RUDN Journal of Agronomy and Animal Industries. ISSN 2312-797X (Print). ISSN 2312-7988 (Online)
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2026;21(1):195—202
http://agrojournal.rudn.ru

DOI: 10.22363/2312-797X-2026-21-1-195-202
EDN GGQBZO
UDC 619:615.1

Research article / Научная статья

Analysis of the pharmacological efficacy of methylprednisolone in targeted delivery using biodegradable carriers in animals

Georges Bannoud[1], Alfiya N. Ibragimova[2], Santiago Mas-Coma[3], Natalya I. Troshina[2], Arfenya S. Karamyan[2] ✉

[1]Vetlife Veterinary Clinic, Moscow, *Russian Federation*
[2]RUDN University, Moscow, *Russian Federation*
[3]University of Valencia, *Valencia, Spain*
✉ karamyan-as@rudn.ru

Abstract. The use of glucocorticosteroids (GCS) in veterinary pharmacotherapy helps reduce inflammation and allergic reactions and has an immunosuppressive effect. Adaptive hormones increase the body's resistance to stress. The widespread use of GCS in surgical interventions involving implantation is driven by a decreased reaction of surrounding tissues, reduced postoperative edema, and improved implant integration. However, oral administration of GCS is often associated with low bioavailability, and dose escalation carries the risk of severe adverse effects. The development of novel targeted delivery systems using biodegradable carriers makes it possible to increase the bioavailability of drugs, minimize side effects, and enhance the efficacy of pharmacotherapy. When choosing targeted delivery, it is important to assess the quantitative parameters of GCS concentration in blood plasma depending on the route of administration. The developed high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) method enables the determination of very low drug concentrations within a range of 2 to 1000 ng/mL.

Keywords: glucocorticosteroids, targeted delivery system, veterinary medicine, pharmacotherapy, high-performance liquid chromatography, HPLC, tandem mass spectrometry

Authors' contribution: Ibragimova A.N. — analysis of obtained information, data processing; Bannoud J. — sampling, sample preparation, data collection and acquisition, writing of the manuscript; Mas-Koma S. — study design; Troshina N.I. — data processing; Karamyan A.S. — manuscript writing, overall supervision of the scientific project. All authors reviewed the final version of the manuscript and approved it.

Conflict of interest. The authors declare no conflict of interests. The methylprednisolone drug was purchased by the authors with their own funds for the purpose of conducting a comparative study.

Article history: received 2 October 2025; accepted 12 January 2026.

For citation: Bannoud J, Ibragimova AN, Mas-Koma S, Troshina NI, Karamyan AS. Analysis of the pharmacological efficacy of methylprednisolone in targeted delivery using biodegradable carriers in animals. *RUDN Journal of Agronomy and Animal and Animal Industries*. 2026;21(1):195–202. doi: 10.22363/2312-797X-2026-21-1-195-202 EDN: GGQBZO

Introduction

The development of novel routes of drug administration is often associated with a number of potential challenges and risks, most are solved through technological improvements, drug stability studies, and preclinical research [1–3]. Anti-inflammatory drugs for veterinary use are widely represented on the pharmaceutical market. The most accessible options, both in terms of convenience for animal owners and economic considerations, are conventional oral formulations [4–7]. Drugs intended for intravenous administration are preferably given in a veterinary clinical setting to minimize the risks of phlebitis, intravenous contamination, patient injury, and stress. However, beyond these obvious problems, there are also issues related to the direct pharmacological action of drugs and strategies to reduce the drug burden on the animal organism. Targeted delivery using modern biodegradable carriers applied to the surface of implants helps address these issues during the provision of high-tech medical care [8–10]. The use of targeted drug delivery is promising because it allows the administration of antibiotics, hormones, and anti-inflammatory agents while monitoring their concentrations, reducing drug loss during passage through tissue barriers, and acting directly at the site of implantation injury [9, 11–13]. The use of glucocorticosteroids (GCS) is justified for reducing inflam-matory edema, decreasing granulation of injured tissue, managing allergic reactions, and achieving immunosuppression, all of which affect the rate and effectiveness of implant integration in the animal body [14–15].

The aim of the study is to perform a pharmacokinetic analysis and evaluate the efficacy of methylprednisolone in targeted delivery.

Materials and Methods

For the quantitative determination of GCS concentrations in serum and blood plasma of laboratory animals (rabbits of the Soviet Chinchilla breed, age 5 months, males), four groups of animals (n = 3 males per group) were formed. The first and second groups received methylprednisolone hemisuccinate in the dosage form of a solution for intramuscular and intravenous injections; the third and fourth groups received a methylprednisolone-based formulation. Rabbits in groups 1 and 2 received methylprednisolone hemisuccinate intravenously at a dose of 20.0 mg/kg, while animals in groups 3 and 4 received an equivalent dose intramuscularly. Megestrol acetate was chosen as an internal standard for the analytical study. The standard sample was added directly to the test samples during sample preparation. The use of a standard

sample during sample preparation serves as a calibration mechanism, allowing the determination of minimal concentrations of the target GCS in the serum and plasma of the experimental animals.

Results and Discussion

The concentrations of methylprednisolone hemisuccinate and methylprednisolone were studied in *in vivo* experiments. Biological material was collected from animals at the same time (10:00 a. m.) after standard procedures. The sample volume was 2.0 mL; blood was collected into Eppendorf tubes. The analysis was performed in two media — plasma and blood serum — to determine differences in drug concentrations and metabolism in the body. The results are presented in Tables 1, 2 and Figs. 1–4.

Table 1

The content of GCS in the plasma and serum of rabbits after intravenous administration of a dose of 20.0 mg/kg

№	Time	Methylprednisolone hemisuccinate, mcg/ml (No. 1)		Methylprednisolone, mcg/ml (No. 3)	
		Plasma	Serum	Plasma	Serum
1	0 min	0.0	0.0	0.0	0.0
2	15 min	12.9	11.5	0.7	0.4
3	30 min	5.3	5.1	1.1	0.9
4	2 hours	2.7	2.0	2.1	1.9
5	4 hours	2.3	2.1	2.1	2.2
6	24 hours	0.2	0.2	0.0	0.0

Source: compiled by A.N. Ibragimova, J. Bannoud, S. Mas-Koma, N.I. Troshina, A.S. Karamyan.

Table 2

The content of GCS in the plasma and serum of rabbits after intramuscular administration of a dose of 20.0 mg/kg

№	Time	Methylprednisolone hemisuccinate, mcg/ml (No. 2)		Methylprednisolone, mcg/ml (No. 4)	
		Plasma	Serum	Plasma	Serum
1	0 min	0.0	0.0	0.0	0.0
2	15 min	3.6	3.4	0.4	0.2
3	30 min	2.9	2.8	0.7	0.5
4	2 hours	1.6	1.3	1.7	1.4
5	4 hours	0.4	0.3	2.0	2.1

Source: compiled by A.N. Ibragimova, J. Bannoud, S. Mas-Koma, N.I. Troshina, A.S. Karamyan.

During the quantitative determination of GCS concentrations in the plasma and serum of the experimental animals, the limit of detection for methylprednisolone hemisuccinate was established at 20 pg/mL in all animals. At the same time, the concentration of methylprednisolone was twice that of methylprednisolone hemisuccinate.

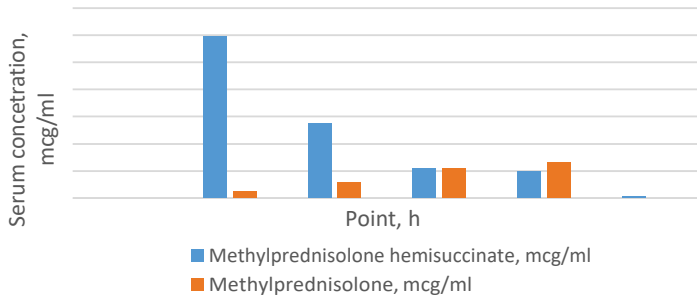

Fig. 1. Dependence of the concentrations of methylprednisolone hemisuccinate and methylprednisolone in the serum of rabbits (intravenous administration of a dose of 20.0 mg/kg)
Source: compiled by A.N. Ibragimova, J. Bannoud, S. Mas-Koma, N.I. Troshina, A.S. Karamyan.

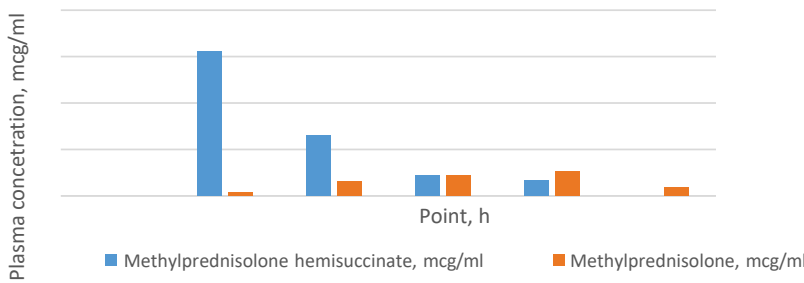

Fig. 2. Dependence of the concentrations of methylprednisolone hemisuccinate and methylprednisolone in the plasma of rabbits (intravenous administration of a dose of 20.0 mg/kg)
Source: compiled by A.N. Ibragimova, J. Bannoud, S. Mas-Koma, N.I. Troshina, A.S. Karamyan.

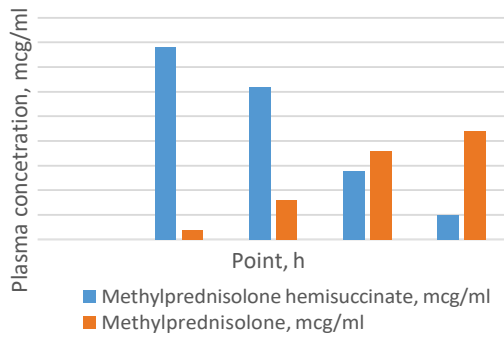

Fig. 3. Dependence of the concentrations of methylprednisolone hemisuccinate and methylprednisolone in the plasma of rabbits (intramuscular administration of a dose of 20.0 mg/kg)
Source: compiled by A.N. Ibragimova, J. Bannoud, S. Mas-Koma, N.I. Troshina, A.S. Karamyan.

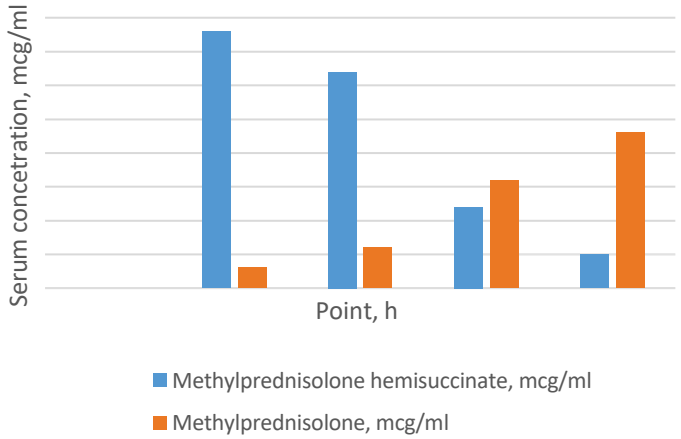

Fig. 4. Dependence of concentrations of methylprednisolone hemisuccinate and methylprednisolone in the serum of rabbits (intramuscular administration of a dose of 20.0 mg/kg)

Source: compiled by A.N. Ibragimova, J. Bannoud, S. Mas-Koma, N.I. Troshina, A.S. Karamyan.

To confirm the stability of the drug on the surface of the biodegradable implant carrier, samples were analyzed using a well-established and standardized method of high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS). The concentration of methylprednisolone was determined by studying the finished carriers. The conducted release allowed us to establish the drug concentration on the film in the first week at 723 ± 18 μg. A repeat analysis of the samples after two weeks revealed a decrease in the drug content on the film, with a loss of 3.59% (less than 5%), which is acceptable (Fig. 5).

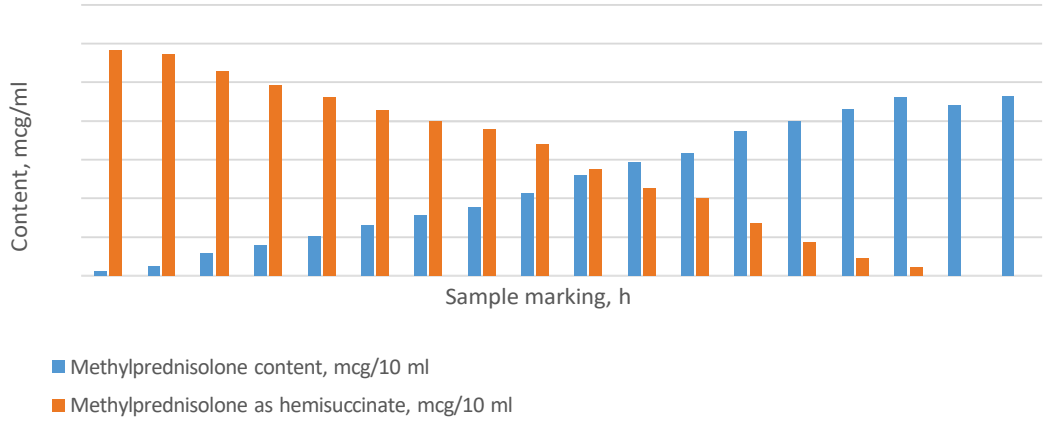

Fig. 5. Graph of the release of methylprednisolone from microcells on biodegradable carriers in phosphate buffer

Source: compiled by A.N. Ibragimova, J. Bannoud, S. Mas-Koma, N.I. Troshina, A.S. Karamyan.

Conclusion

A comparative *in vivo* study of GCS concentrations in the plasma and serum of experimental animals established significantly higher values in plasma. The correlation between the release, biotransformation, and elimination of methylprednisolone depending on the proposed route of administration was investigated. The demonstrated release of GCS from the film applied to the implant supports the expanded use of biodegradable surfaces and the improvement of pharmacotherapeutic approaches using glucocorticosteroids without increasing postoperative risks.

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About the authors:

Bannoud Georges — Veterinarian, Vetlife Veterinary Clinic, 12 Beskudnikovsky Blvd., bldg. 1, Moscow, 127474, Russian Federation; e-mail: george911@mail.ru

Ibragimova Alfiya Nailevna — Candidate of Pharmaceutical Sciences (PhD), Associate Professor, Department of Disaster Medicine, Medical Institute, RUDN University, 6 Miklukho-Maklaya St., Moscow, 117198, Russian Federation; e-mail: Ibragimova-an@rudn.ru
ORCID: 0000-0003-3484-3949 SPIN-code: 3948-5218

Mas-Coma Santiago — Doctor of Veterinary Sciences, Professor, Director of the Department of Veterinary and Animal Science, University of Valencia, Av. de Vicent Andrés Estellés s/n 46100, Burjassot, Valencia, Spain; e-mail: S. Mas.Coma@uv.es
ORCID: 0000-0002-1685-7004

Troshina Natalya Igorevna — Senior Lecturer, Department of Veterinary Medicine, Agrarian and Technological Institute, RUDN University, 6 Miklukho-Maklaya St., Moscow, 117198, Russian Federation; e-mail: troshina-ni@rudn.ru
ORCID: 0009-0003-8230-0153 SPIN-code: 9355-7573

Karamyan Arfenya Semenovna — Doctor of Biological Sciences, Candidate of Veterinary Sciences (PhD), Associate Professor, Associate Professor of the Department of Veterinary Medicine, Agrarian and Technological Institute, RUDN University, 6 Miklukho-Maklaya St., Moscow, 117198, Russian Federation; e-mail: karamyan-as@rudn.ru
ORCID: 0000-0003-2112-673X SPIN-code: 5511-4446

Анализ фармакологической эффективности применения метилпреднизолона у животных при адресной доставке на биодеградируемых носителях

Ж. Баннуд[1], А.Н. Ибрагимова[2], С. Мас-Кома[3], Н.И. Трошина[2], А.С. Карамян[2] ✉

[1]Ветеринарная клиника «Ветлайф», *г. Москва, Российская Федерация*
[2]Российский университет дружбы народов, *г. Москва, Российская Федерация*
[3]Университет Валенсии, *г. Валенсия, Испания*
✉ Karamyan-as@rudn.ru

Аннотация. Применение глюкокортикостероидов (ГКС) в фармакотерапии животных позволяет снизить развитие воспаления, аллергических реакций и оказывает иммуносупрессивное действие. Адаптивные гормоны повышают устойчивость организма к стрессу. Широкое использование ГКС при хирургических вмешательствах, сопряженных с имплантацией, продиктовано снижением реакции окружающих тканей, послеоперационного отека, повышением приживаемости имплантов. Однако, пероральное применение ГКС часто сопряжено с низкой биодоступностью, а повышение дозы препаратов чревато тяжелым проявлением побочного действия. Разработка новых систем адресной доставки с использованием биодеградируемых носителей позволяет повысить биодоступность лекарственных средств, снизить побочное действие и повысить эффективность фармакотерапии. Выбирая адресную

доставку, важно оценить количественные параметры концентрации ГКС в плазме крови в зависимости от выбранного пути введения. Разработанная методика высокоэффективной жидкостной хроматографии с тандемной масс-спектрометрией (ВЭЖХ–МС/МС) позволяет определить самые низкие концентрации лекарственных средств, диапазон методики от 2 до 1000 нг/мл.

Ключевые слова: глюкокортикостероиды, система адресной доставки, ветеринария, фармакотерапия, высокоэффективная жидкостная хроматография, ВЭЖХ, тандемная масс-спектрометрия

Вклад авторов: Баннуд Ж. — отбор проб, пробоподготовка, поиск и сбор данных, написание статьи; Ибрагимова А.Н. — анализ полученной информации, обработка данных; Мас-Кома С. — дизайн исследования; Трошина Н.И. — обработка числовых данных; Карамян А.С. — написание статьи, общее руководство научным проектом.

Заявление о конфликте интересов. Авторы заявляют об отсутствии конфликта интересов. Лекарственный препарат метилпреднизолона был закуплен авторами за собственные средства для проведения сравнительного исследования. Все авторы одобрили окончательную версию статьи.

История статьи: поступила в редакцию 2 декабря 2025 г., принята к публикации 12 января 2026 г.

Для цитирования: *Баннуд Ж., Ибрагимова А.Н., Мас-Кома С., Трошина Н.И., Карамян А.С.* Анализ фармакологической эффективности применения метилпреднизолона у животных при адресной доставке на биодеградируемых носителях // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2026. Т. 21. № 1. С. 195–202. doi: 10.22363/2312-797X-2026-21-1-195-202 EDN: GGQBZO