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The role of PLGA/TPGS nanoparticle on xylazine-ketamine anesthetic activity in male albino rabbits

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Abstract

D-α-tocopheryl polyethylene glycol succinate (Vitamin E TPGS or TPGS) has been approved by food and drug administration (FDA) as harmless adjuvant and is largely used in drug systems delivery. The aim of the study was to use the TPGS polymer as a drug release model to regulate the release of the anesthetic xylazine-ketamine in order to minimize therapeutically reference dose, avoid side effects, and improve efficacy. The study performed on 15 adult local breed male rabbits, divided into 3 groups with same number which injected intramuscularly with single dose of suggested anesthetics. Heart rate, respiratory rate, degree of muscle relaxation, onset of action and stages of anesthesia were evaluated, also induction of anesthesia, surgical anesthesia and recovery time were recorded. Nanoprecipitation technique was optimal method for preparing small particle size as well as reduce dose for therapeutic effect. Small and large dose was showed perfect analgesic and muscle relaxant activity of xylazine-ketamine drugs. Ketamine 30 mg and xylazine 10 mg loaded PLGA showed elevation of conciseness period as well as increase muscle relaxant. Ketamine 30 mg and xylazine 10 mg loaded PLGA reduce heart rate but onset of action delayed when compared with reference drug. The process of nanoprecipitation was ideal for forming small particle sizes and reducing the dosage for therapeutic effects. PLGA loaded with ketamine-xylazine demonstrated improved cycle concentration (walk time) as well as improved muscle relaxant, finally the protocol created an excellent anesthetic combination for induction of general anesthesia.

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Introduction

The polymer is a macromolecule which consists of monomers called multiple repeating units. A copolymer is composed of two or more monomer of different forms. Glycolic and lactic acid are monomers of poly (lactic-co-glycolic acid) (PLGA). The ratio of monomers can differ which had a significant influence on the copolymer 's properties (1). The FDA has given PLGA for utilizing outstanding properties such as biocompatibility, biodegradability and sustained release features a variety of medical (2). In last decate, its use as non-toxic bio-degradable, and nonimmunogenic polymer for the progress controlled, and selective drug systems delivery have ultimately increased with the introduction of the block copolymer concept (3). Easy regulation of mechanical, chemical and physical properties was the greater advantage of synthetic polymers (4). PLGA nanoparticles are inspired by various kinds of medications counting anti-cancer drugs, hormones, anti-hypertensive drugs, vitamins and, macro-molecules as antibodies, peptide (5). Polymers are also classified into two classes; natural polymers which known as

a first class due to found in nature, for instance proteins such as collagen or carbohydrates such as cellulose. Potential biomaterials are all these polymers. In addition, naturally the polymers are existent like chitosan, present in arthropod exoskeletons; agarose which produced by algae and alginate derived from sea-weed, also a bilateral candidate for study (4,6). Synthetic polymers including poly lactic acid (PLA), polystyrene and PLGA were called in the second class. There are various methods for PLGA preparation, such as single emulsion, double emulsion solvent evaporation, nanoprecipitation, drying and salting spray (7). Nanoprecipitation techniques are known to be an easy method for preparing nano drugs which distinguished by the low surfactants available, creating small sizes and low concentrations resulting of them. In addition, this technique provides perfect benefits for preparing polyethylene glycol-poly lactic acid-co-glycolic acid (PEG-PLGA) filled with low hydrophobicity active ingredients atoms encapsulated effectively that have been reported (8). In cardiovascular disease PLGA was also used, such that the coated polymer minimized the initial blast and helped to extend the release of the compounds (9). TPGS, water miscible forms of vitamin E, is consist of a hydrophilic PEG chain matched with a hydrophobic vitamin E part (10). It displays wonderful drug delivery capability according to special amphiphilic structures. Additional researches, it has been proven that TPGS show excessive potential in over-coming multiple drug resistance (MDR) tumor for the P-glycoprotein (P-gp) inhibition, and selective anti-cancer outcome (11). TPGS can simply conjugate with polymers, or therapeutic agents to procedure TPGS based on polymers, or pro-drug. The co-administered ketamine aid two purposes: reducing the advancement of analgesic tolerance to the strong opioid and relieves neuropathic pain as it is strong N-methyl-D-aspartate (NMDA) antagonists (12). Ketamine is a nonbarbiturate anesthetic, a short-acting, makes dissociated state with un-conscious patients, while it seems to be wakeful, and does not sense pain. Dissociative anesthesia offers amnesia, fixity and sedation. In addition, it is considered a potent bronchodilator so that safely to use with asthmatic patient, or cardiogenic, or hypovolemic shocks. On the other hand, it is used with caution with hypertensive patients or with stroke due to the drug is lipophilic passing rapidly to the brain. The coadministration of ketamine-xylazine (kx) is a preferable choice for injecting rabbits' regimen with anesthetic surgery (13). The publicity of ketamine-xylazine is mostly due to supplemental action, analgesic effect, relaxation of muscle and improve sedation. Safety anesthesia can be utilized without needed expert tools (14). As a result, the current project is aimed to find the best polymers for loading XK with lowering the dose level that should be more safety, healthy and yielded surgical tolerance for performing surgical interventions in rabbits.

Materials and methods

PLGA 50:50 (PURASORB PDLG 5010), Vitamin E TPGS National Formulary (NF) Grade, xylazine and ketamine were dissolved in Dimethyl Sulfoxide (DMSO). Nano-particles prepared according to the nano-precipitation method (15) including organic steps alteration of percentages of minh and organic solvent. An organic solution of PLGA 10 mg, liquefied in 4 ml of DMSO and xylazine and ketamine (1 mg) dissolved in 1ml of DMSO, then drug added slowly to PLGA under high vortex to form 5 ml of organic phase. Aqueous phase prepares by 0.03% vitamin E TPGS solution 20 ml, distinct w/v, organic phase was added slowly to aqueous phase under high vortex then transfer solution to probe sonication for 5 minutes. The suspension collected was filtered to discard any precipitate then centrifuged at 14000 rpm at 4ºC Sorvall RC 5 Plus to discard any precipitate. The free drug was separated in the supernatant portion and the pellet collected then washed 3-4 times with deionized water and lyophilized for two days to produce a free-flowing powder. Empty PLGAs have been produced in conjunction with the same process as before (16) (Figure 1).

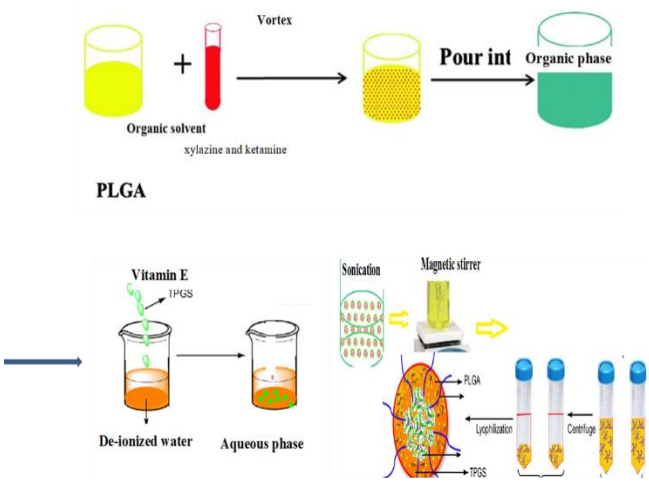

Figure 1: Show schematic of using our sequential nanoprecipitation method to fabricate ketamine-xylazine loaded polymeric nanoparticles. Suspension mixture containing ketamine-xylazine loaded by PLGA-TPGS. The scale bars are 280 nm.

Animal study

The study was done on 15 adult local breed male rabbits, separated into 3 equivalent intramuscular injection groups in a single syringe. Group 1 injected with cocktail from ketamine 30 mg / Kg B.W and xylazine 10 mg / Kg B.W. Group 2 was infused with PLGA-TPGS loaded with ketamine 30 mg / Kg B.W and xylazine 10 mg / Kg B. Group 3 was injected with PLGA-TPGS loaded with ketamine 15 mg / Kg B.W and xylazine 5 mg / Kg B.W.

The following physiological parameters were analyzed, including: heart rate, respiratory rate, level of muscle relaxation, initiation of operation and anesthesia levels before administration of drugs at time 0 then 10, 30, 60 minutes after administration of the drug, anesthesia induction, surgical anesthesia and recovery time were also reported (Figure 2) (17,18).

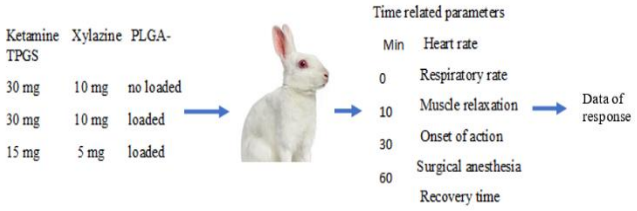

Figure 2: Show the three groups and physiological parameters analyzed.

Statistical analysis

The present study data were analyzed utilizing SPSS version 19 software. all outcome parameters are presented as mean + se. differences between quantitative data were analyzed using one-way ANOVA, followed by turkey's test. P - a value less than 0.01 is statistically significant for all data shown in our results.

Result

Characterization of ketamine-xylazine nanoparticles drug

Based on nanoprecipitation technique, we advanced ketamine-xylazine catalog loaded (TPGS-PLGA) nanoparticles to control the drug release profile and side effect of a large dose. For the preparation of high ketamine-loaded polymer TPGS -PLGA nanoparticles, the same mass of ketamine and xylazine-TPGS-PLGA were dissolved in a solvent mixture of DMSO. Using our TPGS-PLGA method, ketamine precipitated to form uniform ketamine-xylazine nanoparticles. The scanning electron microscope (SEM) image shows a rounded shape particle, and the formulation size of ketamine-xylazine nanoparticles was 280 nm (Figures 3).

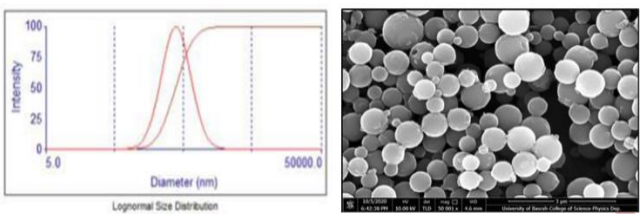

Figure 3: Show zeta sizer of ketamine and xylazine loaded by PLGA-TPGS, and SEM image shows a rounded shape particle.

Effect of ketamine-xylazine nanoparticles on heart rate

There was no-significantly difference, P≤0.05 between group 1 and 3 in the heart rate within 0, 10, 30 and 60 minutes. Moreover, PLGA loaded ketamine-xylazine showed a significant reduction of heart rate under normal level to recorded mean value 185.00 ± 4.76 and 190.80 ± 10 at 30 and 60 minutes respectively for group 2 that received complete dose than compared with half dose 15 mg Ketamine and 5 mg Xylazine filled with PLGA-TPGS that still mimic to reference dose cocktail. In addition, there were no-significantly differences P≤0.05 between all groups of study at 0 and 10 minutes (Figure 4).

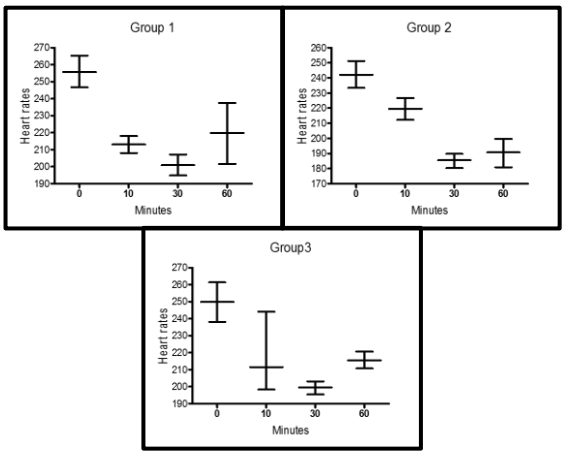

Figure 4: Show the heart rate within 0, 10, 30 and 60 minutes in the three groups.

Effect of ketamine-xylazine nanoparticles on respiratory rate

Our result showed that there were no-significantly differences P≤0.05, between groups 1, 2 and 3 in the respiratory rate within 0, 10, 30 and 60 minutes. Moreover, PLGA loaded Ketamine-Xylazine showed no significant reduction of respiratory rate to recorded mean value 162.40 ± 10.83, 165.60 ± 5.32 and 175.60± 6.79 at 60 min respectively (Figure 5).

Role of Ketamine-Xylazine nanoparticles on muscle relaxant

Our result in table 1 showed there was no significant differences between group 1, 2 and 3 in the degree of muscle relaxant within 0 minutes, but there was a significant difference in G2 comparing with G1 and G3. Degree of muscle relaxant within 10, 20- and 30-minutes show there were no significant differences between G1 and G3. The present study showed that Ketamine-Xylazine nanoparticles appear significant differences of increasing surgical anesthesia at animal received nanoparticle at complete dose in G2 as well as half dose but there was no-significantly differences P≤0.05, between group G1 and G3 in the degree

of surgical anesthesia as well as recovery time was elevated in G1 group. Although delay onset of action in nano drug was very clear depend on release the drug from PLGA, but after short time of releasing them from PLGA, it was enough to induce pharmacological action in pulsatile manner (Figure 6).

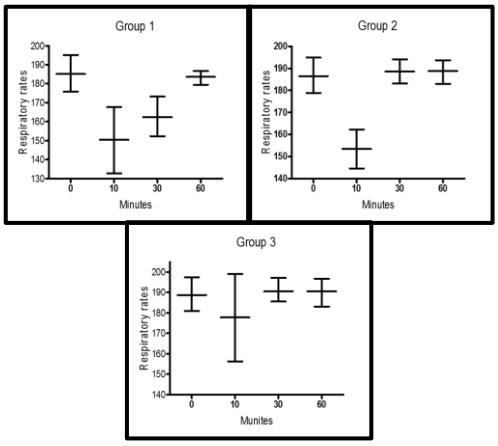

Figure 5: Show the respiratory rate within (0, 10, 30 and 60) minutes in three groups.

Table 1: Show the degree of muscle relaxant within 0, 5,10, 20 and 30 minutes in all anesthetized groups

Time (minutes)	degree of muscle relaxant		
	Group 1	Group 2	Group 3
0	±	±	±
5	+	++	+
10	+	++	+
20	++	+++	++
30	++	+++	++

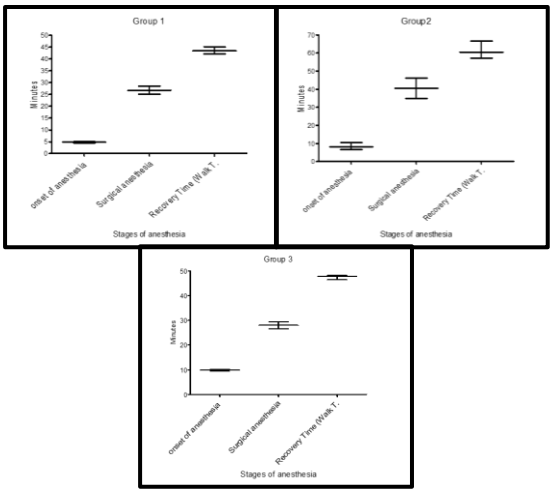

Figure 6: Show the onset of action, surgical anesthesia and recovery time in the three groups of anesthesia.

Discussion

Nanoparticles of around preparation of high ketamine loaded polymer TPGS -PLGA nanoparticles, using our TPGS-PLGA method, ketamine precipitated to form uniform ketamine-xylazine nanoparticles. The SEM image shows rounded shape of particles and the formulation size of particles of ketamine-xylazine nanoparticles was 280 nm, which is optimal for the capacity of nanoparticles to be used after intramuscular injection. Our study used a drug-polymer ratio of 1:10, which is in line with the findings stated by Gaonkar *et al.* (19) who proved that the 1:10 ratio is ideal for a garcinol loading drug delivery device for PLGA rather than 1:20, and for drug loading (DL) and encapsulation efficiency (EE). The technique used here focuses on the synthesis of vitamin E TPGS nanoparticles as the emulsifying agent and DMSO as a solvent. Our analysis has shown that PLGA decreases the size of nanoparticles and inhibits aggregation and sedimentation of nanoparticles. In addition, the cell absorption and delivery restriction in the body due to the mononuclear phagocytic system tag decreased and the drug size of more than 1000 nm expelled (20). Ketamine-encapsulated PEG-PLGA nanoparticles recorded mean value 107.4 nm with highly perfect encapsulation efficiency reach to 71.8 % after utilized of nanoprecipitation method, moreover noted that a sustained-release of drug persist for nearly 21 in vitro and 5 day in mice. Therefore, a prolonged period of analgesia, it may be promise to control cancer or accidental sever pain is an area of future investigation.

There was no effect of xylazine-ketamine loading TPGS-PLGA on the respiratory rate, on the other side, nanoparticles reduced full-dose heart rate relative to ketamine-xylazine nanoparticle and reference ketamine-xylazine. In our study, the development of ketamine-xylazine analgesia is compatible with the results of related studies. However, several workers documented a lack of analgesia in all the rabbits tested, including using higher doses of ketamine-xylazine than the doses used in our research (16). Our data revealed that there are no-significantly differences, $P \leq 0.05$, between group 1 and 3 in the heart rate within 0, 10, 30 and 60 minutes. Moreover, PLGA loaded ketamine-xylazine showed a significant reduction of heart rate under normal level, ketamine increases cardiac output, blood pressure, central venous pressure. Cardiac stimulant characteristics support good induction agent for hypovolemic patient. Ketamine increases sympathetic outflow in the central nervous system, leading to activation of the heart with elevation of blood pressure and cardiac output. In rats, co-injection of ketamine with fentanyl, blocking target receptors channels in the spinal cord which can result in adequate analgesia by reducing analgesic tolerance and increasing pain relief (21). PLGA nanoparticles promoted by three factors includes, polymer degradation, internal-mass transfer, and external-diffusion. On the fundamental of our present data, the external-diffusion and internal-mass

transfer likely limit the extent of ketamine-xylazine release and polymer degradation is the major factor that control the release afterward (22).

Conclusion

PLGA loaded with ketamine-xylazine demonstrated suitable polymer for developing drug delivery for general anesthesia regimen in rabbits. finally, the protocol created an excellent anesthetic combination for induction and maintenance of general anesthesia, it can be used in the future after conducting many researches to develop it in performing surgeries with local and general anesthesia.

Acknowledgments

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Conflict of interest

The authors declare that no conflict of interest exists.

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دور بولي حمض اللاكتيك-كو-جليكوليك / توكوفيرول بولي إيثيلين جلايكول سكسينات النانوية في التأثير المخدر لمزيج الكيتامين والزيلازين في ذكور الأرانب البيضاء

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الخلاصة

اعتمد استخدام توكوفيرول بولي إيثيلين جلايكول سكسينات من قبل إدارة الغذاء والدواء كمساعد آمن وعلى نطاق واسع في أنظمة توصيل الأدوية. هدفت الدراسة على استخدام بوليمر توكوفيرول بولي إيثيلين جلايكول سكسينات كنموذج لتحرير الدواء لتنظيم إطلاق مخدر الزيلازين-كيتامين من أجل تقليل الجرعة العلاجية وتجنب الآثار الجانبية وتحسين الفعالية. أجريت الدراسة على ١٥ أرنب ذكر بالغ ومن السلالة المحلية مقسمة إلى ٣ مجموعات بنفس العدد، تم حقنها عضليا بجرعة واحدة من أدوية التخدير المقترحة. تم تقييم المعايير الفسيولوجية والتي شملت معدل ضربات القلب، معدل التنفس، درجة استرخاء العضلات، بداية التأثير المخدر ومراحل التخدير قبل إعطاء الدواء في الوقت صفر ثم في ١٠ و ٣٠ و ٦٠ دقيقة بعد إعطاء المخدر، تم أيضا تسجيل إدخال التخدير والتخدير الجراحي ووقت الإفاقة. كانت تقنية الترسيب النانوي هي الطريقة المثلى لتحضير حجم الجسيمات الصغيرة وكذلك تقليل الجرعة للتأثير العلاجي. أظهرت الجرعات الصغيرة والكبيرة نشاطا مثاليا مسكنا ومريحا للعضلات لمادتي الكيتامين والزيلازين. أظهر الكيتامين ٣٠ ملغم والزيلازين ١٠ ملغم المحمل ارتفاعا في فترة الإيجاز بالإضافة إلى زيادة ارتخاء العضلات كذلك قلل من معدل ضربات القلب ولكن تأخر بدء التأثير عند مقارنته بالمجموعة الأولى. كانت عملية الترسيب النانوي مثالية لتشكيل أحجام جزيئات صغيرة وتقليل جرعة التخدير. أظهر بولي إيثيلين جلايكول سكسينات المحمل بـالكيتامين والزيلازين تركيزا محسّنا للدورة (وقت المشي) بالإضافة إلى تحسين ارتخاء العضلات، وأخيرا أنشأ البروتوكول مزيجا مخدرا ممتازا لتحريض التخدير العام.