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Research Article

Single Dose Pharmacokinetic and Tissue Distribution of Recombinant Human Galectin-1 Buffered

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Abstract

Galectins are a family of evolutionarily conserved proteins distributed from lower invertebrates to mammals and found in several tissues and cells and they are cited in the literature as multifunctional molecules that participate in several biological processes such as adhesion, proliferation and cell cycle, apoptosis, RNA processing, control of the inflammatory processes, and physiological mechanisms of reproduction. One of them, the Galectin-1 is a 14kDa lectin, with high affinity to carbohydrates (beta-galactoside and lactose), expressed different tissues and with a considerable mRNA and protein expression in endometrium and placenta (trophoblasts, stromal cells, villous endothelium, syncytiotrophoblast membrane and villous stroma), and in fetal membranes (amnion, chorioamniotic mesenchyme and chorion), considering different mammals' species. GAL-1 is implicated to maternal-fetal tolerance, associated with regulating, and modulating the embryo elongation events', immunological responses and adherence to the endometrium, and it also contribute to placentation as they regulate the development, migration, and trophoblastic invasion, essential in early gestational development. GAL-1 has been cited as a mediator involved in preventing early embryonic death in mammals, and already used as an interesting tool to improvement of beef cattle cow's pregnancy rate using an effective dose of rHGAL-1, administrated into the uterus, during the AI procedures. Thinking in a future possibility of usage of rHGAL-1 administration in human reproduction procedures, we are spending efforts to comprehend the pharmacology of rHGAL-1, and this article is part of them. The objective of this study was to determine pharmacokinetic parameters of single dose rHGAL-1 following intravenous (I.V.), intraperitoneal (I.P.), and intrauterine (I.U.) routes of administration and to measure the levels of GAL-1 in uterus tissue homogenate after those administrations, both in female rats. As results, no clinical signs were observed in IV and IP groups after dosing; GAL-1 concentration was observed in plasma and uterus homogenate samples (independently of the route of administration). In plasma samples, higher Cmax (2.774 $ng.ml^{-1}$) was observed in I.V., higher $AUC_{0/t}$ (12.746 hr *$ng.ml^{-1}$) was observed in I.P., and higher Tmax (2.167 hr) was observed in I.U. After I.V. administration, the content of rHGAL-1 in uterus at 0.5h, 2.0h, 4.0h, 8.0h, 24.0h, 48.0h observed were 10.92, 15.25, 17.92, 12.48, 20.50, and 0.00 $ng.gm^{-1}$, respectively. At the same time after I.P. administration contents observed were 29.72, 15.40, 54.25, 23.53, 6.70, and 0.00 $ng.gm^{-1}$ and after I.U. administration contents observed were 128.22, 131.97, 134.43, 136.05, 94.40, and 0.00 $ng.gm^{-1}$.

Keywords: *Tolerana®; Pregnancy; Fetal-Maternal Recognition; Human Reproduction*

Abbreviations

AI: Artificial Insemination Procedure; GAL-1: Galectin-1; rHGAL-1: Human Recombinant Galectin-1; I.V.: Intravenous Route of Administration; I.P: Intraperitoneal Route of Administration; I.U.: Intrauterine Route of Administration; MFC: Maximum Feasible Concentration;

Cmax: Maximum (or Peak) Serum Concentration; Tmax: The amount of time that a drug is present at the maximum concentration in serum; T1/2: Half-life (t1/2) refers to the time taken for half the initial dose of medicine administered to be eliminated from the body; $AUC0_{0\text{-}t}$: The area under the plot of plasma concentration of a drug versus time after dosage (called "area under the curve" or AUC) gives insight into the extent of exposure to a drug and its clearance rate from the body, at time interval; $AUC0_{-\infty}$: Area under the concentration time-curves from time zero to infinity, which corresponds to a calculation of mean concentration levels of a therapeutic agent in the body See Pharmacokinetics; Vd: Volume of distribution (Vd) is a pharmacokinetic parameter representing an individual drug's propensity to either remain in the plasma or redistribute to other tissue compartments; CL: Clearance - in volume/unit time - of a drug or chemical from a body fluid, usually plasma or blood, by specified route(s) and mechanism(s) of elimination; w.r.t.: With reference to

Introduction

Galectins are a family of evolutionarily conserved proteins distributed from lower invertebrates to mammals [5,11], and found in several tissues and cells and they are cited in the literature as multifunctional molecules that participate in several biological processes such as adhesion, proliferation and cell cycle, apoptosis, RNA processing, control of the inflammatory processes, and physiological mechanisms of reproduction [1,2,6,10,12-17,19].

One of them, the Galectin-1 is a 14 kDa lectin, with high affinity to carbohydrates (beta-galactoside and lactose), expressed different tissues (cardiac, smooth, and skeletal muscle, neurons, thymus, kidney, hematopoietic cells) and with a considerable mRNA and protein expression in endometrium and placenta (in trophoblasts, stromal cells, villous endothelium, syncytiotrophoblast apical membrane and villous stroma, maternal decidua and fetal membranes), and in fetal membranes (amnion, chorioamniotic mesenchyma and chorion), considering different mammals species, including humans GAL-1 is implicated to maternal-fetal tolerance, both innate and adaptive, is associated with regulating and modulating the embryo elongation events' immunological responses and adherence to the endometrium, and it also contribute to placentation as they regulate the development, migration, and trophoblastic invasion, essential in early gestational development [1-4,7,8]. Because of that GAL-1 has been cited as a mediator involved in preventing early embryonic death in mammals, and already used as an interesting tool to improvement of beef cattle cow's pregnancy rate using an effective dose of exogenous Galectin-1 (compound by human recombinant GAL-1 - Tolerana®) when administrated into the lumen uterus of cows during the artificial insemination procedures, similarly a semen dose administration [12]. There are so many particularities between placentation and embryo elongation of different mammals' species but, based on Cummings and Liu (2009) [5], Modenutti., *et al*. (2019) [11] and Than., *et al*. (2008) [20], that described that GAL-1 has a high degree of structural conservation, dimerization, and binding properties with carbohydrates and integrins (adhesion proteins), suggesting that these properties are conserved among vertebrates and that they maintain a pattern of gene expression among the different types of the placenta (deciduous or not), our researches were based on rHGAL-1.

Thinking in a future possibility of usage the exogenous rHGAL-1 administration in human reproduction procedures (artificial insemination, embryo transfer ex.), we are spending a lot of efforts in studies to comprehend pharmacokinetics, toxicology, and pharmacological security, all based on ICH Guideline S6R1 (2011) [9], and this article is part of them.

Objective of the Study

The objective of the study was to determine pharmacokinetic parameters of single dose rHGAL-1 following intravenous (I.V.), intraperitoneal (I.P.), and intrauterine (I.U.) routes of administration in female Wistar rats and also, to measure the levels of GAL-1 in uterus tissue homogenate after those administrations.

Materials and Methods

rHGAL-1 production and purification: The method for obtaining rHGAL-1 is determined by the manufacturing process of Tolerana® (Inprenha Biotecnologia®) and completely described on Morani., *et al*. (2021) [12]. Aliquots of *E. coli* strains transformed with the inser-

tion of the vector containing the GAL-1 gene (pET-29a(+)+lgals-1 gene[1]) were grown in systems with LB Broth Base medium containing kanamycin sulfate until obtaining optimal bacterial growth rate, demonstrated by optical density. Induction of expression is done with the addition of Isopropyl-D-Thiogalactopyranoside (Sigma-Aldrich) to the culture. After the induced growth period, the bacterial suspension is retained by microfiltration on a Hollow Fiber membrane (0.22 *µm,* Cytiva) and centrifuged at 5000g for 15 - 20 minutes at 4°C, always with the supernatant being discarded and the " bacterial crude = pellet", which were then subjected to bacterial lysis. For Bacterial lysis, the crude or bacterial pellet was resuspended in Phosphate Saline Lysis buffer (1X PBS - 136.8 mM NaCl, 2.7mM KCl, 6.4 mM Na_2HPO_4, 0.9 mM KH_2PO_4, pH 7.4), containing 14 mM Mercaptoethanol, protease inhibitor EDTA-free, lysozyme-1, RNAse A-Type 3A, and DNAse I Type IV-10. All components are Sigma-Aldrich. The pellet diluted in Lysis buffer (Chemical Lysis) was subjected to constant homogenization for 70 minutes and then sonicated for 3 cycles of 15 seconds each in a Vibra-Cells Sonicator, Sonics (Mechanical Lysis), with intervals of 20 seconds between each cycle. The bacterial lysate was then clarified by centrifugation at 7,000 g for 20 minutes at 4°C and filtered through a 1.0 µm filter (Whatman) with the aid of a peristaltic pump (maximum pressure of 4 BAR). For protein purification the lysate was submitted to 3 steps of purification by chromatography in an AKTA Protein Purification System (Cytiva) to obtain a buffered protein solution containing only GAL-1. The first step is based on affinity chromatography on agarose-lactose columns (Sigma-Aldrich), followed by a "size exclusion" chromatography (Sephadex G-25, Cytiva) and another affinity chromatography (PIERCE High-Capacity Endotoxin Removal Resin column - Thermo Scientific) to removal of bacterial endotoxins (LPS). After all the chromatographic steps, the protein concentration was determined by spectrometry (Abs 280 nm) and expressed in milligrams of protein per milliliter ($mg.mL^{-1}$) and were submitted to sterilizing filtration (0.22 *µm* PES membrane). Purified protein batches were submitted to the last stage of industrialization only if they reached compliance with the quality standard predetermined by the company, including protein concentration (1.05 ± 0.05 $mg.mL^{-1}$), microbiological status, protein bioactivity (Hemagglutination test), molecular weight analysis by SDS-PAGE, and SEC (size exclusion chromatography), protein secondary structure analysis (Circular Dichroism Analysis), aggregate detection and molecular size by DLS (Dynamic Light Scattering) analysis and endotoxin quantification (LPS). Protein identity was confirmed by LCMS (Liquid Chromatography Mass Spectrometry) and nucleotide sequence confirmation of human galectin-1 cDNA - galectin-1 [*Homo sapiens*] Consensus Coding Sequence (CCDS) CCDS13954.1 (https://www. ncbi.nlm.nih.gov/projects/CCDS/CcdsBrowse.cgi?REQUEST=ALLFIELDS&DATA=CCDS13954.1&ORGANISM=0&BUILDS=CURRENTBUILDS).

Animals: Seventy tree (n=73) females of Wistar rats (*Rattus norvegicus*), with 8 to 12 weeks, 181.67 to 268.86gr of Body weight range, were divided into 3 treatments groups Group 1 - Intravenous (I.V.), Group 2 - Intraperitoneal (I.P.), Group 3 - Intrauterine (I.U.), all with 21 rats per group. Animals were marked by cage label and tail marking. An additional 10 animals were used for blood and tissue collection (uterus) for bioanalysis.

Husbandry practices

- **Environmental condition:** Standard Laboratory conditions, temperature was maintained between 19.9 - 21.7°C and relative humidity between 41 - 59% under a 12 Hr. light/dark cycle.
- **Housing:** 3 rats in each polycarbonate cage with stainless steel grill tops, facilitates for feed and water bottle. Provided bedding with corn cobs and the cages were suspended on stainless steel racks for I.V. and I.P. group animals. 1 rat in each polycarbonate cage with stainless steel grill tops, facilitates for feed and water bottle. Provided bedding with corn cobs and the cages were suspended on stainless steel racks for I.U. group animals.

[1]Subcloning of GALl-1 into pET-29a(+) expression vector. GAL-1 Consensus Coding Sequence (CCDS) CCDS13954.1 (length 408nt) was synthesized and subcloned, with juxtaposed insertion of the desired sequence, immediately after the RBS Ribosome binding site sequence of a pET-29a (+) expression vector cut in NdeI / HindIII (GenScript®). This construct was then used for competent transformation of the Rosetta strain of Escherichia coli, maintained in a cell bank

- **Feed:** Irradiated standard pellet rodent feed manufactured by Bajaj Instrument Works, Loni, Ghaziabad was provided *ad libitum*.
- **Water:** Potable water passed through reverse osmosis water filtration system was provided *ad libitum* in autoclaved polycarbonate bottles with stainless steel sipper tubes.

Dose selection and justification: 1.00 $mg.kg^{-1}$ as a dose for intravenous administration; 5 $mg.kg^{-1}$ as dose for Intraperitoneal administration and up to 50 *µL of MFC (1.05 ± 0.05 mg.ml*$^{-1}$ solution of rHGAL-1) for intrauterine was selected for pharmacokinetic study.

Group allocation and dose administration: All the animals of Group 1 and Group 2 received a single dose of rHGAL-1 Buffered Solution through intravenous and intraperitoneal route, respectively. All the animals were provided food and drinking water ad libitum. The administered dose volumes were adjusted based on recorded body weight. All the animals of Group 3 were submitted a surgery to uterus administration of rHGAL-1 doses. For that they were fastened for 16 hrs. All surgeries were performed under anesthesia using a combination of Ketamine and Xylazine and utmost care was taken for asepsis using 70% ethanol as antiseptic and disinfectant. All surgicals were autoclaved to ensure sterility. The area of surgery was wiped clean with 70% Ethanol before making incision. Both sides of flanks near the hump of the rat were shaved. The hairs were clipped with clipper. The abdomen and flank were cleaned. Both sides of flanks were sterilized with a Betadine dipped gauze sponge and Betadine was washed with D.I. water. The left flank skin layer was cut with Adson forceps (in surgeon's left hand) and 12 cm straight blunt/blunt scissors (in surgeon's right hand) along the abdominal line. Then nick cut was made on the muscle layer with 12 cm straight sharp/sharp scissors to form a small hole. Forceps was used to find the fallopian tube surrounded by a variable amount of fat. The fallopian tube was pulled out through the muscle incision. The required amount of test formulation was injected slowly. The fallopian tube was returned into the abdominal cavity. Bleeding is usually slight and inconsequential and soon stops of its own. Suture the incision for closing. The rat was placed back into the cage.

Blood collection and storage of samples: After single dose administration through intravenous, intraperitoneal and intrauterine route in animals, blood and uterus samples were collected at specific time points (0.00; 0.50; 2.00; 4.00; 8.00; 24.00; 48.00 hs after rHGAL-1 doses administration). Immediately after blood collection, animals were humanely sacrificed, and organ (uterus) were collected for further analysis. Blood was collected through retro-orbital sinus under mild anesthesia in micro tube containing saturated solution of Na_2EDTA as an anticoagulant. Plasma was separated by centrifuging the blood samples at 10,000 rpm for 5 mins. Plasma samples were immediately transferred to micro tubes and stored at -80°C until analysis. Samples were analyzed using ELISA.

Bioanalysis (quantification of rHGAL-1 in uterus homogenate): All the tissue sample (Uterus) were homogenized with lysis buffer equivalent to 1:50 (w:v) i.e. 1 ml of lysis buffer (1M Tris-HCl, 0.5M EDTA pH adjusted to 8.0 with 1M NaOH) was added to 20 mg of tissue sample and homogenized. The homogenate was centrifuged for 5 minutes at 10,000 rpm and supernatant was collected and stored at -80°C and was analyzed using ELISA. Bioanalysis was performed by commercially available ELISA kit for determination of GAL-1 (Cloud Clone Inc, ref. SEA321Hu) concentration in rat plasma and uterus homogenate. For ELISA procedure Cloud Clone Corp. instruction manual provided with Kit was followed.

Winnonlin analysis: The following mean pharmacokinetic parameters of Recombinant Human Galectin-1 Buffered Solution such as Cmax, Tmax, T1/2, AUC0-t, AUC0-∞, Vd and CL in rat plasma were determined from the concentration-time data using Non-compartmental Analysis (Phoenix WinNonlin Pro, version 8.1 Pharsight, Inc, USA).

Test facility: DRF Dabur Research Foundation, 22 Site IV, Sahibabad, Ghaziabad, 201010, Uttar Pradesh, India.

Animal welfare: The facility is registered for breeding and experiment of animals with the CPCSEA [(The Committee for the Purpose of Control and Supervision of Experiments on Animals), Registration No. [64/PO/RcBi/S/99/CPCSEA]. Ministry of Environment and Forest, Govt. of India. All the procedures were in compliance with the CPCSEA guidelines of India.

Institutional animal ethics committee (IAEC) approval: This study was approved by Institutional Animal Ethics Committee (IAEC) vide Protocol No. IAEC/66/1300 on 02/JUL/2021.

Institutional biosafety committee (IBSC) approval: The IBSC approval no. for single dose pharmacokinetic study is IBSC/09/010 approved on 27/JUL/2021.

Results

The present study was performed to evaluate single dose pharmacokinetics of test item through I.V, I.P. and I.U. route in plasma and uterus. During the study animals were divided into three groups G1, G2 and G3. Each group contains 21 animals with 3 animals per time point. All animals of G1, G2 and G3 were administered with test item rHGAL-1 Buffered Solution through I.V., I.P. and I.U. route, respectively. Single dose was administered at 1 $mg.kg^{-1}$, 5 $mg.kg^{-1}$ and 51.5 µg for I.V., I.P. and I.U. routes, respectively. After dosing plasma and uterus were collected from each animal at defined time points. At each time point three animals were sacrificed to collect plasma and Uterus.

After I.V., I.P. and I.U. administration Cmax was observed to be 2.774, 1.635 and 0.619 $ng.ml^{-1}$, AUC0-t was observed to be 5.073, 12.746 and 1.330 $hr*ng.ml^{-1}$, Tmax was observed to be 0.5, 2.0 and 2.167h, respectively in plasma. Elimination half- life for I.P., I.V. and I.U. routes cannot be calculated due to insufficient points to draw elimination curve (Table 6).

After I.V. administration content of Recombinant Human Galectin-1 in uterus at 0.5h, 2.0h, 4.0h, 8.0h, 24.0h, and 48.0h contents observed were 10.92, 15.25, 17.92, 12.48, 20.50, 0.00 $ng.gm^{-1}$, respectively. At the same time after I.P. administration contents observed were 29.72, 15.40, 54.25, 23.53, 6.70, 0.00 $ng.gm^{-1}$ and after I.U. administration contents observed were 128.22, 131.97, 134.43, 136.05, 94.40, 0.00 $ng.gm^{-1}$ (Table 8 and figure 2).

Calibration curves back calculated parameters for rHGAL-1 Buffered Solution are summarized from table 1. The time-concentration profile and WinNonlin calculated PK parameters of rHGAL-1 Buffered Solution for I.V, I.P. and I.U. groups are summarized in tabular form in table 2-5 and figure 1.

We can cite salient findings during the study as (i) no mortalities were observed during the study; (ii) no clinical signs were in any of the rats with I.V. and I.P. administration. With IU route of administration, we have observed clinical signs and symptoms related to uterine surgery as sedation, lethargy, piloerection, abdominal pain and ataxia; (iii) Intrauterine dosed animals were recovered between 0.75h to 2.0h post-surgery and dosing; (iv) GAL-1 concentrations were detected in plasma uterus samples.

Back Calculated Standards of rHGAL-1 Buffered Solution from Calibration Curve							
Calibration curve Matrix		**Rat Plasma**		**Rat I.V., I.P. tissue**		**Rat I.U. tissue**	
Conc ($ng.ml^{-1}$)		**% Accuracy**	**Conc ($ng.ml^{-1}$)**	**%Accuracy**	**Conc ($ng.ml^{-1}$)**	**%Accuracy**	
Cal 1	0.312	0.329	105.45	0.304	98.06	0.32	102.56
Cal 2	0.625	0.599	95.84	0.672	107.52	-	-
Cal 3	1.25	1.224	97.92	1.157	92.56	1.189	95.12
Cal 4	2.50	2.704	108.16	2.575	103	2.635	105.4
Cal 5	5.00	5.126	102.52	5.262	105.24	5.122	102.44
Cal 6	10.00	9.546	95.46	9.946	99.46	10.23	102.3
Cal 7	20.00	20.821	104.11	20.707	103.54	20.088	100.44
r^2		0.999		0.999		1	

Table 1: *Curve parameter summary and back calculated concentration of calibration curve standards during sample analysis for rHGAL-1 buffered solution.*

rHGAL-1 Buffered Solution Conc. (ng.mL^{-1}) I.V.				
Time point (h)	**Set-1**	**Set-2**	**Set-3**	**Mean**
0	BLQ	BLQ	0.000	0.000
0.5	1.516	3.482	3.323	2.774
2	BLQ	0.677	1.030	0.854
4	0.000	BLQ	0.589	0.295
8	0.000	BLQ	0.424	0.212
24	0.000	0.000	BLQ	0.000
48	0.000	0.000	0.000	0.000

Table 2: *Time-concentration profile of rHGAL-1 buffered solution following I.V. administration at dose of 1 mg kg^{-1} in wistar rats (n=3) of group 1.*

BLQ: Below Limit of Quantification.

rHGAL-1 Buffered Solution Conc. (ng.mL^{-1}) I.P.				
Time point (h)	**Set-1**	**Set-2**	**Set-3**	**Mean**
0	0.000	0.000	0.000	0.000
0.5	0.638	0.000	2.092	0.910
2	1.197	0.000	3.132	1.443
4	0.369	0.000	3.709	1.359
8	0.475	27.126*	1.001	0.738
24	0.000	0.000	0.000	0.000
48	0.000	0.000	0.000	0.000

Table 3: *Time-concentration profile of rHGAL-1 buffered solution following I.P. administration at dose of 5 mg kg^{-1} in wistar rats (n = 3) of group 2.*

BLQ: Below Limit of Quantification.

**Outlier-not considered in calculation.*

rHGAL-1 Buffered Solution Conc. (ng.mL^{-1}) I.U.				
Time point (h)	**Set-1**	**Set-2**	**Set-3**	**Mean ± SD**
0	0.000	0.000	0.000	0.000 ± 0.00
0.5	BLQ	BLQ	0.677	0.677 ± 0.39
2	0.664	BLQ	0.429	0.547 ± 0.34
4	0.632	0.516	BLQ	0.574 ± 0.34
8	BLQ	0.000	0.000	0.000 ± 0.00
24	0.000	BLQ	0.000	0.000 ± 0.00
48	0.000	0.000	0.000	0.000 ± 0.00

Table 4: *Time-concentration profile of rHGAL-1 buffered solution following I.U. administration at dose of NMT 50 µL in wistar rats (n=3) of group 3.*

BLQ: Below Limit of Quantification.

rHGAL-1 Buffered Solution Conc. ($ng.mL^{-1}$)			
Time point (h)	**I.V.**	**I.P.**	**I.U.**
0	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00
0.5	2.774 ± 1.09	0.910 ± 0.88	0.677 ± 0.39
2	0.854 ± 0.52	1.443± 1.29	0.547 ± 0.34
4	0.295 ± 0.34	1.359 ± 1.67	0.574 ± 0.34
8	0.212 ± 0.24	0.738 ± 0.26	0.000 ± 0.00
24	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00

Table 5: *Mean time-concentration profile of rHGAL-1 buffered solution in plasma through I.V, I.P. and I.U. routes.*

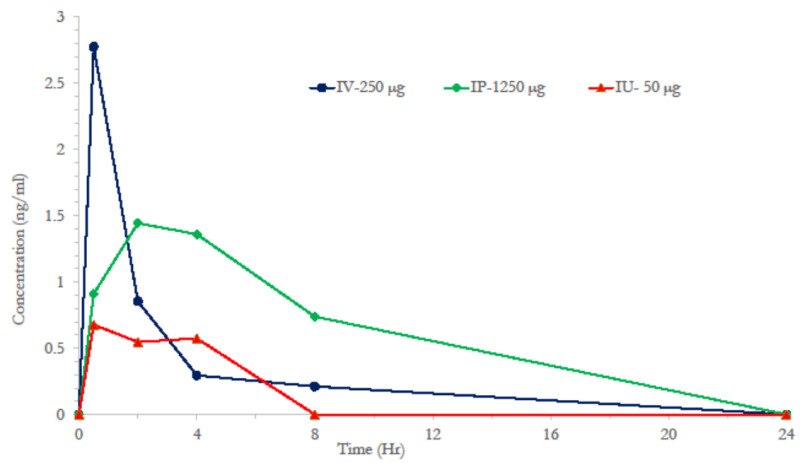

Figure 1: *Mean time-concentration profile of rHGAL-1 buffered solution in plasma through I.V, I.P. and I.U. routes.*

Parameter	Unit	I.V.	I.P.	I.U.
C_{max}	ng ml^{-1}	2.774	1.635	0.619
T_{max}	hr	0.500	2.000	2.167
AUC_{0-t}	hr*ng ml^{-1}	5.073	12.746	1.330
$AUC_{0-\infty}$	hr*ng ml^{-1}	NC	NC	NC
$T_{1/2}$	hr	NC	NC	NC
Vd	ml	NC	NC	NC
CL	ml hr^{-1}	NC	NC	NC

Table 6: *Mean pharmacokinetic parameters of rHGAL-1 buffered solution in plasma through I.V, I.P. and I.U. routes.*

NC- Not calculated due to insufficient points to draw elimination curve.

Parameter	Unit	Matrix	I.V.	I.P.	I.U.
Bioavailability w.r.t IV	%	Plasma	-	50 %	127 %
Bioavailability w.r.t IU	%	Plasma	79%	40%	-

Table 7: *Comparation of bioavailability (%) of rHGAL-1 buffered solution between different routes of administration (I.P.; I.U.; and IV).*

Route of Administration	Organ; Uterus Animal no.	Time Point Hr	Wt. of organ (gm)	Galectin-1 Content (ng gm^{-1})	Mean
I.V.	Rat-1	0.00	0.330	BLQ	0.00
	Rat-2		0.480	0.00	
	Rat-3		0.360	0.00	
	Rat-4	0.50	0.480	15.60	10.92
	Rat-5		0.470	0.00	
	Rat-6		0.460	17.15	
	Rat-7	2.00	0.440	0.00	15.25
	Rat-8		0.300	22.55	
	Rat-9		0.930	23.20	
	Rat-10	4.00	0.790	17.70	17.92
	Rat-11		0.780	18.75	
	Rat-12		0.840	17.30	
	Rat-13	8.00	0.490	21.60	12.48
	Rat-14		0.290	15.85	
	Rat-15		0.460	0.00	
	Rat-16	24.00	0.370	24.60	20.50
	Rat-17		0.800	18.75	
	Rat-18		0.370	18.15	
	Rat-19	48.0	0.390	0.00	0.00
	Rat-20		0.800	0.00	
	Rat-21		0.390	0.00	
I.P.	Rat-22	0.00	0.640	BLQ	0.00
	Rat-23		0.400	0.00	
	Rat-24		0.620	0.00	
	Rat-25	0.50	0.620	48.75	29.72
	Rat-26		0.630	18.15	
	Rat-27		0.500	22.25	
	Rat-28	2.00	0.860	21.60	15.40
	Rat-29		0.410	24.60	
	Rat-30		0.500	0.00	
	Rat-31	4.00	0.360	31.00	54.25
	Rat-32		0.440	16.45	
	Rat-33		0.530	115.30	
	Rat-34	8.00	0.590	26.25	23.53
	Rat-35		0.350	27.75	
	Rat-36		0.550	16.60	
	Rat-37	24.00	0.440	0.00	6.70
	Rat-38		0.370	20.10	
	Rat-39		0.630	0.00	
	Rat-40	48.0	0.460	0.00	0.00
	Rat-41		0.330	0.00	
	Rat-42		0.360	0.00	

I.U.	Rat-43	0.00	0.590	BLQ	0.00
	Rat-44		0.510	BLQ	
	Rat-45		0.460	BLQ	
	Rat-46	0.50	0.380	113.30	128.22
	Rat-47		0.490	117.30	
	Rat-48		0.690	154.05	
	Rat-49	2.00	0.480	107.65	131.97
	Rat-50		0.660	147.20	
	Rat-51		0.540	141.05	
	Rat-52	4.00	0.350	107.65	134.43
	Rat-53		0.440	165.45	
	Rat-54		0.660	130.20	
	Rat-55	8.00	0.990	139.80	136.05
	Rat-56		1.070	167.40	
	Rat-57		0.490	100.95	
	Rat-58	24.00	0.420	94.40	94.40
	Rat-59		0.510	97.10	
	Rat-60		0.340	91.70	
	Rat-61	48.0	0.765	0.00	0.00
	Rat-62		0.760	0.00	
	Rat-63		0.450	0.00	

Table 8: *Concentration of rHGAL-1 buffered solution in tissue homogenate (uterus) through I.V, I.P. and I.U. routes. Data presented as total content (ng) per gr of organ.*

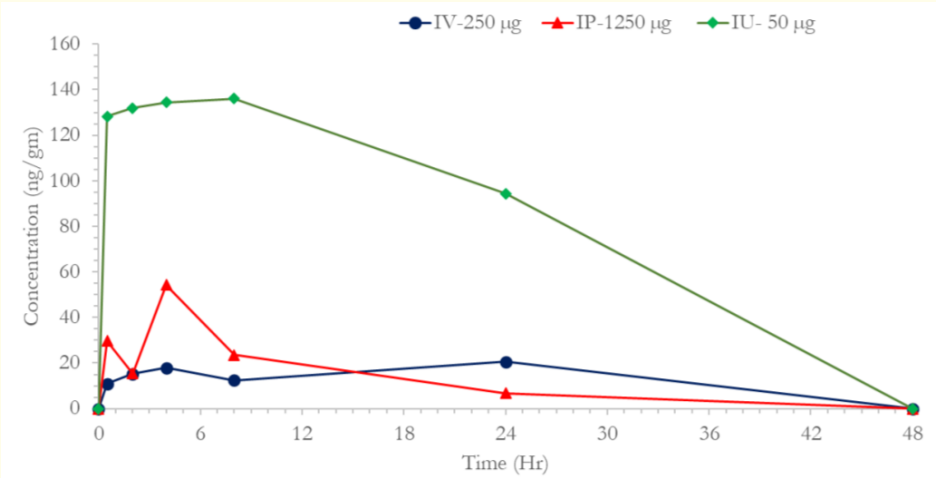

Figure 1: *Mean time-concentration profile of rHGAL-1 buffered solution in plasma through I.V, I.P. and I.U. routes.*

Discussion

The aim of this study was to complement the pharmacological knowledge of rHGAL-1, as an active ingredient in products and/or potential products, intended for assisted reproduction, whether in production animals or humans. The hypothesis of using GAL-1 as a tool to increase fertility was supported by looking at the reports by Blois., *et al*. (2007) [2], who demonstrated a higher rate of pregnancy loss in female knockout mice for the LGALS1 gene, the gene responsible for the expression of GAL-1. Based on this report, the authors of this article had the idea of administering an effective dose of exogenous rHGAL-1 (recombinant GAL-1, from the human gene, based on the phylogenetic conservation of the molecular structure of this protein within the animal kingdom and especially among mammals), imagining that the supplementation of an effective amount of this protein would bring the desired effect on reproduction. The specie selected to the first studies was the bovine, for any reasons, with the theorical support and literature reports, demonstrating an intrinsic participation of GAL-1 in important physiological events related to maternal recognition of pregnancy, either by the immunological aspect, or by biological aspects such as embryonic elongation and adhesion, and trophoblastic development and placentation [1-4,7,12,14,19].

Thus, since from 2008, they worked on issues such as (i) the definition of the optimal dose versus uterine lumen area (species particularities); (ii) definition of the optimal time for its administration - intrauterine administration, by simple deposition of the protein solution at a defined dose and time; (iii) protein stability; (iv) production scalability; (v) manufacturing compliance according to international biopharmaceutical regulations, and (vi) efficacy studies in bovine females, which demonstrated an increase of 8.68 percentage points in the probability of obtaining pregnancy in inseminated bovine females using rHGAL-1 as a supplement to the procedure, as demonstrated by Morani., *et al*. (2021) [12].

As part of the Non-Clinical Studies needed to start Clinical Trials (Phase-1 onwards), this article presents pharmacological aspects (pharmacokinetic parameters of single dose rHGAL-1 following intravenous (IV), intraperitoneal (IP), and intrauterine (IU) routes of administration in female Wistar rats and also, to measure the levels of GAL-1 in uterus tissue homogenate after those administrations). The choice of this species was based on bioethical questions of study in animals and aiming to meet the recommendations of the ICH Guides, as Tests Preclinical Safety Evaluation of Biotechnology-derived pharmaceuticals, as is the case presented here - recombinant protein-based product.

It was through this study that important answers to questions intrinsic to recombinant protein-based biopharmaceuticals were obtained. It was noted that exogenous rHGAL-1 administered by IV, IP or UI routes could be detected in blood plasma and in lysed uterine tissue (through ELISA assays performed), in lower concentration and for a shorter period of time in plasma samples seminal, when compared with uterine lysate samples (pharmacokinetic parameters of single dose rHGAL-1 following intravenous (I.V.), intraperitoneal (I.P.), and intrauterine (I.U.) routes of administration in female Wistar rats and also, to measure the levels of GAL-1 in uterus tissue homogenate after those administrations). After I.V. administration, the maximum content of rHGAL-1 in uterus was observed at 24.0h (20.50 $ng.gm^{-1}$). After I.P. administration the maximum content observed at 4.0hs (54.25 $ng.gm^{-1}$) and after I.U. administration the contents observed were higher than observed by another administration routes considering any time of observation (at 0.5, 2.0, 4.0 and 8.0hs, 128.22, 131.97, 134.43, and 136.05 $ng.gm^{-1}$., respectively). After 48hs were observed 0.00 $ng.gm^{-1}$, independently of administration route, which may suggest a degradation of the protein molecule in this interval between 24 - 48h of administration, an AUCmax of 8h after the I.U. administration, and a tropism of rHGAL-1 for uterine tissue (most likely the endometrium), independent of the route of administration of the exogenous protein, since levels of human GAL-1 were detected in uterine lysate in times from 0.00 to 24 hours of administrations. It is noteworthy that the I.U. administration route was the one that demonstrated the highest amount of exogenous protein per gram of lysed uterine tissue, which reinforces the hypothesis that the rHGAL-1 administration route chosen for the purpose of increasing reproductive efficiency is coherent.

Soon, we will present the Toxicology, Reproductive Toxicology and Pharmacological Safety results that will complement the Non-Clinical Testing for this innovation. As additional information, but not least, no undesirable clinical signs were observed in studies of administration of rHGAL-1 in female rats, regardless of the form of administration, remembering that with IU route of administration, we have observed only clinical signs and symptoms related to uterine surgery (the same observed on rats that received placebo). It is also worth mentioning that in the efficacy study carried out in bovine females, no unwanted clinical signs were observed in the females' cows worked, as well as there was no report of born calves showing congenital abnormalities and/or neonatal mortality [12].

Conclusion

Based on these results it was proved that rHGAL-1 can be detectable in plasma and uterus homogenate independently of route of administration tested (I.P., I.V. or I.U.) and no clinical signs were observed related with the rhGAL-1 solution administration, independently of the route used after dosing. In plasma samples, higher Cmax (2.774 $ng.ml^{-1}$) was observed in I.V., higher $AUC_{0/t}$ (12.746 hr *$ng.ml^{-1}$) was observed in I.P., and higher Tmax (2.167 hr) was observed in I.U. After I.V. administration, the maximum content of rHGAL-1 in uterus was observed at 24.0h (20.50 $ng.gm^{-1}$). After I.P. administration the maximum content observed at 4.0hs (54.25 $ng.gm^{-1}$) and after I.U. administration the contents observed were higher than observed by another administration routes considering any time of observation (at 0.5, 2.0, 4.0 and 8.0hs, 128.22, 131.97, 134.43, and 136.05 $ng.gm^{-1}$., respectively). After 48hs were observed 0.00 $ng.gm^{-1}$, independently of administration route. Those results can support the hypothesis that an effective exogenous rHGAL-1 doses administrated on lumen uterus can be difference on fetal-maternal recognition and improve the changes of a pregnancy to be terminal.

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Conflict of Interest

We declare that no is any conflict of interest.

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