LOMLS was approximately 2.3 times more than that of the IV LOM solution, and the brain Tmax of IN LOMLS was approximately 4.3 times more than that of the IV LOM solution. Five rats died in the IV group, whereas there were no deaths in the IN of LOMLS. The newly developed intranasal lomustine-loaded solusomes (LOMLS) are a better nanocarrier for improving local efficacy and safety, although previously reported intravenous nanocarriers of MET nanoparticles successfully reduced the dose-limiting bone marrow toxicities of lomustine through changing the systemic biodistribution. Solusomes are rapidly and accurately targeted to the brain via the olfactory and trigeminal neuronal pathways, thus bypassing the blood-brain barrier. An amphiphilic polymer, Soluplus®, is used to stabilize solusomes to improve the stability of the medication. The DTE% is 486.22, with the brain Cmax being 2.3-fold higher with a peak at 0.5 h. Such a direct nose-to-brain delivery route not only exceeds the pharmacokinetic efficacy and accumulation of systemic nanocarriers but also diminishes the major systemic mortality risks, as no deaths were observed in the intranasal group compared to five in the intravenous control group, while safely preserving local mucosal integrity. A histopathological study showed evidence of the safety of the excipients and drug in the solusomes formulation. Therefore, it is possible to consider the ingredients of the formulation as biocompatible and that they will not lead to a major histological change in the nasal mucosa when used over an extended period of time. [36].

Conclusion

The intranasal LOMLS treatment significantly increased the Cmax, Tmax, and AUC values of the drug in the brains of the animals compared to the intravenous LOM solution, according to the investigation of the *in vivo* distribution of LOM in rats. Applying LOMLS intranasally improved the permeability of the nasal membrane to LOM, enabling an efficient procedure for LOM brain delivery by the trigeminal and olfactory pathways. An effective and safe formulation for the brain targeting of LOM as an alkylating agent for the treatment of brain tumors can be considered the optimal LOMLS formulation.

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Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

The data that supports the findings of this study are available from the corresponding author upon a reasonable request.

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