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Original Article

TOLERABILITY OF EXCIPIENTS OF A VACCINE FORMULATION IN OUTBRED WHITE LABORATORY MICE

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Abstract. The present study evaluated the local and systemic tolerability of vaccine formulation components in the absence of antigen following intranasal and sublingual administration in outbred white laboratory mice. The investigated substances included excipients of the vaccine formulation mannitol and gelatin at various concentrations as well as phosphate-buffered saline and water for injection, which are used as technological components during the development of vaccine candidates. The control group consisted of intact animals.

Tolerability was assessed over a 10-day observation period based on clinical condition, body weight dynamics, and key blood biochemical parameters. All tested substances demonstrated good tolerability: no clinically significant local or systemic reactions were observed, and the evaluated biochemical parameters remained within reference ranges. Based on the overall assessment, mannitol at a concentration of 5% and gelatin at 0.5% were identified as optimal.

The obtained results indicate the safety of the investigated excipients and technological components and confirm their suitability for use at the preclinical stage of development of vector-based anti-brucellosis vaccine candidates utilizing an influenza virus platform.

Keywords:vaccine; vaccine formulation; excipients; gelatin; mannitol; tolerability; mice

Introduction

Excipients of vaccine formulations, including stabilizers, carriers, and buffering systems, play a critical role in ensuring the safety, stability, and reproducibility of vaccine products. The protein stabilizer gelatin is widely used to enhance the resistance of viral particles to temperature fluctuations and lyophilization processes. However, in certain cases, it has been associated with adverse reactions, necessitating a thorough evaluation of its tolerability [1,2]. Mannitol is commonly employed as a stabilizer and cryoprotectant, while phosphate-buffered saline (PBS) serves as a buffering medium that maintains physiological pH and osmolarity within the vaccine formulation [2].

Although data are available regarding the safety of these substances following parenteral administration, the literature lacks systematic evidence concerning their local and systemic tolerability after mucosal administration (intranasal and sublingual routes), particularly when administered in the absence of an antigenic component. This represents a significant knowledge gap, as the impact of excipient and technological components of vaccine formulations on the host organism following mucosal delivery remains insufficiently characterized. Such limitations hinder the scientific justification of their use at early stages of preclinical research.

This issue is especially relevant in the context of developing vector-based vaccines utilizing influenza virus platforms, for which mucosal routes of administration are considered promising in terms of inducing local immune responses. The practical importance of assessing excipient tolerability lies in enabling their safe incorporation into vaccine formulations for vector-based anti-brucellosis vaccine candidates derived from influenza virus platforms. Moreover, such evaluation reduces the risk of confounding interpretations of reactogenicity and immunogenicity data attributed to the vector constructs themselves during preclinical investigations [3,4].

The aim of the present study was to evaluate the local and systemic tolerability of vaccine excipients (gelatin and mannitol), as well as the buffering medium and technological solvent, following intranasal and sublingual administration in outbred white laboratory mice, based on body weight gain, clinical observations, and blood biochemical parameters.

Materials and Methods

A total of 110 outbred white laboratory mice aged 6-8 weeks were used in the study and allocated into 11 groups of 10 animals each. The study was conducted as a controlled preclinical experiment, with animals randomly assigned to experimental and control groups. The control group consisted of intact animals that did not receive any administration.

The tested substances included excipients of the vaccine formulation mannitol (3%, 5%, and 7%) and gelatin (0.3%, 0.5%, and 0.7%) as well as phosphate-buffered saline (PBS; 0.01 M, pH 7.2-7.4) and water for injection, which was used as a technological solvent in the preparation of vaccine candidates. The substances were administered once via the sublingual and intranasal routes. Animals were monitored for 10 days following administration.

The following parameters were assessed: body weight gain (Day 0 and Day 10), local tolerability, and systemic clinical signs (including activity, appetite, and general condition), evaluated using a semi-quantitative scoring scale (0-3 points). In addition, key biochemical blood parameters were measured, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, total bilirubin, glucose, and total protein levels. Blood samples were collected from the tail vein on Day 10 of the experiment.

Statistical analysis was performed using descriptive statistical methods. Results are presented as mean ± standard deviation (M ± SD). Intergroup comparisons were conducted using parametric or nonparametric tests depending on data distribution. Differences were considered statistically significant at $p < 0.05$. Reference values were derived from the intact control group within the present study.

Results

All investigated components of the vaccine formulation including mannitol, gelatin, phosphate buffered saline, and water for injection demonstrated good tolerability in outbred white laboratory mice following both sublingual and intranasal administration. The obtained results are presented in Tables 1-4.

Following sublingual administration, physiological body weight gain was observed in all groups. No local reactions at the mucosal site or systemic clinical manifestations were detected.

After intranasal administration, mild transient local reactions characterized by slight mucosal hyperemia were observed in the groups receiving gelatin, whereas the remaining components were well tolerated without signs of irritation or systemic effects.

Biochemical blood parameters including alanine aminotransferase, aspartate aminotransferase, total bilirubin, glucose, and total protein remained within the reference values obtained from intact control animals in all groups. These findings indicate the absence of hepatic dysfunction or disturbances in general metabolic status.

Formal statistical comparisons between groups were not performed, as the objective of the study was descriptive evaluation of tolerability. The limited sample size and the nature of the data did not allow the application of parametric statistical methods.

Analytical conclusion. Based on the overall assessment of clinical observations, body weight dynamics, and biochemical parameters, mannitol at a concentration of 5% and gelatin at 0.5 %were identified as optimal for further investigations. The mild local reactions observed after intranasal administration of gelatin were not clinically significant and did not affect the overall tolerability profile.

Table 1- Body weight, weight gain, and tolerability following sublingual administration

Component	Concentration (%)	Number of animals	Body weight Day 0 g mean ± SD	Body weight Day 10 g mean ± SD	Weight gain g mean ± SD	Weight gain % mean ± SD	Local tolerability score	Systemic clinical score
Mannitol	3	10	20,3 ± 0,2	21,8 ± 0,3	1,5 ± 0,3	7,4 ± 1,5	0	0
Mannitol	5	10	20,4 ± 0,2	22,1 ± 0,2	1,7 ± 0,3	8,3 ± 1,6	0	0
Mannitol	7	10	20,5 ± 0,2	22,3 ± 0,3	1,8 ± 0,3	8,8 ± 1,7	0	0
Gelatin	0,3	10	19,2 ± 0,2	21,6 ± 0,3	2,4 ± 0,4	12,5 ± 2,1	0	0

Gelatin	0,5	10	19,3 ± 0,2	21,9 ± 0,3	2,6 ± 0,4	13,5 ± 2,4	0	0
Gelatin	0,7	10	19,1 ± 0,2	21,8 ± 0,4	2,7 ± 0,5	14,1 ± 2,7	0	0
Phosphate buffered saline 0.01 M pH 7.2 -7.4	-	10	19,2 ± 0,1	20,9 ± 0,2	1,7 ± 0,2	8,9 ± 5,5	0	0
Water for injection	-	10	18,4 ± 0,3	20,1 ± 0,4	1,7 ± 0,6	9,5 ± 3,2	0	0
Control	-	10	19,2 ± 0,7	21,9 ± 0,3	2,7 ± 0,6	14,0 ± 3,8	-	0
A score of 0 indicates absence of local reactions including hyperemia, edema, or mucosal erosion and absence of systemic clinical manifestations including depression, reduced appetite, or behavioral changes. Scores from 1-3 indicate increasing severity of the reaction.								

Table 1 presents body weight dynamics, weight gain, and tolerability indicators of different concentrations of mannitol and gelatin, as well as phosphate buffered saline and water for injection, following sublingual administration. In all experimental groups, physiological body weight gain was observed throughout the 10 day observation period.

Local reactions at the level of the oral mucosa were absent or minimal, and no systemic clinical manifestations were recorded.

These findings indicate good local mucosal and systemic tolerability of all tested substances at the selected concentrations and route of administration.

Table 2- Body weight, weight gain, and tolerability following intranasal administration

Component	Concentration (%)	Number of animals	Body weight Day 0 g mean ± SD	Body weight Day 10 g mean ± SD	Weight gain g mean ± SD	Weight gain % mean ± SD	Local tolerability score	Systemic clinical score
Mannitol	3	10	20,4 ± 0,2	22,0 ± 0,3	1,6 ± 0,3	7,9 ± 1,5	0	0
Mannitol	5	10	20,5 ± 0,2	22,1 ± 0,3	1,6 ± 0,3	7,9 ± 1,5	0	0
Mannitol	7	10	20,6 ± 0,3	22,2 ± 0,3	1,6 ± 0,3	7,8 ± 1,6	0	0
Gelatin	0,3	10	19,2 ± 0,1	21,8 ± 0,7	2,6 ± 0,7	13,7 ± 3,7	1	0
Gelatin	0,5	10	19,2 ± 0,1	21,9 ± 0,6	2,7 ± 0,6	14,0 ± 3,5	1	0
Gelatin	0,7	10	19,3 ± 0,2	22,0 ± 0,7	2,7 ± 0,7	14,0 ± 3,6	1	0
Phosphate buffered saline 0.01 M pH 7.2-7.4	-	10	19,3 ± 0,1	21,9 ± 0,2	2,6 ± 0,2	13,6 ± 0,9	0	0
Water for injection	-	10	21,3 ± 0,2	22,5 ± 0,3	1,2 ± 0,3	5,8 ± 1,6	0	0
Control	-	10	19,2 ± 0,7	21,9 ± 0,3	2,7 ± 0,7	14,0 ± 3,8	-	0
A score of 0 indicates absence of local reactions including hyperemia, edema, or mucosal erosion and absence of systemic clinical manifestations including depression, reduced appetite, or behavioral changes. Scores from 1-3 indicate increasing severity of the reaction.								

Table 2 presents body weight dynamics, weight gain, and tolerability indicators of the investigated substances following intranasal administration. In all experimental groups, physiological body weight gain was observed throughout the 10 day observation period.

Mild local reactions at the level of the nasal mucosa were recorded only in individual animals in the groups receiving gelatin and were transient in nature. No systemic clinical manifestations were observed in any of the groups.

These findings indicate good local mucosal and systemic tolerability of the investigated components and technological media following intranasal administration.

Table 3- Biochemical blood parameters following sublingual administration

Component	Concentration (%)	Number of animals	ALT (U/L) M±SD	AST (U/L) M±SD	Total bilirubin (mg/dL) M±SD	Glucose (mmol/L) M±SD	Total protein (g/L) M±SD
Mannitol	3	10	45 ± 3	85 ± 5	0,12 ± 0,01	15,2 ± 1,0	52 ± 2
Mannitol	5	10	46 ± 4	87 ± 4	0,13 ± 0,02	15,5 ± 0,9	53 ± 2
Mannitol	7	10	47 ± 4	88 ± 6	0,12 ± 0,02	15,3 ± 1,1	52 ± 3
Gelatin	0,3	10	48 ± 3	90 ± 5	0,13 ± 0,01	15,6 ± 1,2	54 ± 2
Gelatin	0,5	10	46 ± 4	88 ± 5	0,12 ± 0,01	15,4 ± 1,0	53 ± 2
Gelatin	0,7	10	47 ± 5	89 ± 6	0,13 ± 0,02	15,5 ± 1,1	53 ± 3
Phosphate buffered saline 0.01 M pH 7.2-7.4	-	10	46 ± 3	87 ± 4	0,12 ± 0,01	15,4 ± 0,9	53 ± 2
Water for injection	-	10	45 ± 3	85 ± 5	0,12 ± 0,01	15,3 ± 1,0	52 ± 2
Control	-	10	46 ± 4	86 ± 5	0,12 ± 0,01	15,5 ± 1,0	53 ± 2

Table 3 presents the biochemical blood parameters of laboratory mice following sublingual administration of all investigated substances. The values of alanine aminotransferase activity, aspartate aminotransferase activity, total bilirubin, glucose, and total protein in all experimental groups remained within the reference ranges typical for laboratory mice.These findings indicate the absence of impaired liver function or disturbances in general metabolism and confirm good systemic tolerability of the investigated excipients, buffering medium, and technological solvent following sublingual administration.

Table 4- Biochemical blood parameters following intranasal administration

Component	Concentration (%)	Number of animals	ALT (U/L) M±SD	AST (U/L) M±SD	Total bilirubin (mg/dL) M±SD	Glucose (mmol/L) M±SD	Total protein (g/L) M±SD
Mannitol	3	10	46 ± 3	86 ± 5	0,13 ± 0,01	15,3 ± 1,0	52 ± 2
Mannitol	5	10	47 ± 4	88 ± 4	0,14 ± 0,01	15,6 ± 0,9	53 ± 2
Mannitol	7	10	48 ± 4	89 ± 6	0,13 ± 0,02	15,4 ± 1,1	52 ± 3
Gelatin	0,3	10	49 ± 3	91 ± 5	0,14 ± 0,01	15,7 ± 1,2	54 ± 2
Gelatin	0,5	10	47 ± 4	89 ± 5	0,13 ± 0,01	15,5 ± 1,0	53 ± 2
Gelatin	0,7	10	48 ± 5	90 ± 6	0,14 ± 0,02	15,6 ± 1,1	53 ± 3
Phosphate buffered saline 0.01 M pH 7.2-7.4	-	10	47 ± 3	88 ± 4	0,13 ± 0,01	15,5 ± 0,9	53 ± 2
Water for injection	-	10	46 ± 3	86 ± 5	0,13 ± 0,01	15,4 ± 1,0	52 ± 2
Control	-	10	47 ± 4	87 ± 5	0,13 ± 0,01	15,6 ± 1,0	53 ± 2

Table 4 presents the biochemical blood parameters of laboratory mice following intranasal administration of the investigated substances. In all experimental groups, the values of alanine aminotransferase activity, aspartate aminotransferase activity, total bilirubin, glucose, and total protein remained within the reference ranges typical for laboratory mice.

These findings indicate the absence of impaired liver function or disturbances in general metabolism and confirm good systemic tolerability of the investigated excipients, buffering medium, and technological solvent following intranasal administration.

Discussion

The results of the experiment demonstrate good local and systemic tolerability of the investigated components of the vaccine formulation including mannitol, gelatin, phosphate buffered saline, and water for injection following sublingual and intranasal administration in outbred white laboratory mice. Body weight gain remained within physiological limits, local reactions were minimal and transient, and no systemic clinical manifestations were observed. Biochemical blood parameters including alanine aminotransferase, aspartate aminotransferase, total bilirubin, glucose, and total protein corresponded to the control values of intact animals, indicating the absence of liver dysfunction or disturbances in general metabolism.

The mild local reactions observed after intranasal administration of gelatin are consistent with previously published studies [1-4], which describe the potential irritative activity of protein stabilizers following mucosal administration. The investigated components were administered once, the observation period lasted 10 days, and no morphological analysis of mucosal tissues was performed.

Despite these limitations, the findings confirm the safety of the investigated components and technological media at the stage of preclinical evaluation of vector vaccines based on an influenza virus platform.

Conclusion. The excipients of the vaccine formulation including mannitol and gelatin, as well as the buffering medium and technological solvent, demonstrate good local and systemic tolerability following sublingual and intranasal administration. Body weight gain remains within physiological limits, local reactions are minimal and transient, and systemic clinical manifestations are absent.

Based on the overall assessment, mannitol at a concentration of 5% and gelatin at 0.5% were identified as optimal for further investigations. The next stages of preclinical development include repeated administration, incorporation of the antigen component, and histological examination.

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ВАКЦИНАЛЫҚ ПРЕПАРАТТЫҢ ҚОСАЛҚЫ КОМПОНЕНТТЕРІНІҢ ЗЕРТХАНАЛЫҚ АҚ ТЫШҚАНДАРДАҒЫ ТӨЗІМДІЛІГІ

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Аннотация. Бұл зерттеуде антигендік компонентсіз вакциналық композицияның құрамдас бөліктерінің интраназальды және сублингвальды енгізілуі кезінде зертханалық ақ тышқандарда

жергілікті және жүйелік төзімділігі бағаланды. Зерттеу нысандары ретінде вакциналық композицияның қосалқы компоненттері - әртүрлі концентрацияларда маннитол мен желатин, сондай-ақ вакциналық кандидаттарды әзірлеуде технологиялық компоненттер ретінде қолданылатын фосфатты-буферлі ерітінді және инъекциялық су пайдаланылды. Бақылау тобына интактты жануарлар енгізілді.

Төзімділігі 10 тәулік бойы жануарлардың клиникалық жағдайы, дене салмағының динамикасы және қанның негізгі биохимиялық көрсеткіштері бойынша бағаланды. Барлық зерттелген заттар жақсы тзімділік көрсетті: клиникалық мәні бар жергілікті немесе жүйелік жағымсыз реакциялар байқалмады, ал зерттелген биохимиялық көрсеткіштер референттік мәндер шегінде қалды. Параметрлердің жиынтық бағасы бойынша маннитолдың 5 % және желатиннің 0,5 % концентрациялары оңтайлы деп анықталды.Алынған нәтижелер зерттелген қосалқы және технологиялық компоненттердің қауіпсіздігін көрсетеді және оларды тұмау вирусы негізіндегі векторлық бруцеллезге қарсы вакциналар-кандидаттарының вакциналық композицияларын әзірлеудің доклиникалық кезеңінде қолдану мүмкіндігін негіздейді.

Түйінді сөздер: вакцина, вакциналық композиция, қосалқы компоненттер, желатин, маннитол, төзімділік, тышқандар.

ПЕРЕНОСИМОСТЬ ВСПОМОГАТЕЛЬНЫХ КОМПОНЕНТОВ ВАКЦИННОГО ПРЕПАРАТА У ЛАБОРАТОРНЫХ БЕЛЫХ БЕСПОРОДНЫХ МЫШЕЙ

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Аннотация. В настоящем исследовании проведена оценка местной и системной переносимости компонентов вакцинной композиции в отсутствие антигенного компонента при их интраназальном и сублингвальном введении у белых беспородных лабораторных мышей. В качестве объектов исследования использовали вспомогательные компоненты вакцинной композиции - маннитол и желатин в различных концентрациях, а также фосфатно-буферный раствор и инъекционную воду, применяемые в качестве технологических компонентов при разработке вакцинных кандидатов. Контрольную группу составляли интактные животные.

Переносимость оценивали в течение 10 суток по показателям клинического состояния животных, динамики массы тела и основным биохимическим параметрам крови. Установлено, что все исследованные вещества характеризовались хорошей переносимостью: клинически значимые местные и системные реакции отсутствовали, а исследуемые биохимические показатели крови не выходили за пределы референтных значений. По совокупности оцениваемых параметров оптимальными были признаны маннитол в концентрации 5 % и желатин в концентрации 0,5 %.Полученные результаты свидетельствуют о безопасности исследованных вспомогательных и технологических компонентов и подтверждают возможность их применения на доклиническом этапе разработки вакцинных композиций векторных противобруцеллезных вакцин-кандидатов на основе вируса гриппа.

Ключевые слова: вакцина, вакцинная композиция, вспомогательные компоненты, желатин, маннитол, переносимость, мыши.