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Safety pharmacology of human endogenous retrovirus-enveloped baculoviral DNA vaccines against SARS-CoV-2 in Sprague-Dawley rats and beagle dogs

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ABSTRACT

The coronavirus disease 2019 (COVID-19) emerged as a major global health crisis, posing significant health, economic, and social challenges. Vaccine development has been a crucial response to the severe-acute-respiratory-syndrome-related coronavirus-2 pandemic owing to the critical role of immunization in controlling infectious diseases, leading to the expedited development of several effective vaccines. Although mRNA platform-based COVID-19 vaccines authorized under emergency-use authorization have been administered globally, concerns regarding the vaccines have increased owing to the occurrence of various side effects. The present study aimed to evaluate the safety of a non-replicating recombinant baculovirus expressing the human endogenous retrovirus envelope gene (AcHERV) vaccine encoding SARS-CoV-2 antigens. Owing to the limited number of existing safety pharmacology studies on AcHERV as a viral vector vaccine, we conducted neuro-behavior (Modified Irwin's Test), body temperature, and respiratory function studies in rats and cardiovascular system studies in male beagle dogs, which were administered the AcHERV-COVID-19 vaccine using telemetry. The safety assessment revealed no significant toxicological alterations. However, in rats, both sexes administered with the AcHERV-COVID-19 vaccine exhibited a temporary increase in body temperature, which normalized or showed signs of recovery. In conclusion, AcHERV-COVID-19 demonstrates a sufficient safety profile that supports its potential evaluation in future clinical trials.

1. Introduction

Coronavirus disease (COVID-19), first reported in Wuhan, China, in December 2019, has rapidly emerged as a major global health crisis, posing significant health, economic, and social challenges worldwide [1]. COVID-19, initially recognized for its respiratory symptoms, such as pneumonia and acute respiratory distress syndrome, can also affect multiple organs and systems, extending beyond the lungs to impact the heart, various organs, blood vessels, and the central nervous system [2]. Vaccine development has been a key response to the severe-acute-

respiratory-syndrome-related coronavirus-2 (SARS-CoV-2) pandemic, leading to the expedited establishment of several effective vaccines [3,4]. This rapid progress in vaccine technology signifies a major stride in public health efforts to mitigate the impact of COVID-19 and demonstrates the critical role of immunization in controlling infectious diseases [5–7]. However, the challenges in the adoption of COVID-19 vaccines are partly due to concerns over rare but serious adverse events, such as vaccine-induced myocarditis and thrombosis [8–10]. Notably, myocarditis, particularly associated with mRNA vaccines, such as Pfizer-BioNTech and Moderna, has been reported in various studies

[11–13].

The development of vaccines against COVID-19 involves various approaches, including the use of viral vector platforms [14,15]. These platforms use different types of viruses as vectors to deliver genetic material into human cells. The Oxford-AstraZeneca COVID-19 vaccine, one of the most widely administered vaccines in the world, is engineered to harbor a DNA sequence that encodes the spike (S) protein of SARS-CoV-2 [16]. Recently, baculoviruses of the family Baculoviridae have been developed as viral vector vaccines. These vaccines comprise recombinant baculoviruses expressing the envelope glycoprotein of human endogenous retrovirus (HERV) [17,18]. Compared to subunit vaccines, which contain only a part of the pathogen, such as a protein or sugar, live vector vaccines, such as those using baculoviruses, tend to exhibit better immunogenicity. Hence, live vector vaccines are more effective in stimulating the immune system to produce a strong and long-lasting response [19]. In a previous study, a non-replicating recombinant baculovirus expressing the HERV envelope gene (AcHERV) was engineered as a DNA vaccine vector for efficient gene delivery into human cells. This vaccine, named AcHERV-COVID19-S, demonstrated significant efficacy, providing remarkable protection against SARS-CoV-2 [20].

Safety pharmacology focuses on understanding the risks and benefits of pharmaceuticals, particularly concerning rare lethal events. Unlike toxicology, it specifically predicts the risk of adverse effects on vital functions [21]. In terms of vaccine safety, it assesses the effects beyond the immune system, including effects on the nervous, respiratory, and cardiovascular systems. Given the limited existing safety pharmacology studies on the AcHERV viral vector vaccine, in the present study, we aimed to conduct neurobehavior (Modified Irwin's Test), body temperature, and respiratory function studies in rats and studies examining the effects on the cardiovascular system in male beagle dogs administered the AcHERV-COVID19 vaccine using telemetry. To the best of our knowledge, this is the first report of a safety pharmacology study of a vaccine using the AcHERV platform.

2. Materials and methods

2.1. Ethics statement

Animal facilities were approved by the American Association for Accreditation of Laboratory Animal Care International. All procedures for the animal vaccination studies were approved by the Institutional Animal Care and Use Committee of the Korea Institute of Toxicology [IACUC Approval No.: 2107–0006 (neurobehavior and body temperature study in rats), 2107—0007 (respiratory function study in rats), and 2106–0022 (cardiovascular function study in dogs)]. All experiments were conducted in accordance with the guidelines of the Korea Ministry of Food and Drug Safety Notification No. 2018–93 Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, and the US Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies 21 CFR Part 58.

2.2. Vaccine development

The AcHERV-COVID-19 vaccine was developed and provided by KR Biotech Co. (Seoul, Republic of Korea), as previously reported [20]. Briefly, a recombinant baculoviral vector, pFastBac1-HERV, was created by integrating a synthetic codon-optimized envelope gene of HERV type W (GenBank accession number NM014590; GenScript, Piscataway, NJ, USA) into the pFastBac1 vector (Invitrogen, Carlsbad, CA, USA). To construct recombinant AcHERVs that encode SARS-CoV2 antigens (AcHERV-COVID-19), a codon-optimized SARS-CoV2 *S* gene, designed for enhanced expression in mammalian cells, was synthesized by GeneArt (Regensburg, Germany). Subsequently, the full-length *S* gene

was individually cloned into pcDNA3.1 (+) plasmids comprising a CMV promoter (Invitrogen). AcHERV-GFP was developed as a vehicle control by incorporating green fluorescent protein (GFP) into a recombinant baculoviral vector. Distilled water (DW) was used as the negative control.

2.3. Animal housing

Specific pathogen-free (SPF) Sprague-Dawley (SD) rats were obtained from Orient Bio, Inc. (Seongnam-si, Republic of Korea). Beagle dogs, approximately ten months old, were obtained from Beijing Marshall, China. All animals were observed daily for clinical signs during the pre-administration period, and the health status of the beagle dogs was assessed by performing hematological tests and urinalysis. After transplantation of the telemetry system into the dogs, maintenance tests were conducted to identify and select healthy animals that exhibited stable blood pressure, heart rate, and electrocardiogram (ECG) readings. Subsequently, the animals were randomly assigned to treatment groups in a stratified manner using the Pristima system (Version 7.4, Xybion Medical System Co., Princeton, NJ, USA) based on their most recent body weights. The housing conditions for the animals were consistent with those described previously [22–24].

2.4. Neurobehavior and body temperature study in SD rats

Neurobehavioral evaluations were conducted in accordance with the ICH S7A safety pharmacology guidelines (established by the US Food and Drug Administration in 2001), adapting the methods previously described by Irwin in 1968, with certain modifications [25]. Sixty-four SPF SD rats with an approximate age of five weeks were obtained from Orient Bio, Inc. (Seongnam-si, Republic of Korea). The rats were randomly assigned to four groups and were separated based on sex (eight animals per group): negative control (DW), vehicle control (AcHERV-GFP), and low-, and high-dose test items (AcHERV-COVID19) of 1.0×10^8 and 1.0×10^9 FFU/animal, respectively. The clinical dose of the test item in humans is approximately 1×10^9 FFU/human (1.67×10^7 FFU/kg, 3.34×10^6 FFU/animal based on approximately 200 g rats). Based on this, 1×10^9 FFU/animal was set as the high dose, which was approximately 300 times the intended clinical dose, and 1×10^8 FFU/animal was set as the low dose by applying a power factor of 10. The animals were administered the vaccine once intramuscularly to the femoral muscle in the left and right hind limbs at a volume of 250 μL/site (total 500 μL/animal). Neurobehavioral observations, including death, startle reflex, locomotion, respiratory rate, tail elevation, eyelid reflex, piloerection, exophthalmos, righting reflex, pinna reflex, abdominal tone, skin pigmentation, salivation, lacrimation, tremor, convulsion, diarrhea, catalepsy, and traction, were performed before dosing (0) and at 1, 2, 4, 6, and 24 h (±10 %) after dosing. Rectal body temperature measurements were obtained concurrently with the neurobehavioral assessments. All observation parameters were evaluated according to standard operating procedures, and the degree and judgment of the observations were recorded. After completion of the study, all animals were euthanized using CO_2 inhalation.

2.5. Examination of the respiratory function in SD rats

Respiration was evaluated in compliance with the ICH S7A safety pharmacology guidelines established by the FDA in 2001. A total of 32 male SPF SD rats were randomly assigned to four groups (n = 8/group) and administered the vaccine once, as described in the previous section. Respiratory parameters, including respiratory rate, tidal volume, and minute volume, were measured using a respiration analyzer (unrestrained whole-body plethysmograph system; BUXCO Electronics Inc., Wilmington, NC, USA). The animals were allowed a minimum of 30 min of stabilization before commencing data collection in the chamber. Respiratory parameters were recorded for 10 min at each time point

(prior to dosing (0), at 1, 2, 4, 6, and 24 h after dosing, with a tolerance of ± 10 %). After completing the measurements at the 6-h time point, the animals were removed from the chamber and provided *ad libitum* access to the pellet food and water. Subsequently, they were reintroduced to the chamber for another stabilization period of 30 min before recording the respiratory parameters after 24 h of dosing. Data were collected and stored using the BioSystem XA program (version 2.11.2; BUXCO Electronics Inc.) and saved as BioSystem files. The program calculates the average value over 10 min for each time point. Finally, all animals were euthanized using CO_2.

2.6. Examination of the cardiovascular function in beagle dogs

Cardiovascular function was evaluated based on previously established methods in accordance with the ICH safety pharmacology guidelines (ICH 2009) [26–28]. Telemetry transmitters (Data Sciences International, St. Paul, MN, USA) were implanted into four dogs to monitor their cardiovascular function remotely. All dogs were administered 500 μL of DW, AcHERV-GFP, and AcHERV-COVID19 at a dose of 1.0×10^9 PFU/animal and were observed for washout periods of 5, 7, and 28 days. Subsequent administrations were performed according to the schedule described in Supplementary Table S1. Blood pressure (including diastolic, systolic, and mean blood pressure), heart rate, and lead II ECG signals from the implanted transmitter in the dogs were continuously monitored via telemetry 1 and 24 h after each administration. NOTOCORD-hem software (version 4.2, NOTOCORD, Paris, France) was used to analyze the cardiovascular parameters. Data points were extracted at specific time intervals, including pre-administration and at 4 (with a tolerance of ± 10 %) and 24 h (with a tolerance of ± 1h) after administration. These data points represent the average values over one-minute intervals at each specified time slot. Data extraction and calculations were performed using NOTOCORD-HEM. The parameters concerning blood pressure and heart rate were derived using the APR module in the NOTOCORD-hem program. Similarly, parameters associated with the ECG data were acquired using the VME module of the NOTOCORD-hem program. The body temperature data were retrieved and processed using the NOTOCORD-hem program. Metrics, such as diastolic blood pressure, systolic blood pressure, mean blood pressure, and heart rate, were measured or computed using the blood pressure data. The parameters obtained from ECG lead II included the PR, QRS, RR, QT, and QTc intervals. The QTc interval, which was used to adjust for heart rate changes, was automatically calculated using Van de Water's formula (QTcV) within the NOTOCORD-hem program. The ECG waveforms of each animal were assessed based on 10 continuous waveforms at each time point.

2.7. Statistical analyses

Statistical analyses (SAS/STAT Version 9.4, USA) were performed to compare the item-treated and control groups. Data were initially assessed for homogeneity of variance using Bartlett's test. For homogeneous data, analysis of variance (ANOVA) was used to evaluate intergroup differences, and significance analysis was conducted using Dunnett's test. When data heterogeneity was observed, the Kruskal–Wallis test was employed, and intergroup differences between the control and treated groups were assessed using Dunns rank-sum test. For comparisons between two groups, a homogeneity of variance check was performed using the *F*-test. In cases of homogeneous data, the Student's *t*-test was employed, whereas the Wilcoxon rank-sum test was used for data with heterogeneity. One-way ANOVA was utilized for comparisons between the control groups and the test substance administration group, with a significance level of $p < 0.05$ or $p < 0.01$.

3. Results

3.1. Neurobehavior (Modified Irwin's Test) and body temperature study in SD rats

No abnormal neurobehaviors were observed in any of the groups at any time point (Supplementary Table S2). In the group administered a high dose of the AcHERV-COVID-19 vaccine, a significant increase in body temperature was observed at 4 and 6 h after administration in both sexes compared to that of the negative or vehicle control (Fig. 1). Subsequently, the males recovered to normal temperatures, whereas the females did not exhibit a complete recovery but showed a tendency toward normalization.

3.2. Examination of respiratory function in SD rats

The respiratory rate was significantly decreased in the vehicle control group (127.89 ± 10.56 BPM) compared to that in the negative control group (196.23 ± 62.28 BPM) 6 h after administration. No differences were observed between the negative control and vehicle control groups at the other measurement times. No statistically significant changes were observed between the test item, negative control substance, and vehicle control substance administration groups at any time point (Fig. 2a). Tidal volume was significantly decreased in the vehicle control group (1.06 ± 0.09 mL) compared to that in the negative control group (1.17 ± 0.09 mL) 1 h after administration. No differences were observed between the negative control and vehicle control groups at other measurement time points. No significant changes were observed between the test item, negative control substance, and vehicle control substance administration groups at any time point (Fig. 2b). Minute respiratory volume was significantly decreased in the vehicle control group (142.57 ± 16.85 mL/min) compared to that in the negative control group (216.34 ± 62.57 mL/min) 6 h after administration. No differences were observed between the negative control and vehicle control groups at other measurement time points. No statistically significant changes were observed between the test item, negative control substance, and vehicle control substance administration groups at any time point (Fig. 2c).

3.3. Telemetry evaluation of cardiovascular function in male beagle dogs

Systolic blood pressure, diastolic blood pressure, and mean blood pressure showed no significant differences at each time period in each test group administered the test item once or twice compared with those at the same time periods in the negative and vehicle control groups (Fig. 3a-c). Heart rate measurements at all time points exhibited no significant differences in each test group administered the test item once or twice compared with that at the same time periods in the negative and vehicle control groups (Fig. 3d).

ECG measurements displayed no significant changes in the PR, QRS, RR, QT, and QTcV intervals at each time point in each test group administered the test item once or twice compared to those at the same time point in the negative and vehicle control groups (Fig. 4). No abnormal findings, such as abnormal ECG waveforms or arrhythmia, were observed at any experimental time point in the animals administered the test item once or twice, nor in the negative and vehicle control groups (Supplementary Table S3).

4. Discussion

The present study investigated the effects of AcHERV on pharmacological safety parameters in SD rats and beagle dogs. This investigation included neurobehavioral observations and analysis of body temperature, respiratory function, and cardiovascular parameters.

Notably, no neurobehavior changes due to the test item were observed in any administration group; however, both sexes in the high-
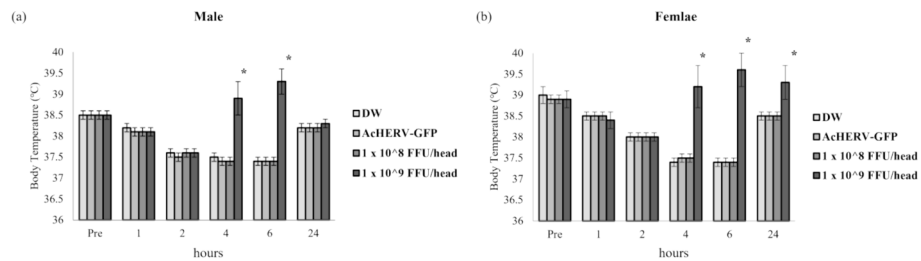
Fig. 1. Effects of AcHERV on body temperature in rats. Bar graph illustrating the data, with error bars representing mean ± standard deviation. (a) Males. (b) Females. * Significantly different from the negative control and vehicle control at $p < 0.01$.
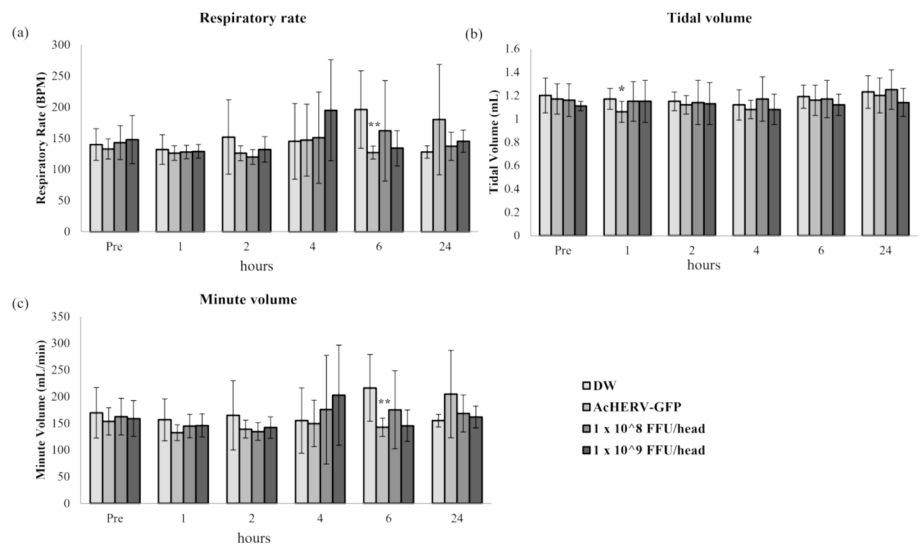

Fig. 2. Effects of AcHERV on respiratory function in rats. Bar graph illustrating the data, with error bars representing mean ± standard deviation. (a) Respiratory rate. (b) Tidal volume. (c) Minute volume. * Significantly different from the negative control at $p < 0.05$; # significantly different from the negative control at $p < 0.01$.
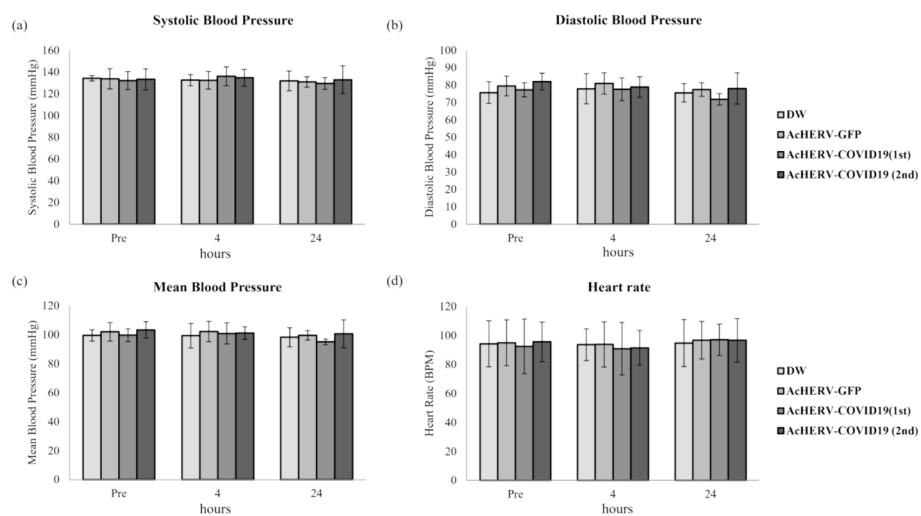

Fig. 3. Effects of AcHERV on blood pressure and heart rate in beagle dogs. Bar graph illustrating the data, with error bars representing means ± standard deviation. (a) Systolic blood pressure. (b) Diastolic blood pressure. (c) Mean blood pressure. (d) Heart rate.

dose group of the test item exhibited a temporary increase in body temperature, attributed to the effect of the test item. An increase in body temperature following vaccination is a frequent adverse reaction, with most reported vaccine-associated reactions being non-serious and often not immunologically mediated or reproducible upon re-exposure [29–31]. Most acute-onset reactions are attributed to type I

hypersensitivity reactions involving preformed immunoglobulin E antibodies against a component of the vaccine and normally occur within 4 h [30]. However, these reactions can occasionally escalate to serious anaphylactic responses, and acute reactions, such as febrile seizures, are common and serious, especially in children [32,33]. In the present study, body temperature slightly and temporarily increased 4 h after
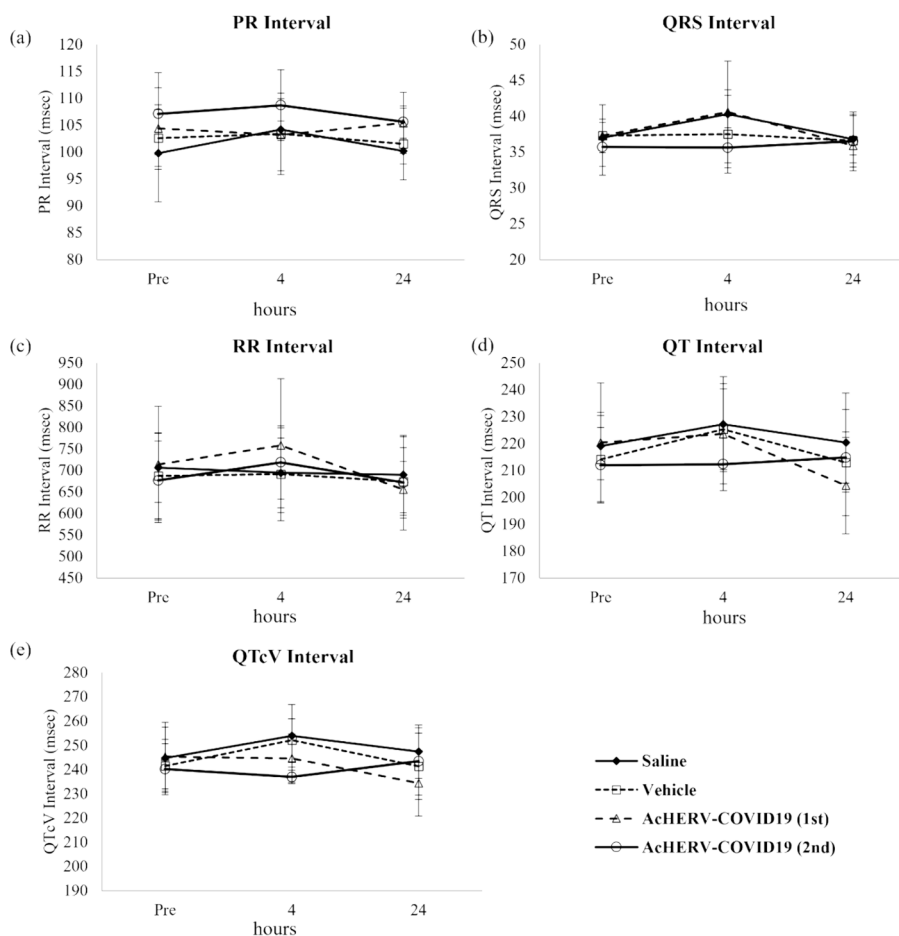
Fig. 4. Effects of AcHERV on ECG parameters in beagle dogs measured using telemetry. Bar graph illustrating telemetry data, with error bars representing mean ± standard deviation. (a) PR interval. (b) QRS interval. (c) RR interval. (d) QT interval. (e) QTcV interval.

vaccine administration and recovered or showed a tendency to recover within 24 h. However, because vaccinated rats were aged 5–6 weeks, evaluating the potential risks in neonates or infants would require a corresponding assessment of body temperature using an animal model that reflects the age group.

In rare instances following COVID-19 vaccination, a correlation has been observed between vaccine-induced immune responses and the onset of interstitial lung disease (ILD) [34–36]. Additionally, ILD occurs after vaccinations for other respiratory viral infections, such as influenza [37,38]. Although the precise mechanism is unclear, the immune response to vaccinations may occasionally trigger lung inflammation and ILD, potentially due to an overactive immune system or individual susceptibility [39]. Therefore, assessment of respiratory function is essential for evaluating vaccine safety. In the present study, tidal volume measurements exhibited a temporary decrease in the vehicle control group 1 h after administration. However, this was not consistent, as the values increased at 2 h and decreased again at 4 h, suggesting that the decrease cannot be attributed to the vehicle control substance. No significant differences were found between the test item groups and control groups at any time, indicating that the test item had no impact. Similarly, a temporary decrease in the respiratory rate and minute respiratory volume was noted in the vehicle control group at 6 h, likely due to a concurrent increase in the negative control group rather than in the vehicle control group. Overall, no changes associated with the test item were observed in the respiratory rate or minute respiratory volume at any time point.

Blood pressure measurements exhibited no significant differences in systolic, diastolic, or average blood pressure between the test groups administered the test item once or twice and the control groups.

Similarly, the heart rate measurements revealed no changes across these groups. ECG assessments also indicated no significant differences in the PR, QRS, RR, QT, and QTcV intervals, and no abnormal waveforms or arrhythmias were observed. These results suggest that the test items did not mediate changes in blood pressure, heart rate, or electrocardiographic parameters.

Adverse effects with suspected relation to COVID-19 vaccines after their global administration have been reported, particularly cardiovascular complications [40,41]. Increases in blood pressure after COVID-19 vaccination may be linked to the interaction of the S protein with angiotensin-converting enzyme 2 receptors, which disrupts the renin-angiotensin system and leads to an imbalance between Ang2 and Ang1-7, thereby affecting blood pressure regulation [41–43]. In this study, we did not observe adverse cardiovascular reactions during the evaluation of a vaccine containing a gene inserted to produce the S protein as an antigen. However, we used healthy and very young beagle dogs (ten months old), whereas the risks to the cardiovascular system following COVID-19 vaccination have been reported more in the older adult population [44,45]. Therefore, geriatric animal models may be needed to evaluate the risks in older adults, particularly for cardiovascular safety assessments.

In summary, the safety assessment of AcHERV-COVID-19, including evaluations of neurobehavioral, respiratory, and cardiovascular parameters, revealed no toxicologically significant alterations, with the notable exception of a temporary increase in body temperature observed in animal studies. This particular finding underscores the importance of establishing stringent monitoring protocols for adverse reactions in initial human trials, particularly for identifying optimal dosing that balances immunogenicity with minimal side effects. However, it is

important to acknowledge the limitations of this study. The assessments were conducted exclusively on young animals. Consequently, future studies should focus on examining the effects of vaccines in specific high-risk groups, particularly in neonatal or geriatric models. Nevertheless, despite the limitations of the design of our study, we demonstrated the safety profile of AcHERV-COVID-19, which supports its potential evaluation in future clinical trials.

Funding

Data statement

The data supporting the findings of this study are available from Konkuk University; however, restrictions apply to the availability of these data, which were used under license for the current study and hence are not publicly available. Data are, however, available from the authors upon reasonable request and with the permission of Konkuk University.

CRediT authorship contribution statement

Sang-Jin Park: Data curation, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Joung-Wook Seo:** Investigation. **Kang-Hyun Han:** Supervision. **Byoung-Seok Lee:** Supervision. **Chanyeong Lee:** Investigation. **Bong Young Kim:** Supervision. **Kyong-Cheol Ko:** Project administration. **Yong-Bum Kim:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvacx.2024.100545.

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