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The Impact of Dexmedetomidine-loaded Nano-microsphere Combined with Percutaneous Acupoint Electrical Stimulation on the Postoperative Cognitive Function of Elderly Patients with Hip Fracture

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ABSTRACT

To explore the impact of nano-microsphere loaded with dexmedetomidine (DEX) combined with percutaneous acupoint electrical stimulation on the postoperative cognitive function of elderly patients with hip fracture. The free base was prepared by the alkali precipitation method in this research, and then the drug was loaded into PLGA microspheres to construct the drug sustained-release system. The PLAG microspheres loaded with DEX (MS/DEX) were prepared by the O/W emulsion volatilization method and then Gel-(DEX-MS/BUP) suspension was obtained. A scanning electron microscope (SEM) was used to analyze the characterization of the prepared drug-loaded nano-microsphere, rheological analysis was performed on the copolymer solution, and in vivo release and degradation, experiments were carried out. Wistar rats were randomly divided into four groups (n=ten). After the sciatic nerve block model was established, the block time was observed after the injection of each sustained-release agent. The results showed that the gel-forming temperature of Gel and Gel-(DEX-MS/BUP) were 27.3°C and 26.3°C, respectively. Both MS/BUP and Gel-(DEX-MS/BUP) drugs could completely enter the blocking state. There was no loss of motor function in the rats after GEL-DEX. The clinical trials showed that Gel-(DEX-MS/BUP) system had good in situ and sustained release effects, and the analgesic effect of local anesthesia was significantly improved.

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Introduction

Postoperative cognitive dysfunction (POCD) refers to some moderate central nervous system complications such as personality, social cognitive ability, and social ability after surgery. Studies showed that the incidence of POCD in the elderly was about 47%, and the ability of patients to care for themselves was decreased or even lost, which increases the burden on the family and society (1). Hip fracture in the elderly refers to hip intertrochanteric fracture and femoral neck fracture. The incidence of hip fracture in the elderly is about 1/1000, and nearly 90% of the patients are caused by falls. The incidence also increases with the increase of age, and it doubles every ten years after the age of 50 (2). In recent years, the incidence of hip fracture shows a growing trend, the study of hip fracture and treatment have made great progress internationally, and there has been a major shift in fracture risk assessment and rehabilitation interventions. How to accurately predict postoperative development and help patients recover as much as possible to their pre-fracture state is an urgent problem to be solved (3,4). POCD is a common complication in elderly patients after surgery, and inhaled anesthetics are the main inducing factor. It is also related to factors such as age, surgical trauma, metabolic function, and changes in the central nervous system. If the appropriate anesthetic is selected during the operation, it is not only the patient's respiratory circulation can be maintained smoothly, but also the incidence of POCD can be reduced (5,6).

Dexmedetomidine (DEX) has a highly selective α2 receptor agonist, which has a good effect on the treatment of anxiety, sedation, and analgesia (7). Some researchers found that DEX had a protective effect on nerves and could anti-inflammatory pathways by activating cholinergic, and nuclear factor

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Kappa B inflammatory pathway and the expression of inflammatory factors in mononuclear macrophages were inhibited to reduce the body's inflammatory response. The inflammatory response of the body was reduced so that the nerve damage was reduced, and finally, the incidence of POCD was effectively reduced (8,9). Percutaneous acupoint electrical stimulation is a treatment method based on stimulating acupoints, placing electrodes on acupoints, and then inputting a specific low-frequency pulse current into the body and acting through acupoint current. In related studies, the constant current device can be used repeatedly, it was not easy to tolerate the analgesic effect, and it had the advantages of non-invasiveness and good compliance (10).

Studies showed that DEX mixed with bupivacaine for supraclavicular block and ropivacaine for intermuscular groove block can effectively extend the duration of the block by eight hours and four hours, respectively, which had a good improvement effect on postoperative analgesia (11). Studies also showed that peripheral nerve block of about 200min can be prolonged by 1mcg/kg DEX, which was very suitable for patients with bradycardia or hypotension. The efficacy was also stable in hemodynamics (12,13). Poly (lactic acid-glycolic acid copolymer) microspheres (PLGA MS) are biodegradable and biocompatible, and their stable physical and chemical properties in production and storage have been widely used. Lidocaine, bupivacaine, ropivacaine, and other drugs were successfully carried by the microspheres, which can last as long as 6-24 hours in terms of nerve tissue. For Thermo-sensitive hydrogels in the local drug delivery system, drugs can be uniformly dispersed in a polymer solution and injected into the body through a syringe (14). A simple mixture of local anesthetics and polymers can also achieve long-term analgesia. The nanosystem can improve the permeability of cells and achieves reliable and controllable release of local anesthetics. The drug is encapsulated in a biocompatible mononuclear biodegradable nano-result system, which accurately controls the drug release rate and minimizes tissue reaction (15). The drug release rate of the nano-scale drug delivery system is equivalent to the production rate of non-toxic degradation products. Nano liposomes refer to liposomes with particle sizes less than 100nm, which have the characteristics of relative targeting, long-term release, and reducing adverse reactions to skin tissue. Nano liposomes are mainly used for transdermal delivery of local anesthesia (16). POCD has a serious impact on the life and work of patients. At present, the occurrence of POCD has become the focus of clinical research.

Therefore, The PLAG microspheres loaded with DEX (MS/DEX) were prepared by alkalized precipitation method. The prepared DEX-loaded nano-microsphere was subjected to fluid testing, in vitro release, and detection analysis and the rat sciatic nerve model was constructed. Then, drug loading of nano-spheres combined with electrical stimulation was applied to the treatment of fractured patients, and postoperative MMSE scores of patients were analyzed. To further explore the clinical applicability of the prepared nano microspheres on the cognitive function after fracture surgery.

Materials and methods

Preparation of dexmedetomidine base

The alkalization precipitation method was used to prepare DEX in this study, and the PLGA microspheres were prepared by the volumetric volatilization method of an oil-in-water emulsion, which was suitable for liposoluble drugs. The first step was to convert DEX hydrochloride to DEX. Firstly, 1.0g of sodium hydroxide powder was dissolved in 50 mL of ultra-pure water with 2.0wt% of NaOH solution. Then, the nearly saturated solution of 5.0 GDEX-HCl was weighed, and the prepared sodium hydroxide solution was slowly added to the DEX-HCl solution. Then, the mixture was left standing for an appropriate time so that the DEX was completely precipitated. After the mixture was pumped and filtered, the drug was repeatedly washed with ultrapure water until the filtrate was neutral. Finally, the freeze-dried DEX powder was obtained for use (17).

Preparation and characterization of PLAG microspheres loaded with dexmedetomidine

PLAG microspheres (MS/DEX) loaded with DEX were prepared by the O/W emulsion evaporation method. First, 0.1g of PLGA and DEX were weighed, respectively, and they were dissolved in 2.0 mL of dichloromethane solution and treated with ultrasound

in a water bath for one minute. The mixture was finally clarified as an organic phase. In the process of the high-speed shearing speed of 2000r/min and emulsification time of two minutes, 2.5mL oil phase was added into 20mL PVA aqueous solution (ice water bath) with a mass fraction of 1.5wt% by a syringe. Then, the obtained colostrum was quickly poured into a 60mL beaker with 0.5wt% PVA aqueous solution, at room temperature, and magnetically stirred for 6-8 hours to volatilize the organic solvent. After volatilization, the microspheres were completely solidified, the microspheres larger than 100 microns were screened out, and the products were collected after centrifugation at 4500r/min for five minutes. The product was resuspended in the water again, washed three times for the same time and rotation speed, and then freeze-dried in a vacuum.

Preparation of Gel-MS composite drug delivery system

The Gel-MS composite drug delivery system referred to the preparation of drug-loaded PLGA microspheres (MS/BUP) and PLGA-PEG-PLGA gel through low-temperature stirring. In PLGA-PEG-PLGA, Mn=4200g/mol, LA/GA=75mol:25mol. An appropriate amount of PEG was added to the reaction flask for vacuum extraction and filtered water was removed. Then, 3:1 La/Ga was added, and the oil was heated at 130°C for complete melting. After that, toluene and catalyst were added to the reaction flask for an oil bath for 24 hours. The precipitation of the product in ether was collected, and the product was dried in a vacuum. Then, 20wt% PLGA-PEG-PLGA copolymer and 10wt% MS/BUP were added to 0.01M PBS solution, and the mixture was stirred at 4°C for 24 hours to obtain Gel-MS/BUP suspension (18).

Preparation of Gel-(DEX-MS/BUP)

In the preparation of GEL-(DEX-MS/BUP), the dosage ratio of DEX to BUP was 1:2000 according to clinical dosage. PLGA-PEG-PLGA was dissolved in PBS to form a 20wt% copolymer solution. 1.0 mL copolymer solution was added to 74.1mg MS/BUP and 33.3μg DEX, and Gel-(DEX-MS/BUP) suspension can be obtained after the copolymer solution was stirred at 4°C for 24 hours.

Rheological testing

The rheological analysis of Gel-(DEX-MS/BUP) and Gel of the copolymer solution were carried out using an MCR rheometer. Firstly, 350μL PLGA-PEG-PLGA copolymerization solution was selected and placed on the instrument test bench for five minutes so that the internal structure could be restored. Then, a thin layer of silicone oil was added to the edge of the sample to prevent surface moisture from evaporating. The sample was tested at temperatures ranging from 20°C to 60°C, the instrument amplitude was 1.0%, the angular frequency parameter was 1.0 Hz, and then the change in gel modulus with temperature was recorded (19).

Release and degradation in vitro

The direct release method was used to detect the release and degradation. The MS/BUP powder was added to the centrifuge tube and 3.0 mL phosphate buffer solution with or without elastase was added two times. The tube oscillated at a constant speed of 70rpm in an incubator at 37°C, and the tube was centrifuged at 5000rpm for four minutes at a specified time interval. The upper liquid was stored in the refrigerator for later use. The samples of microspheres were freeze-dried and the degradation was observed. Then, the same volume of buffer solution was added and vibrated in an incubator at a constant speed.

0.5g of the polymer solution was placed in round-bottomed vials, and the round-bottomed vials were placed in an incubator for ten minutes until Gel-BUP or Gel-MS/BUP was formed. Then, 3.0 mL phosphate buffer without or with 2.0 mg/ mL elastase was added to the GEL-BUP or GEL-MS /BUP surface with a trace amount of oxygen. The upper liquid was stored in the refrigerator. The residual water was sucked dry by the filter paper and the remaining gel was weighed. Fresh buffers were added at wavelength 263nm to measure gel and microsphere degradation.

Experimental animals

A total of 40 healthy adult SD rats (male or female) with an average body weight of 250±23g were selected as the research objects.

All rats were fed normally for one week. They drank water freely, they were kept at temperatures between 24°C and 26°C and the humidity was 40 to 60 percent. Sixty rats were divided into groups A, B,

C, and D by the random number table method. The animals were placed in natural light/dark conditions, they drank and ate freely under ventilation and moderate temperature. The animals were held before the experiment so that the animals could adapt to the experimental environment and operation as soon as possible.

Animal treatment

The rats were anesthetized with 2% isoflurane. They lay on their sides but their femurs were perpendicular to the trunk. Then, the greater trochanter and ischial tuberosity were found, and the needle was inserted from the posterior medial side of the greater trochanter and proceeded inside. When it touched the surface of the ischial bone, the needle was moved back 1mm, and then 0.6mL of the drug was injected. The Gel-MS/NR drugs prepared in the study were injected around the sciatic nerve in the form of a suspension. Five rats in each group were injected with the drug for 10 minutes, 10 hours, 20 hours, and 30 hours, and then deeply anesthetized with sodium pentobarbital solution. Then, the heart was perfused with phosphate buffer and paraformaldehyde solution. After the sciatic nerve and left hind limb muscles of rats were exposed, the sciatic nerve and surrounding muscles after injection were selected. The samples were fixed overnight in paraformaldehyde and dehydrated in sucrose solution, then the samples were made into frozen sections and observed under a microscope.

Wistar rats were randomly divided into four groups (n=ten). After the sciatic nerve block model was established, the block time was observed after the injection of each sustained release agent. The injection dose was as follows: a. 0.3mg bupivacaine hydrochloride phosphate buffer. b. 40mg BUP Gel suspension. c. 40mg BUP MS/BUP suspension. d. Gel-MS suspension of 40mg BUP and 20μg DEX.

The right limb was selected as the control, and the left sciatic nerve was injected around the main trunk at a dose of 0.6mL.

Clinical application

With the consent of the hospital ethics committee and the consent of the patients' family members, 20 elderly patients were proposed to undergo artificial femoral head replacement surgery. 20 elderly patients were randomly divided into two groups, and there was no significant difference in all the data of the two groups. The patients in group A were treated with common DEX combined with electrical acupoint stimulation, and the patients in group B with hip fracture were treated with DEX combined with electrical stimulation of acupoints. The depth of anesthesia was detected according to the bispectrality index of EEG in the experiment. The mini-mental state examination (MMSE) was scored 12 hours before surgery, 24 hours, and 72 hours after surgery. Cognitive dysfunction was assessed in terms of orientation, memory, recall, language, attention, and computer skills.

Statistical processing

SPSS19.0 software was used for the statistical processing of experimental data, and all experimental data were expressed as mean ± standard deviation ($\bar{x}\pm s$). The differences between groups were compared using a one-way ANOVA analysis. When $P<0.05$, the differences between the groups analyzed were statistically significant.

Results and discussion

SEM images of DEX and DEX-HCL

After the morphology changes of the alkali precipitated DEX, the surface morphology of DEX-HCl and DEX nano-powders was observed by electron microscopy (SEM). The free alkali-form DEX prepared by the alkali precipitation process can obtain better drug efficacy and encapsulation efficiency in the drug sustained-release system. In figure 1A, the DEX base was a polygon with a side length of about 10μm under SEM. The shape of DEX-HCL shown in figure 1B was also a rectangular crystal shape, with a length and width of about 30μm. Figure 1C is a microscope image (5 μm) with a rectangular crystal shape. This also showed that the dissolution and morphology of DEX changed to a certain extent during the precipitation process. Highly hydrophobic drugs were more likely to be embedded in a hydrophobic matrix by hydrophobic biological gifts, and drug release was better than hydrophilic drugs.

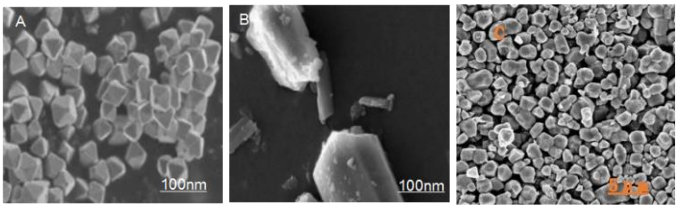

Figure 1. SEM images.

MS/DEX preparation and characterization

After emulsification and microspheres, the particle size of the nanometers filtered by the standard sieve was less than 30 microns. From Figure 2, the appearance of the microspheres measured by the electron microscope was smooth and spherical, and the particle size distribution was uniform. The average particle size was about 15μm.

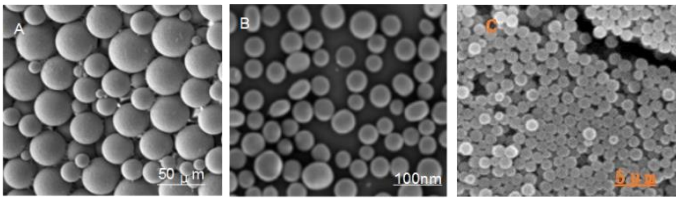

Figure 2. Microscope image of PEG-BC lipid nanoparticles. (×400)

Preparation of Gel-MS composite drug delivery system

PLGA-PEG-PLGA hydrogels, GEL-BUP, and GEL-MS/BUP were prepared under low-temperature agitation, and then the appearance and structure of the gels were observed under SEM, as shown in figure 3A was the SEM image of PLGA-PEG-PLGA, figure 3B was the SEM image of GEL-BUP, and figure 3C was the SEM image of GEL-MS /BUP. From figure 3A, the PLGA-PEG-PLGA hydrogel presented a porous network structure under the microscope, and the pore size was relatively uniform. Figure 3B showed that the drug presented a needle-like structure and was interspersed in the matrix of the hydrogel. Figure 3C showed that in the Gel-MS drug delivery system, the microspheres were uniformly dispersed on the surface of the gel, and the appearance and structure were not changed due to the participation of nano-microspheres. This showed that the prepared drug-carrying system had a complete porous structure, the encapsulation of drugs was very good, and it played a good role in long-term drug-carrying systems.

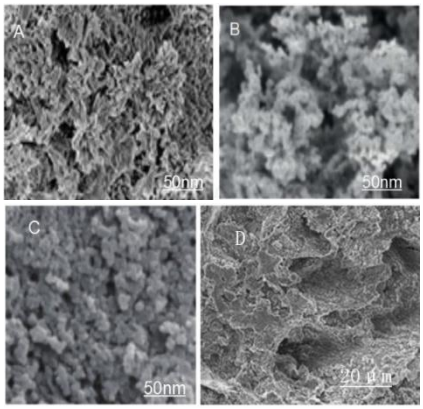

Figure 3. SEM image.

Rheological analysis

The left picture in figure 4 was the GEL-(DEX-MS/BUP) rheological curve, and the right picture was the Gel rheological curve. Rheology was used to detect the solution-gel phase transition behavior of the polymer solution. The elastic and viscous characteristics of the polymer were represented by G1 and G2, respectively. The range of detected temperature in the figure was 20-60°C. Figure 4 showed that as the temperature increased, the changing trend of elasticity and viscosity was roughly the same. If G2 was superior toG1, it indicated that the solution-gel phase transformation process was completed by Gel and Gel-(DEX-MS/BUP), and it was changed to the gel state. In the figure of GEL-(DEX-MS/BUP), the maximum values of G1 and G2 were both superior to those of GEL, it indicated that the mechanical strength of GEL was enhanced after the addition of DEX and MS/BUP. The Gel forming temperatures of Gel and Gel-(DEX-MS /BUP) was 27.3°C and 26.3°C, respectively. The results showed that the composite had good temperature sensitivity and the gel temperature and strength could also be used as a long-term anesthetic drug.

Drug release and degradation

The release rate of DEX was stable and uniform in GEL -(DEX-MS/BUP) drug-loaded composites. The in vitro release curve of the protease complex showed in figure 5 on the right. The in vitro degradation curve of the non-elastase complex is shown in the figure on the left. From figure 8 the drug loading release rate of DEX increased with the change of time. However, Gel-(DEX-MS/BUP) had no in vitro degradation curve of elastase in phosphate-buffered saline (PBS).

The release behavior of DEX was related to the loading position in the Gel-MS system. When DEX was in the gel, the drug and the medium in the gap were contacted by the gel surface, and the drug was released quickly.

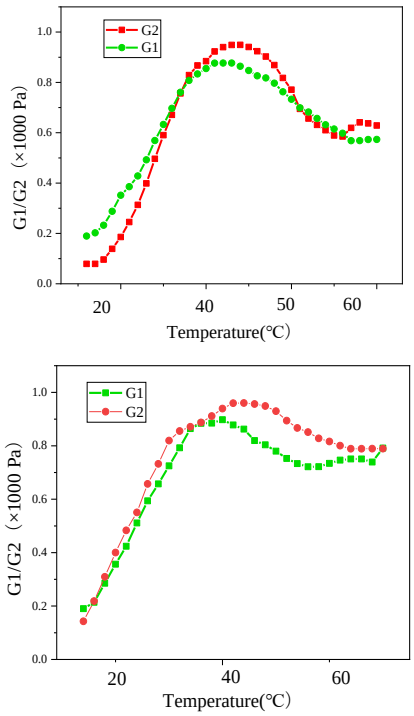

Figure 4. Rheological curve.

Exercise block and maximum proportional effect

In the right figure of figure 6, the maximum proportional effect (MPE) of MS/BUP and GEL-(DEX-MS/BUP) had reached 100% in less than ten minutes. It showed that both drugs can completely enter the state of the block, and there was no significant difference in the onset time of nerve block in each group. The left figure of figure 6 showed the detection results of motion arrest in rats. In the GEL-DEX group, there was no loss of motion function during the process.

Comparison of clinical effects

The MMSE score is an index commonly used in the clinical evaluation of cognitive dysfunction, and the operation is simple. From the left figure of figure 7, there was no significant difference in MMSE between the two groups 12 hours before surgery, and the MMSE score of group B was significantly superior to that of group A at 24 hours and 72 hours after the operation. This showed that the nano-drug-loaded DEX can effectively reduce inflammation and nerve damage more than conventional DEX.

The right figure of figure 7 showed the awakening time of the two groups. It showed that the awakening time of group B was less.

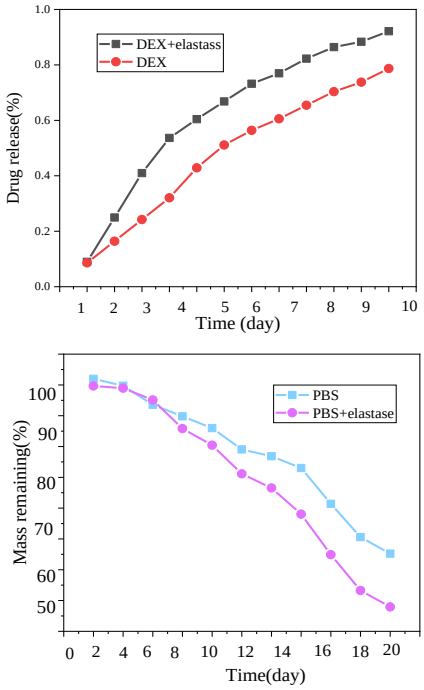

Figure 5. Results of drug release and degradation curves.

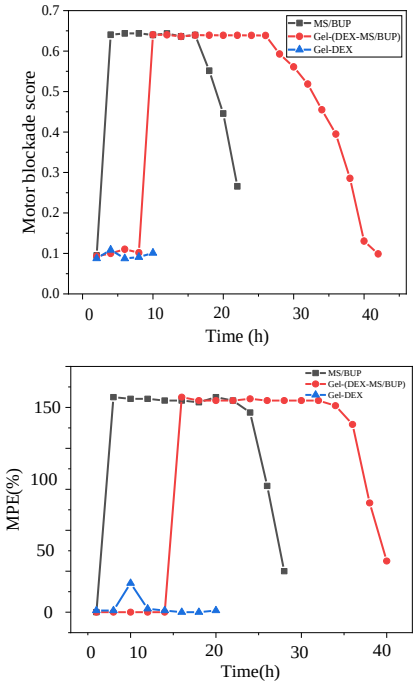

Figure 6. The effect of the motor score and maximum ratio at different times after injection of preparations around the sciatic nerve.

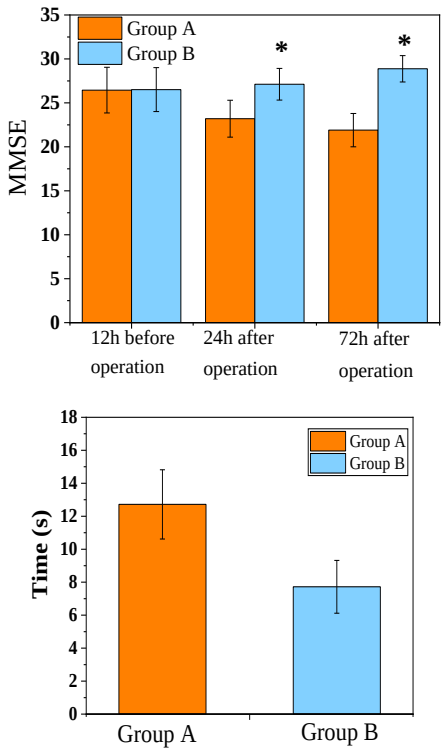

Figure 7. MMSE score and time comparison. (* indicated that the difference was statistically significant (P<0.05)

Anesthetics can reversibly combine with the voltage-gated sodium ion channel on the nerve cell membrane to block nerve conduction, and it has been widely used in the biomedical field (20). The drug-loaded nanospheres are magnetically responsive. The method is simple and the raw materials are easily available. The particle size of the microspheres and the drug loading rate showed good results (21). Studies showed that the stronger the drug was when the nanoparticle size was at the level of 50-100nm (22-23). Therefore, it can play a better therapeutic effect. Nanomedicine microspheres are good drug carriers with good drug properties and sustained release effects (24).

Li et al. (25) compared the effect of dexmedetomidine and levobupivacaine co-loaded with transcribed trans-activated peptide modified nanostructured lipid carriers or lipid-polymer hybrid nanoparticles in local anesthesia, and DEX improved the effect of drugs on a large extent. Yang (26) used the combination of ropivacaine and dexmedetomidine as analgesic therapy in local anesthesia: a long-acting lipid carrier with a nano-structure modified by hyaluronic acid, containing a skin osmotic promoter. The dual drug delivery can play a synergistic effect, reduce drug dosage, further reduce systemic toxicity, and improve the efficiency of anesthesia against injury. Therefore, the alkalization precipitation method was used to prepare DEX in this study. The prepared nano microspheres containing the drug DEX were coated with lipid-soluble drugs by PLAG microspheres for the fluid test, in vitro release, and detection analysis, and then the rat sciatic nerve model was constructed. This lay the foundation for the later development of nano-microsphere drug-carrying research. The drug was released slowly into the body through the nanosphere and local nerve impulse transmission was blocked (26-27). The Gel-MS composites material had good biodegradable Hull biocompatibility. The duration of analgesia can be greatly prolonged by the use of anesthetics with a local release via the GEL -(DEX-MS/BUP) system. DEX was added to the Gel-MS system, DEX can be released from the gel preferentially and the absorption of BUP was organized, which had a high effect on local anesthesia. The MPE of MS/BUP and GEL -(DEX-MS/BUP) had reached 100% in less than ten q minutes. It showed that both drugs can completely enter the state of the block, and there was no significant difference in the onset time of nerve block in each group. The detection results of motion arrest in rats showed that there was no loss of motion function during the process in the GEL-DEX group.

Conclusions

In conclusion, the prepared DEX was successfully loaded into PLGA microspheres, and then MS/BUP was embedded into GEL by the low-temperature stirring method. The highly selective α2 receptor agonist DEX was added to the GEL-MS system with the auxiliary agent of anesthetic to form GEL-(DEX-MS/BUP). In vitro drug release and degradation, experiments showed that DEX could be released for a week. MS/BUP and GEL-(DEX-MS/BUP) drugs could completely enter the state of blockade safely, and no motion loss was found in GEL -DEX in motion block detection. In the future, it is necessary to further expand the sample size and increase the clinical application of nano-spheres. The results of this study are expected to provide a theoretical basis for the application and promotion of drug loading in clinical practice.

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

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