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Research Article

Theme: Lipid-Based Drug Delivery Strategies for Oral Drug Delivery
Guest Editor: Sanyog Jain

Oral Delivery of Methylthioadenosine to the Brain Employing Solid Lipid Nanoparticles: Pharmacokinetic, Behavioral, and Histopathological Evidences

Pramod Kumar,[1,2] Gajanand Sharma,[3] Varun Gupta,[4] Ramanpreet Kaur,[5] Kanika Thakur,[3] Ruchi Malik,[1] Anil Kumar,[4] Naveen Kaushal,[5] Om Prakash Katare,[3] and Kaisar Raza[1,6]

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Abstract. The present study aimed to orally deliver methylthioadenosine (MTA) to the brain employing solid lipid nanoparticles (SLNs) for the management of neurological conditions like multiple sclerosis. The stearic acid–based SLNs were below 100 nm with almost neutral zeta potential and offered higher drug entrapment and drug loading. Cuprizone-induced demyelination model in mice was employed to mimic the multiple sclerosis–like conditions. It was observed that the MTA-loaded SLNs were able to maintain the normal metabolism, locomotor activity, motor coordination, balancing, and grip strength of the rodents in substantially superior ways *vis-à-vis* plain MTA. Histopathological studies of the corpus callosum and its subsequent staining with myelin staining dye luxol fast blue proved the potential of MTA-loaded SLNs in the remyelination of neurons. The pharmacokinetic studies provided the evidences for improved bioavailability and enhanced bioresidence supporting the pharmacodynamic findings. The studies proved that SLN-encapsulated MTA can be substantially delivered to the brain and can effectively remyelinate the neurons. It can reverse the multiple sclerosis–like symptoms in a safer and effective manner, that too by oral route.

KEY WORDS: multiple sclerosis; brain delivery; lipid-based systems; remyelination; safety.

INTRODUCTION

Multiple sclerosis is a disease of the central nervous system in which the myelin sheath is destroyed, resulting in symptoms like difficulty in locomotion, numbness, fatigue, muscle spasms, stiffness, pain, and compromised vision. Although the disease can affect population of any age group, it is more commonly diagnosed in young adults (20–30 years). However, the disease is not directly linked with patient mortality, but more than 60% of the affected population do not remain ambulatory after 20 years on the disease onset (1). This autoimmune disease is reported to have a global burden of around 2.5 million (2). There is no cure for multiple sclerosis, but a few drugs are used for the management of the disease. The present treatment includes methyl prednisolone, INF-beta, glatiramir acetate, dimethyl fumarate, and other symptomatic options (2,3). Most of the drugs are associated with enumerable concerns (2). Recently, methylthioadenosine (MTA), a polyamine pathway natural metabolite, has been established to be beneficial in preclinical models of MS (4,5). Being a nucleoside, the studies of MTA in rodents have been performed by intraperitoneal route.

Looking into the nature of the MTA, its promises in MS and the non-availability of studies on the development of nanocarriers loaded with MTA for the oral delivery, it was envisioned to encapsulate MTA in the core of solid lipid nanoparticles (SLNs). SLNs are known to enhance the oral bioavailability of a variety of drugs and also have been established to deliver a wide range of drugs to the brain, *i.e.*, the site of action for neuroprotective agents (2,6–10). Therefore, the present study aimed to orally deliver a nucleoside bioactive by means of lipid-based nanoparticles

Guest Editor: Sanyog Jain

[1] Department of Pharmacy, School of Chemical Sciences and Pharmacy, Central University of Rajasthan, Bandar Sindri, Distt., Ajmer, Rajasthan 305817, India.
[2] Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER), Guwahati, Assam 781125, India.
[3] Division of Pharmaceutics, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, 160014, India.
[4] Division of Pharmacology, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, 160014, India.
[5] Department of Biophysics, Panjab University, Chandigarh, 160014, India.
[6] To whom correspondence should be addressed. (e–mail: drkaisar@curaj.ac.in; razakaisar_pharma@yahoo.co.in)

and evaluate the preclinical outcomes in rodents *vis-à-vis* plain MTA for the management of MS-like conditions.

MATERIALS AND METHODS

Materials

Methylthioadenosine (MTA) and luxol fast blue were purchased from M/s Sigma-Aldrich, Bangalore, India. Phospholipid 90 G (PL) was a generous gift sample from the IPCA Laboratories, Mumbai, India. Stearic acid (SA) and buffer salts were procured from M/s Central Drug House, New Delhi, India. Dialysis bag was purchased from M/s Himedia Laboratories, Nashik, India. HPLC column was supplied by M/s Merck Specialities, Mumbai, India, whereas the HPLC solvents were obtained from M/s Spectrochem, Mumbai, India. Filter papers were provided by M/s GE Healthcare, Buckinghamshire, UK. M/s Alfa AsserLimited, New Delhi, was the source of cuprizone. Throughout the studies, double distilled water was employed and no further purification of the procured chemicals was performed.

Methods

Preparation of MTA-Loaded SLNs

The SLNs were prepared by well-reported microencapsulation technique (11). In actual, stearic acid (1 g) was molten at 70°C, and MTA (250 mg) was completely dissolved in it. Tween 80 (4.55 g) was added isothermally to the molten oily phase. In a separate container, PL (0.442 g) was dispersed in normal saline (14 mL). This system was maintained at 70°C, and both the phases were mixed isothermally and stirred to fetch with a clear and hot microemulsion. This hot microemulsion was added to a pre-stirred ice-cold beaker containing normal saline (q.s. to 100 mL) in a streamlined manner. This system was mechanically stirred for further 15 min. The frothy system was stored overnight and the resulting milky dispersion was refrigerated till further use.

Characterization of SLNs

The average hydrodynamic diameter, poly dispersity index (PDI), and zeta potential of the developed systems were determined using Nano ZS (M/s Malvern Instruments Limited, Worcestershire, UK) (7). The entrapment efficiency and drug loading of the SLNs were determined using the dialysis method, well explained elsewhere (2,6,9).

Pharmacokinetic and Pharmacodynamic Evaluation

Animals. Healthy Wistar rats and Laca mice were used for the pharmacokinetic and pharmacodynamic studies, respectively. The animals were procured from the Central Animal House facility of Panjab University, Chandigarh. The mice were housed as per the standard protocol in ventilated cages with standard dark and light conditions with *ad libitum* supply of food and water. All the animal groups were accustomed to the laboratory conditions prior to incorporation in the experimentation. None of the approved animal experiments along with the training was performed before 10:00:00 and after 17:00:00. All the animal protocols were duly approved by the Animal Ethics Committee, Panjab University, Chandigarh, and the studies were performed in strict accordance to the guidelines laid by the University in accordance with the apt national regulations.

Animal Model, Animal Grouping, Dose, and Dosing Schedule. For pharmacokinetic studies, only two groups of rats with four rats in each group were employed. Each group received a single oral dose of MTA (120 mg/kg) and equivalent amount of MTA-SLNs, respectively. A blood sample of 0.2 mL was withdrawn from the retro-orbital plexus of each pre-anesthetized rat at pre-determined time intervals. The plasma was separated and the drug was quantified by HPLC (4). The plasma concentration-time data was processed as per one compartment open body model, and various pharmacokinetic parameters were determined (7).

Cuprizone-induced demyelination animal model was employed for the pharmacodynamic studies. It is a practical and frequently employed animal model that mimics the etiology of MS. Oxalic acid bis(cyclohexylidene hydrazide), *i.e.*, cuprizone, is a copper-chelating agent that initiates demyelination in various regions of the brain including the corpus callosum, the most widely studied white matter among animal models (12). The animals were divided in a total of five groups of six animals each. The animals of each group were systematically stained with 1% picric acid for recognition, dosing, and training. The grouping of animals was as group 1 (once-a-day oral dose of normal saline), group 2 (once-a-day 6 mg/kg oral dose of cuprizone suspension), group 3 (once-a-day 6 mg/kg oral cuprizone suspension and once-a-day 120 mg/kg MTA oral suspension), group 4 (once-a-day 6 mg/kg cuprizone oral suspension and once-a-day MTA-SLNs equivalent to 120 mg/kg of MTA), and group 5 (once-a-day 6 mg/kg cuprizone oral suspension and once-a-day equivalent amount of blank SLNs) (7). The cuprizone/MTA suspension was prepared by triturating cuprizone/MTA with carboxymethylcellulose (1% by mass) and adding polysorbate 80 (0.2% by mass), and making the volume to 100 mL with normal saline. All the animals were administered the assigned dose/regimen continuously for 30 days and the parameters discussed in the subsequent sections were observed.

Body Mass. Every week, the mass of the individual animal of each group was observed. The average of the mass for each group was determined on a weekly basis. The reference for reporting the change in the body mass was the average mass of all the animals of the group on day 1 of the experimentation.

Behavioral Studies. As the demyelination of neurons is associated with marked changes in the locomotor activities and motor coordination, the animals of each group were subjected to a set of behavioral assessment on a weekly basis. The tests included locomotor activity, rotarod test, and functional observation battery test.

Locomotor Activity:

The locomotor activity of the mice receiving the assigned treatments was assessed employing an Actophotometer (IMCORP, Ambala, India). The infrared beams above the floor of the testing area were used to observe the locomotor

performance. Prior to the experimentation, the rodents were acclimatized for 3 min. The actophotometer observations were recorded for 5 min and the results were reported as the counts in 5 min (13).

Rotarod Test:

Rotarod test is the widely employed animal model to quantify and assess the motor coordination and grip strength in the animals (14). In the present study also, these tests were employed on a rotarod apparatus (IMCORP, Ambala, India). As a standard procedure, the animals were acquainted to the test prior to the dosing, and the cut-off was set as 120 s. The triplicate trials were performed over the intervals of 5 min. The speed of the rotating rod was fixed at 25 rpm and the time of the fall of the animal was recorded. The average of each group was calculated and expressed as the number of falls per 120 s (7).

Functional Observation Battery Test:

The neurotoxicity is effectively measured in animals by means of a functional battery test. For the reported experiment, a box of dimensions 40 cm × 60 cm was employed. The animals were placed in this box and were observed for the abnormal responses like ataxia, tremors, seizures, weakness, and convulsions, as reported earlier (7,13).

Histopathological Evaluation

After 30 days, the animals of each group were sacrificed and the brains of the rodents were harvested and stored in formalin. Microtomy was performed and the brain slices were stained with luxol fast blue ethanolic solution (1%). The photographs of the slides were captured and the evidence-based inferences were drawn (15,16).

RESULTS AND DISCUSSION

Characterization of SLNs

The average hydrodynamic diameter of the developed SLNs was observed to be 89.79 ± 4.67 nm with a PDI of 0.186. The size of the developed nanoparticles was well within the range to offer a promise of enhanced blood brain barrier permeability and bypassing the reticuloendothelial system (RES). The literature reports unequivocally support the better brain permeability and long retention of such SLNs (17). The PDI value < 0.3 assured a more reliable size distribution of the polydispersity phase and inherits the assurance of repeatability of the hydrodynamic diameter in the set conditions (18).

The zeta potential value was found to be − 8.43 ± 0.63 mV. In literature, there are many instances, where the systems derived from non-ionic surfactants like polysorbates (as in the present case) result in nanocarriers with near-neutral charge. However, such systems do not impart stability due to the electrostatic mechanism; rather, complex interactions are responsible for stability of such systems (19). On the other hand, the nanoparticles with 0 to ± 10 mV of zeta potential values are generally pharmacokinetically neutral.

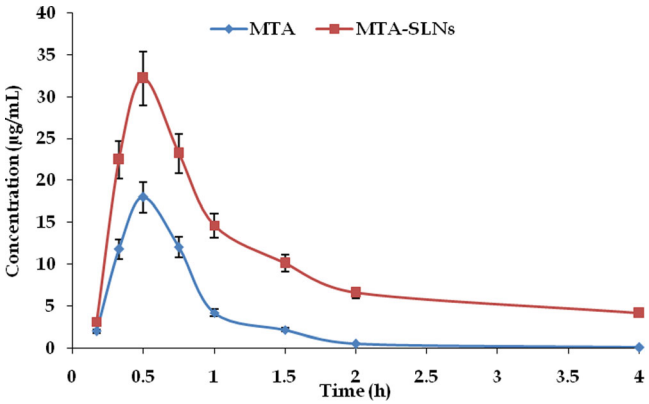

Fig. 1. The variation of plasma concentration of MTA in two animal groups as a function of time, after single oral dose administration of MTA and MTA-SLNs

Nanoparticles with zeta potential ≤ ± 10 mV are reported to bypass the RES and offer higher circulation time (20).

The entrapment efficiency was observed to be 93.74 ± 5.09% with a drug loading of 16.30 ± 1.01%. Even at a higher drug to lipid ratio of ~ 1:4, the substantial drug loading and drug entrapment ensured the complete loading of the desired dose in the developed nanoparticles.

Pharmacokinetic Studies

The pharmacokinetic data obtained from single oral dose administration of equivalent amounts of MTA and MTA-SLNs have been represented as Fig. 1. As conspicuous from the graph itself, the SLNs were able to offer enhanced concentrations of MTA at every single time point *vis-à-vis* plain drug. The C_{max}, *i.e.*, maximum plasma concentration, achieved was 18 µg/mL and 32.17 µg/mL from plain MTA and MTA-SLNs, respectively. The corresponding time (T_{max}) for both the treatments was approx. 0.5 h. The elimination half-life of MTA in the group receiving plain MTA was observed to be around 28 min, which increased up to 1.25 h when encapsulated in SLNs. There was also an enhancement of around four folds in the bioavailability of MTA by means of SLNs. The pharmacokinetic studies proved that the MTA was better absorbed from SLNs *vis-à-vis* plain MTA and the biological residence was substantially enhanced so as to reach the target site.

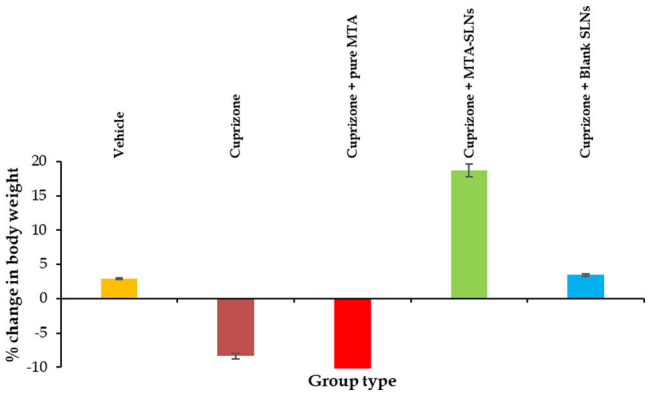

Fig. 2. Percent variation in the average mass of the animal groups on day 30 w.r.t day 1

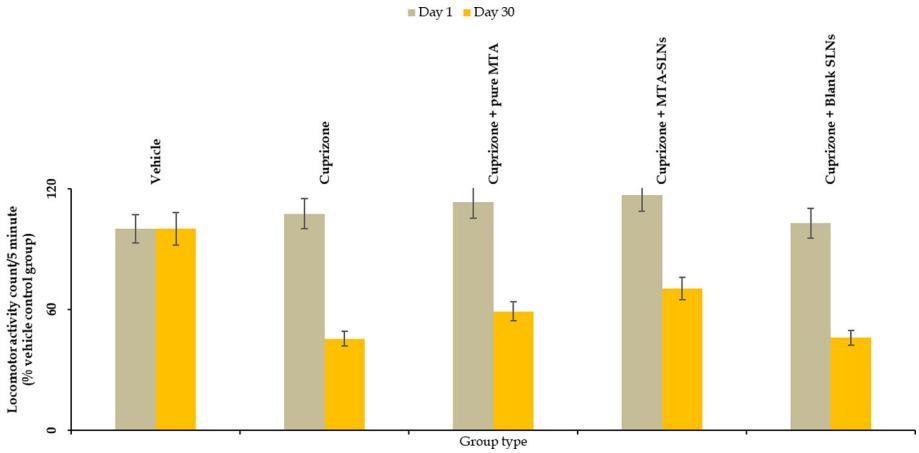

Fig. 3. Percent variation in the locomotor activity on day 30 in the animal groups receiving various treatments w.r.t. day 1

Body Mass

The variation in the average body mass of the animals of the five studied groups, receiving various treatments, is depicted in Fig. 2. As vivid from the figure, administration of plain cuprizone and cuprizone along with MTA resulted in the decrease of the average body mass of the animals. There was no significant difference in the observations of these two groups ($p < 0.05$). In plain cuprizone group, a mortality of 16.67% was observed. No deaths were observed in the other groups. As our previous studies have established that cuprizone is poorly tolerated by the animals, the decrease in the weight and the mortality incidents are easily understandable (7). On the other hand, the group receiving the blank lipid–based nanoparticles along with cuprizone showed a normal course of body mass analogous to the saline-controlled group ($p < 0.05$). More than 15% weight gain was observed in the group receiving the cuprizone and MTA-loaded SLNs. In nutshell, the study proved that the designed nanocarrier alone or with MTA was observed to be safer; however, the SLN version of MTA resulted in slight weight gain in mice.

Behavioral Studies

Locomotor Activity

The results obtained from the actophotometer for the locomotor activity have been presented in Fig. 3. The results clearly vouch that the group receiving cuprizone only exhibited the maximum deviation from the normal locomotor function on day 30 to that of on day 1. The results clearly infer the substantial demyelination resulting in this deviation ($p < 0.05$). The locomotor activity was also substantially decreased in the group receiving the cuprizone and plain SLNs; however, within these two groups, there was no significant difference ($p < 0.05$). In comparison to these groups, the groups receiving plain MTA and MTA-SLNs, there was substantial improvement in the locomotor activity ($p < 0.05$). MTA-loaded SLNs improved the locomotor activity to the maximum extent of the order of 71%, whereas the plain MTA only improved up to 49% in comparison to the cuprizone-only group. The findings provide the evidence of superior activity of MTA-SLNs composed of economic materials in circumventing the MS-like conditions in a more effective manner.

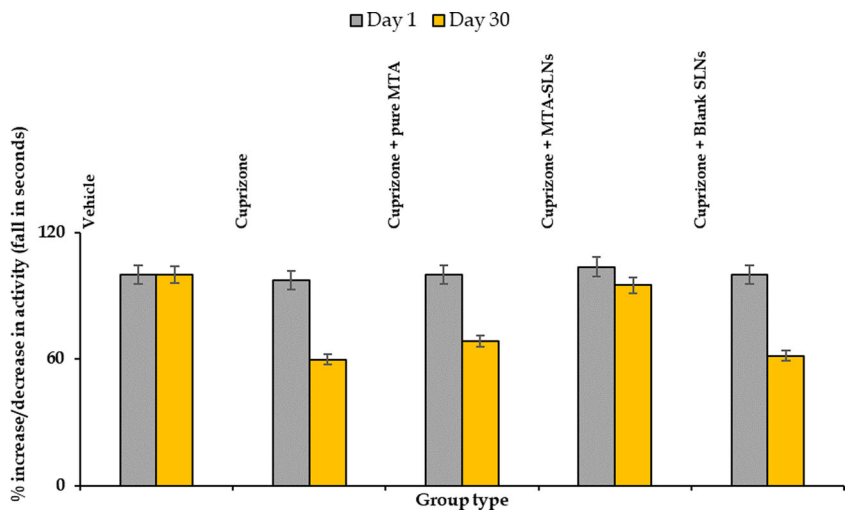

Fig. 4. Percent variation in activity (fall in seconds) of the animals of various groups on rotarod

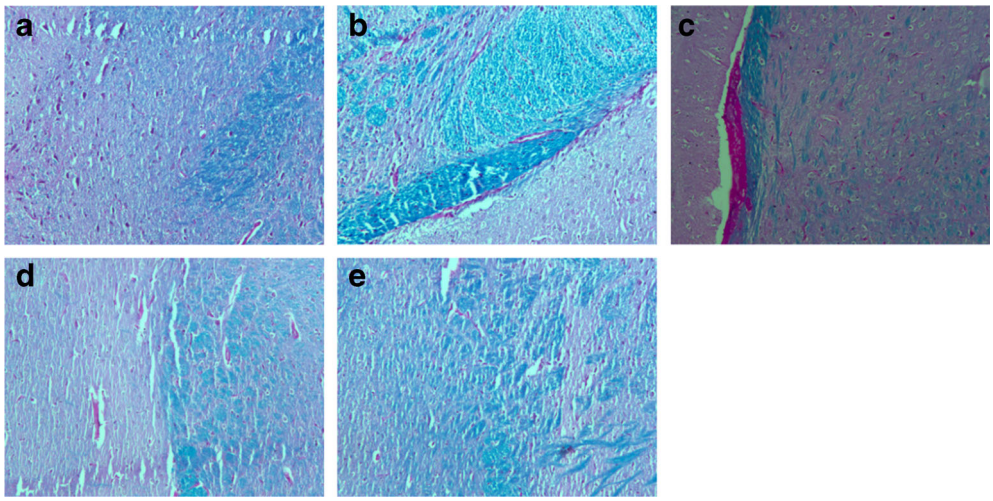

Fig. 5. Histopathological slides of the brain of the animals receiving: saline (**a**); cuprizone (**b**); cuprizone + pure MTA (**c**); cuprizone + MTA-SLNs (**d**); and cuprizone + blank SLNs (**e**)

Rotarod Test

The changes in the motor coordination, balance on rod, and grip strength of all the animals of the five groups on day 30 w.r.t. day 1 have been shown as Fig. 4. The substantial reduction in the coordination, grip strength, and balancing of the animals of the group receiving pure cuprizone and cuprizone plus plain SLNs indicated the suitability of the model and induction of the disease in the animals. The results clearly indicate the superiority of MTA-SLNs over all the treatments indicating a clear-cut benefit of the system over the plain MTA ($p < 0.05$). The MTA-SLNs were able to maintain the motor coordination up to 95% of the vehicle-controlled group whereas the plain MTA was able to maintain the coordination only up to 68%.

Functional Observation Battery Test

No noticeable abnormality in the behaviors like tremors, seizures, slow motion, and weakness was observed in the control animals as well as in the animals receiving MTA and MTA-SLNs. However, a few instances of seizures and laziness were observed in the blank SLN group and in the cuprizone-treated group.

Histopathological Evaluation

As the disease as well as the animal model employed deals with the demyelination of the neurons, henceforth, the histopathological studies of the corpus callosum region of the brain was performed to quantify the extent of demyelination/remyelination in the animals receiving various treatments (21). The microphotographs of the brain sections of the animals of various groups have been presented as Fig. 5, whereas Fig. 6 showcases the percent change in the myelination on day 30 w.r.t. day 1.

Image J software was employed to scan the percent intensity of blue-stained area, *i.e.*, luxol fast blue stained region, that only stains the myelin sheath. On a scale of 100% myelination of the saline/vehicle control group, the average myelination in the cuprizone-only group was around 57%,

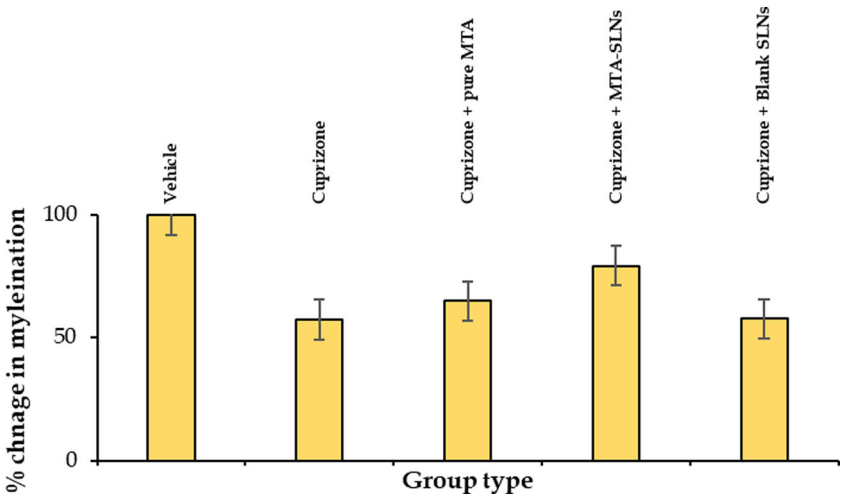

Fig. 6. Percent change in myelination in the brains of the groups receiving various treatments

indication of almost half demyelination of the neurons. It clearly explains the results obtained from the animals of this group in various pharmacodynamic studies. The group receiving plain SLNs and cuprizone also exhibited the same extent of demyelination on day 30. However, the animal group receiving plain MTA exhibited myelination of the order of 65%, substantially higher than the two discussed groups in this section, indicating the efficacy of MTA in reversing the cuprizone-induced demyelination ($p < 0.05$). The myelination observed in the brains of the group receiving cuprizone and MTA-SLNs was the highest and of the order of 80% ($p < 0.05$), indicating almost 80% reversal of the cuprizone-induced MS like neurological disorder. These histopathological findings clearly explain the delivery of MTA by oral route to the brain, which is substantially facilitated by lipid-based nanocarriers of appropriate size, charge, composition, and drug loading.

CONCLUSIONS

The present study provides an evidence of oral delivery of a nucleoside by means of lipid-based nanoparticles to the brain. The multiple sclerosis–like demyelination induced by a copper-chelating agent successfully demonstrated the symptoms of the disease in the rodents, which were monitored to some level by plain MTA and to a major extent by the solid lipid nanoparticle version of this nucleoside. The substantial remyelination by MTA-SLNs *vis-à-vis* plain MTA unequivocally proves the importance of lipid-based carriers in the brain delivery of bioactives administered by oral route. The pharmacokinetics also corroborated the pharmacodynamic findings as not only the C_{max} and bioavailability were enhanced but the biological half-life was also substantially increased, enhancing the better chances to interact and concentrate at the site of action. These results provide a preliminary evidence for the nucleoside delivery, that too, to the brain employing solid lipid nanoparticles developed by simple technique.

FUNDING INFORMATION

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COMPLIANCE WITH ETHICAL STANDARDS

All the animal protocols were duly approved by the Animal Ethics Committee, Panjab University, Chandigarh, and the studies were performed in strict accordance to the guidelines laid by the University in accordance with the apt national regulations.

Conflict of Interest The authors declare that they have no conflict of interest.

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