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Research Article

In vivo Evaluation of Galantamine Injectable *in situ* Gel for Management of Alzheimer's Disease in Controlled Manner

¹Hala Mohammed Alkhalidi and ²Amr Sami Mouminah

¹Department of Clinical Pharmacy, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia

²Department of Neurology, King Abdulaziz Medical City (KAMC), Jeddah, Saudi Arabia

Abstract

Background and Objective: Alzheimer's disease is a neurodegenerative disease characterized by deterioration of cognitive and memory functions. Galantamine is one of the most commonly used drugs for treatment of Alzheimer's disease and the main challenge in its use is the frequent dosing: Patients with Alzheimer's usually suffer from dementia and often forget to take medication. The aim of this research was to evaluate the *in vivo* biodegradability and pharmacokinetic behavior of galantamine when administered as intramuscular, *in situ*, controlled-release depot injection. **Materials and Methods:** *In situ* gel was prepared by using Poly-DL-lactide-coglycolide and its *in vivo* biodegradability rate was evaluated as a novel technique in rats. The pharmacokinetic behavior of galantamine after administration of the *in situ* gel as an intramuscular injection was assessed in rabbits. **Results:** The prepared galantamine *in situ* gel showed an *in vivo* biodegradability rate estimated at 11% every 7 days in rats and the pharmacokinetic evaluation revealed that galantamine mean residence time extended to 71 days in rabbits. **Conclusion:** Preparation and administration of galantamine as an intramuscular *in situ* gel formula is an excellent approach for providing an extended release of more than 2 months, which will eliminate the forgetting of doses associated with dementia.

Key words: Galantamine, intramuscular *in situ* gel, *in vivo* biodegradability, pharmacokinetic, Alzheimer

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Corresponding Author: Hala Mohamed Alkhalidi, Department of Clinical Pharmacy, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia
Tel: 00966544848546

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alzheimer's disease is a neurodegenerative disease that is characterized by deterioration of cognitive and memory functions and continuous impairment of simple daily-living activities that drastically affects the social and behavioral skills of patients who have the disease¹. It occurs most frequently in the elderly and is usually considered one of the most common types of irreversible dementia^{2,3}. Several drugs are used for the treatment of Alzheimer's disease. Galantamine (Reminyl) is one of the most commonly used drugs available commercially. It exerts its action through cholinesterase inhibition^{4,5}. It is a tertiary alkaloid and occurs naturally in the daffodil, snowdrop and snowflake⁵.

Galantamine is available as twice-daily tablets (4 and 8 mg), solution (4 mg mL⁻¹) and daily extended-release capsules⁶. It is able to augment cholinergic function, specifically on the nicotinic receptors⁷, leading to neuro-protection against neurotoxicity and improved cognitive functions^{8,9}, which improve memory functions in patients with Alzheimer's disease¹⁰. However, the patient is required to take doses two times daily and this is a major problem in patients with Alzheimer's who usually suffer from dementia and forget their medication¹¹.

In situ gelling systems have several applications as a controlled-release drug delivery system. Many novel *in situ* gel-based matrices have been designed and fabricated to use as drug delivery systems. These formulations are liquid solutions that convert to gel when in contact with body fluids. They are also regarded as a mucoadhesive drug delivery system. The transformation from solution to gel depends on several factors according to the type of polymer used, which may convert to gel due to temperature, presence of ions, pH change or precipitation of the polymer, leading to controlled drug diffusion and release¹².

In contrast to strong gels, *in situ* gel can be easily injected; it then swells and forms a strong gel in the muscle. This gel matrix can release the drug in a controlled manner and prolong its residence time¹². *In situ* gels are administered by oral, injectable, rectal, ocular, vaginal and intraperitoneal routes¹³.

This study aimed to evaluate an intramuscular *in situ* gel loaded with galantamine through two approaches: *In vivo* biodegradability rate in rats and pharmacokinetic behavior assessment in rabbits.

MATERIALS AND METHODS

Galantamine hydrobromide and Poly-DL-lactide-coglycolide grade 50:50, N-Methyl-2-Pyrrolidone were

purchased from Sigma Chemical Company (St. Louis, Missouri, USA). Phosphate-buffered saline kit was purchased from Fluka Chemicals (Buchs, Switzerland). All other chemicals used were of analytical reagent grade. This study was conducted from 10th of February 2018 and finished completed at 2nd of September, 2018.

Preparation of galantamine *in situ* gel: For preparation of the *in situ* gel formula, 250 mg of galantamine was dissolved in 2 mL of NMP containing 600 mg PLGA. The solution was sonicated by using a prob-sonicator and then allowed to completely dissolve in a water bath shaker at 25 °C for 3 days. The resultant *in situ* gelling solution was stored at 8 °C for further *in vivo* evaluation¹⁴.

Evaluation of galantamine intramuscular *in situ* gels
***In vivo* biodegradability of intramuscular *in situ* gel formula:** Twenty-four male wistar rats, each weighing 200-250 g were divided randomly into eight groups of three rats and used for the experiment. The test method and steps were revised and approved by the King Abdulaziz University animal ethical committee (approval no. 222-2018 on 15 May, 2018).

The tested formula was injected in the left flank region of the muscle at a dose of 32 mg kg⁻¹. The groups of rats then were subjected to the approved surgical procedure at 1, 7, 14, 21, 28, 42, 56 and 60 days after administration. At each of the previously mentioned time points, a group of rats was sacrificed by administration of an overdose of euthasate and the marketed site of injection was surgically excised to detach the implant from its place within the muscle. The detached implants were analyzed and the galantamine content was measured to determine the *in vivo* rate of biodegradability in male Wistar rats and to ensure that galantamine remained within the implant for the time of the test.

***In vivo* pharmacokinetic study:** A second approach in the evaluation of the intramuscular *in situ* gel was the *in vivo* pharmacokinetic animal study. The design and steps of this study were revised and approved by King Abdulaziz University animal ethical committee (approval no. 223-2018 on 15 May, 2018). The study protocol includes the use of 12 healthy male albino rabbits, each weighing 2-2.5 kg, divided randomly into 2 groups of 6 animals. The specific amount of the prepared *in situ* gel that provides a dose of 20 mg kg⁻¹ b.wt., was injected intramuscularly in each rabbit in group 1. Rabbits in group 2 were injected with a galantamine aqueous suspension. Blood samples of 500 µL were withdrawn before administration of the dose, every 6 h during the first day and then every 7 days for 2 months in the

first group. In the second group, samples were collected before administration, every 6 h during the first day and then after 3 and 7 days. The blood samples were collected, immediately centrifuged and stored at -20°C until the time of analysis.

Pharmacokinetic analysis: Useful pharmacokinetic parameters that serve the aim of the research were calculated and are presented as Mean $\pm$ SD. The pharmacokinetic solver program was used to calculate the area under the plasma concentration time curve and the mean residence time for each formulation¹⁵.

Statistical analysis: The results of the pharmacokinetic study were analyzed statistically by one-way analysis of variance at a significance level of $p \leq 0.05$ using the SPSS program and are presented as Mean $\pm$ SD.

RESULTS

In vivo biodegradability: The *in vivo* biodegradability results indicated that the galantamine *in situ* gel degraded by a rate of 11% every 7 days. The results are illustrated in Fig. 1, which shows the zero-order degradation rate with a degradation rate constant equal to 1.405% per day. This biodegradability curve was obtained by plotting the weight (%) of the implant that still remained within the muscle at each time point of the test across the time intervals of the study protocol.

In vivo pharmacokinetic studies: Figure 2a showed the relation between the galantamine plasma level and time after intramuscular administration of the prepared *in situ* gel, the results of *in vivo* pharmacokinetics indicated that the MRT for galantamine was extended to 71 ± 6 days.

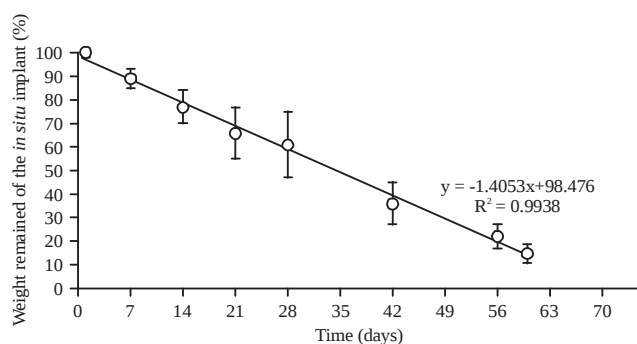


Fig. 1: Relation between weight (%) of implant detached intact from the rat muscle and time intervals

Figure 2b showed the relation between the galantamine plasma level and time after intramuscular administration of the aqueous suspension in rabbits. The MRT for aqueous suspension was only 5 ± 2 days. In addition, the AUC increased by about 3-4 times after administration of galantamine as *in situ* gel compared to aqueous suspension as shown in (Table 1).

Table 1: Pharmacokinetic parameters of galantamine obtained after administration of intramuscular *in situ* gel and aqueous suspension

Parameters	Galantamine <i>in situ</i> gel	Galantamine aqueous suspension
$\text{AUC}_{0-\infty} (\text{ng mL}^{-1} \text{ day}^{-1})$	969 ± 101	367 ± 43
$\text{AUC}_{0-\text{inf_obs}} (\text{ng mL}^{-1} \text{ day})$	1231 ± 121	390 ± 52
$\text{MRT}_{0-\text{inf_obs}} (\text{day})$	71 ± 6.0	5 ± 2.0

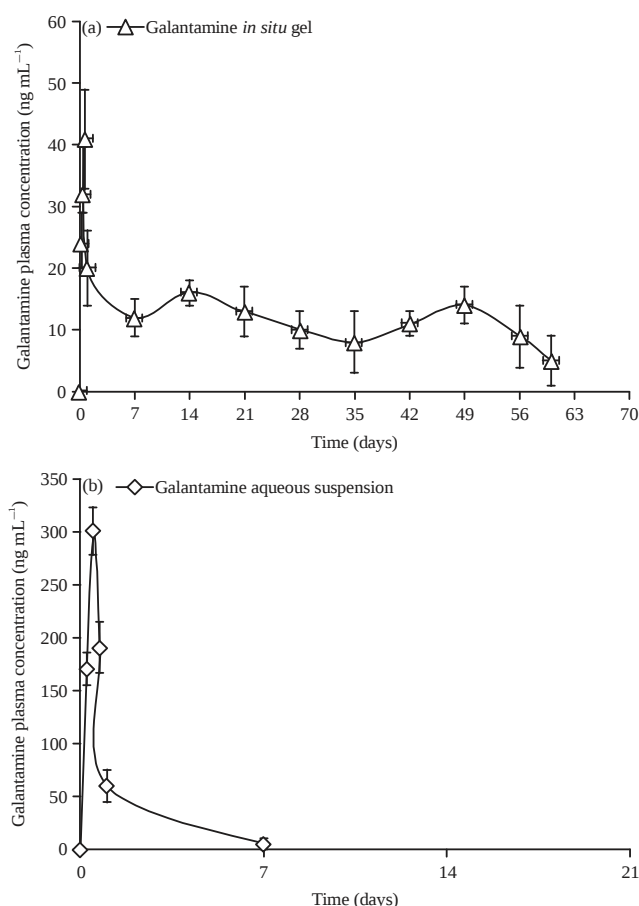


Fig. 2(a-b): Plasma concentration-time profiles for galantamine after intramuscular administration of (a) *In situ* gel formulation and (b) Aqueous suspension

All data are presented as Mean $\pm$ SD (n = 6)

DISCUSSION

This study aimed to evaluate intramuscular *in situ* gel loaded with galantamine through two evaluation approaches: *In vivo* biodegradability rate in rats and pharmacokinetic behavior assessment in rabbits. Ensuring the controlled and prolonged *in vivo* release of the drug from the intramuscular implant will help patients who require a dose two times daily, which is a major problem for Alzheimer's patients, who usually suffer from dementia and forget their medication. These drawbacks from the commercially available product have reported in many previously published researches^{16,17}. The rate of release can be measured easily *in vitro* by laboratory apparatus such as dissolution and diffusion apparatus and those two methods was previously used to study the release behaviours for many types of *in situ* gel bases loaded with drugs as alendronate¹⁸, atorvastatin¹⁴ and bone morphogenetic protein¹⁵, but in the case of intramuscular implant, physiological factors within the body may affect the release rate either positively or negatively¹⁶. For that reason, a very accurate method to test the release and degradation of the intramuscular *in situ* implant is to measure the rate of biodegradability within the body. The results of this study indicated that the intramuscular *in situ* gel formula succeeded in controlling the release of galantamine and the rate of biodegradability of the polymeric implant was slow, extending to more than 2 months. These results agreed with the work of Hosny and Rizg¹⁸, who found that alendronate sodium *in situ* gel degraded by a rate that allowed the implant to remain in the muscle for about 3 months. Also, this results was agreed with the work of Yu *et al.*¹⁹, who evaluate the biocompatibility and 6-month *in vivo* release of bevacizumab from a hyaluronic acid/dextran-based *in situ* hydrogel after injection.

Another approach applied in this research to confirm the controlled and prolonged release of the drug from the implant was the pharmacokinetic study. The results of this study confirmed that the bioavailability of galantamine was enhanced by more than 3- to 4-fold when formulated and administered as an intramuscular *in situ* gel compared to an aqueous suspension. Furthermore, the prolonged action was confirmed by the lengthened mean residence time, which extended to 70 days. This extension in the MRT could be attributed to the slow biodegradation rate of the PLGA polymer used during preparation of the *in situ* gel²⁰⁻²².

CONCLUSION

Preparation and administration of galantamine as an intramuscular *in situ* gel formula is an excellent approach for

providing extended release of medication for more than 2 months, thus eliminating the forgetting of doses associated with dementia.

Research confirmed the controlled and prolonged release of galantamine from the prepared intramuscular *in situ* gel by two different approaches: *In vivo* biodegradability rate and *in vivo* pharmacokinetics. The two approaches confirmed that the intramuscular implant prolonged the release of galantamine for more than 2 months. Of course, this will not obviate the need for further clinical evaluation regarding the bioactivity and toxicity of this novel formula, which may provide clinicians with other important data.

SIGNIFICANCE STATEMENT

This study suggests two approaches to confirm the controlled release of intramuscular *in situ* gel loaded with galantamine as a novel delivery system for management of Alzheimer's disease. This novel delivery system will help patients who require doses two times daily, a major problem because Alzheimer's patients usually suffer from dementia and forget to use their medication. This novel intramuscular *in situ* gel will be given once every 2 months instead of normal pills, which require daily administration.

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