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PRECLINICAL STUDIES

Bio-distribution study of Reolysin® (pelareorep) through a single intravenous infusion in Sprague-Dawley rats

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Summary Numerous pre-clinical and clinical studies on reovirus have generated valuable information which supports the use of this orphan virus as an investigational drug for cancer treatment. Reolysin® (pelareorep) is a clinical formulation of the human Reovirus Type 3 Dearing strain. The clinical safety and efficacy of Reolysin® in humans is being tested on an assortment of cancer indications as a mono and/or combination therapy. Reovirus has many inherent characteristics that make it a potential candidate for virotherapy, including: the rapid and natural spread through the haematogenous route, the ability to overcome immunological barriers thereby reaching tumor sites, and being replication-competent. The purpose of this study was to elucidate the bio-distribution pattern of Reolysin® in healthy Sprague-Dawley rats. Following a single 15-min intravenous infusion via the tail vein in Sprague-Dawley rats, the levels of virus genome were determined in 16 organs/tissues by RT-qPCR (Reverse Transcriptase- Quantitative Polymerase Chain Reaction) over a 336 h (Day 15) incubation regime. Consistent with previous studies, maximal reovirus RNA levels were observed in the spleen; indicating its involvement in viral uptake and clearance, followed by heart, ovaries, tail (infusion site), liver and lungs. All the organs/tissues demonstrated unquantifiable levels of reovirus genome at the end of incubation, suggesting substantial to complete viral clearance. Several studies in the last decade have described the use of reovirus for treating ovarian cancers. An increase of reovirus genome in ovaries at 24 h post infection was noted. The results will aid in the design of additional exploratory clinical trials for Reolysin®.

Keywords Reolysin® · Pelareorep · Reovirus · Bio-distribution · Sprague-Dawley Rats · RT-qPCR

Abbreviations

ANOVA	Analysis-of-variance
BLOQ	Below the Lower Limit of Quantification
EGFR	Epidermal Growth Factor Receptor
K2-EDTA	K2- Ethylene diamine tetra-acetic acid
LLOQ	Lower Limit of Quantification
RNA	Ribonucleic acid
RT-qPCR	Reverse Transcriptase-Quantitative Polymerase Chain Reaction
$TCID_{50}$	50 % Tissue Culture Infective Dose
ULOQ	Upper Limit of Quantification
USP	United States Pharmacopeia

Introduction

Pre-clinical, clinical and research studies have shown reovirus as a potential therapeutic agent for treatment of cancer [1, 2]. The oncolytic capability and tumor specificity of this orphan virus has been established in on-going clinical trials [3, 4]. Virotherapy may offer advantages over conventional cancer therapeutics such as demonstrated efficacy against cancers that historically have had a dismal prognosis and overcoming the limitations of standard chemotherapy treatments, such as lack of specificity resulting in widespread toxicity [5]. Reovirus is a ubiquitous, naturally occurring virus, commonly found in untreated sewage, stagnant water, and rivers worldwide [6].

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Community-acquired reovirus infections in humans are mild and restricted to the respiratory and gastrointestinal tract; however on occasion, reovirus may cause flu-like upper respiratory tract symptoms or mild diarrheal illness. Reoviruses are non-enveloped viruses, with a double-shelled capsid, each with icosahedral symmetry, containing 10 linear double stranded (ds) RNA genome segments [7]. Electropherotyping studies demonstrated that these ten dsRNA segments can be divided into three size classes; three large (L1, L2, L3), three medium (M1, M2, M3), and four small (S1, S2, S3, S4) segments. The homologous segments from each of the three serotypes exhibit distinct electrophoretic mobility allowing for identification of each reovirus serotype. Reolysin® (pelareorep) is a clinical isolate of the human Reovirus Type 3 Dearing strain (natural isolate and replication-competent).

Reovirus is naturally oncotropic, as it does not require molecular engineering in order to target neoplastic tissue. Instead, reovirus specifically targets cells containing an activated Ras pathway [1, 8, 9], which can occur through mutation of the *ras* oncogene or overexpression/mutational activation of EGFR. Previous studies demonstrated that cells expressing high levels of EGFR and v-ErbB, a truncated mutant of EGFR, were susceptible to reovirus infection and cytotoxicity, whereas no cytopathic effects were witnessed in normal cells [10]. In addition to reovirus possessing an innate ability to replicate specifically in transformed cells possessing an activated Ras signalling pathway, reovirus replication is entirely cytoplasmic and replication does not include any intranuclear events [11]. The activating Ras mutation represents more than 30 % of all human tumors [12], therefore making reovirus-induced oncolysis a potential therapy for a wide variety of cancers.

Bio-distribution studies are important parameters to consider when testing novel oncolytic virotherapy, as results may help to guide further clinical study design. Verdin [13] used ^{123}I-radiolabelled reovirus (serotypes 1 and 3) to examine viral clearance in a live animal. After the labeled reovirus was administered intravenously into adult Sprague-Dawley rats, instant imaging of organ-specific uptake of the ^{123}I-radiolabelled reovirus within the rat was monitored using a scintillation camera. Reovirus clearance was rapid and complete, as more than 90 % of the input had been cleared from the bloodstream within 5–10 min after the injection [13, 14]. Reovirus Type 3 Dearing (T3D) strain accumulated in specific organs, mainly the liver and spleen, with some minor viral clearance in the lungs.

The aim of this study was to determine the bio-distribution of Reolysin® in healthy Sprague-Dawley rats. A single 15-min intravenous infusion of Reolysin® was delivered via the tail vein and traces of reovirus genome were quantified in 16 organs/tissues using RT-qPCR at different time points to determine the path of Reolysin® uptake and clearance.

Material and methods

Preparation of Reolysin® test and Control articles

Reolysin® (Oncolytics Biotech Inc., Canada), lot number 160-10006 (manufactured at SAFC®, Carlsbad, USA as per current Good Manufacturing Practice) with a labeled viral titre of 4.5×10^{10} $TCID_{50}$/ml (50 % Tissue Culture Infective Dose) was used as a test article after diluting to 2.6×10^{9} (high dose), 2.6×10^{8} (medium dose) and 2.6×10^{7} (low dose) $TCID_{50}$/ml (or 6.5×10^{9}, 6.5×10^{8} and 6.5×10^{7} $TCID_{50}$/animal, respectively). The Control articles/dosing solution (0.9 % sterile Sodium Chloride USP, lot number W1C23C1) used in this study was obtained from Baxter, USA. The prepared solutions were passed through 0.2 μm syringe filters and the dose aliquots were maintained at 4 °C/wet ice before administration into the Sprague-Dawley rats. The dose level in the rodent was selected based on the equivalent human clinical dose and administered intravenously.

Diet, housing and acclimatization of Sprague-Dawley rats

The Sprague-Dawley rats (90 males and 90 females, including 10 spare rodents per sex) were obtained from Charles River Canada Inc., Quebec, Canada. The age of the animals ranged from 46 to 50 days and body weights (as on day-1) from 204 to 298 g and 153 to 228 g for males and females, respectively. Upon arrival, the health of the rodents was assessed and results examined by a clinical veterinarian to ensure suitability for the study. Animals had a unique identification and were housed in polycarbonate bins under a monitored and controlled room environment (Temperature: 21±3 °C, Humidity: 50±20 %. Photoperiod: 12 h light/dark cycle and 10–15 air change per hr). Standard commercial chow (Harlan Teklad Certified Global Rodent Diet number 2018C) and municipal tap water (exposed to ultraviolet light and purified by reverse osmosis at CiToxLAB North America, Quebec, Canada) was provided to the rodents ad libitum. The Sprague-Dawley rats were acclimatized for 7–11 days prior to the start of treatment. Cage-side clinical observations and mortality checks (twice daily during all phases of the study), body weights (prior to group allocation, 1 day prior to initiation of treatment and on the day prior to necropsy) and food consumption (twice weekly) were recorded during the study. During acclimatization, five rats per gender were assigned for each time point (8.5 min, 2 h, 24 h, 72 h and 336 h, Control group had only one time point at 8.5 min) and dose groups (control and test groups). Male and female Sprague-Dawley rats were randomized separately.

Single intravenous infusion of the dose formulation

The test and control dose formulations (2.5 ml) were administered via the tail vein to Sprague-Dawley rats (based on average

body weight of 225 g) using a temporary peripheral vein catheter over a period of 15 min, at a rate of 10 ml/h with a medical grade infusion pump. The catheter was pre-filled with 0.1 ml of dose formulation to ensure complete administration of the intended dose. Weight of the infusion syringe was recorded before and after infusion for the purpose of accountability (data not shown).

Treatment schedule and sample collection upon termination

Post-infusion, the animals went through 8.5 min, 2 h, 24 h, 72 h and 336 h (Day 15) of incubation (except for the Control group which were terminated at 8.5 min). Upon reaching the termination time point, the Sprague-Dawley rats were anesthetized (by Isoflurane) and blood samples were immediately collected (in tubes containing K2-EDTA) via intra-cardiac puncture. The animals were then completely perfused with 0.9 % saline (USP) and tissues were collected. The organs/tissues included were as follows: blood, brain, bone marrow, heart, tail (infusion site), kidney, large intestine, liver, lungs with bronchi, ovaries, prostate, small intestine, spleen, stomach, testes and uterus. This was followed by a rinse of the organs/tissues, as applicable, with phosphate buffered saline (pH 7.4) then cut into 3–6 pieces, each measuring approximately 0.5 cm. The bone marrow was extracted using a syringe with phosphate buffered saline (pH 7.4).

Table 1 Summary of clinical observations in control and test groups of Sprague-Dawley rats

Clinical sign	Male		Female	
	Control (*N*=5)	Test (*N*=25)	Control (*N*=5)	Test (*N*=25)
Fur, Stained Black				
Number of observations	0	0	NA	NA
Number of animals	0	0	NA	NA
Days from–to	NA	NA	NA	NA
Fur, Stained Brown				
Number of observations	0	0	NA	NA
Number of animals	0	0	NA	NA
Days from–to	NA	NA	NA	NA
Fur, Stained Red				
Number of observations	0	2	0	1
Number of animals	0	2	0	1
Days from–to	NA	−7 to −7	NA	4 to 4
Fur, Thin				
Number of observations	0	11	0	0
Number of animals	0	3	0	0
Days from–to	NA	−7 to 1	NA	NA
No Abnormalities detected				
Number of observations	17	71	10	57
Number of animals	5	24	5	24
Days from–to	−7 to 1	−7 to 15	−7 to 1	−7 to 15
Skin, Dry				
Number of observations	0	4	1	4
Number of animals	0	2	1	2
Days from–to	NA	−1 to 15	−1 to −1	−1 to 15
Skin, Red				
Number of observations	0	2	0	1
Number of animals	0	2	0	1
Days from–to	NA	−1 to 2	NA	15 to 15
Skin, Scab				
Number of observations	0	11	0	8
Number of animals	0	7	0	7
Days from–to	NA	2 to 15	NA	−1 to 4

NA Not Applicable, *N* Number of animals

Table 2 Body weight (g) summary of Control and Test groups of Sprague-Dawley rats

Groups	Days					
	−8/−6[a]	−1	1	3	7	14
Male- Control						
Mean weight (g)	176	224	NA	NA	NA	NA
Standard Deviation	8.7	15.5	NA	NA	NA	NA
N	5	5	0	0	0	0
Male- Test						
Mean weight (g)	179	226	235	254	303	376
Standard Deviation	8.1	12.9	16.7	9.6	25.5	37.7
N	25	25	5	5	5	5
Female- Control						
Mean weight (g)	159	198	NA	NA	NA	NA
Standard Deviation	3.2	7.6	NA	NA	NA	NA
N	5	5	0	0	0	0
Female- Test						
Mean weight (g)	157	195	192	220	221	251
Standard Deviation	10.5	12.3	4.8	13.5	6.5	17.2
N	25	25	5	5	5	5

[a] −8 days for females and −6 days for males

NA, Not Applicable, *N* Number of animals

RNA isolation and RT-qPCR for bio-distribution study

RNA isolation, quantification and reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) were conducted using previously validated methods established by CiToxLab North America, Quebec, Canada. The collected organ/tissue samples were kept in RNAlater solution (Qiagen) for at least 12 h, and then removed from solution and stored at −70 °C pending further analysis, except for bone marrow and blood samples, for which RNA was extracted on the same day as the collection. The RNA from all samples was extracted from three different organ/tissue aliquots (except for bone marrow and ovaries samples for which generally two aliquots were used) using an adapted method based on the RNeasy kit (Qiagen). Subsequent analysis for RNA concentration determination and RT-qPCR were performed in triplicate (duplicate for bone marrow and ovaries). The RNA concentration was quantified by Ribogreen® (Life Technologies). Copies of the reovirus transcripts from each organ/tissue and time interval were then quantified based on the standard curve by RT-qPCR, using a dual-labelled probe system and an Mx3005P Real-time PCR instrument. The standard curve used in this study ranged from 25 copies/well (LLOQ: Lower Limit of Quantification; equivalent to 125 copies/ug of RNA) to 2.5×10^7 copies/well (ULOQ: Upper Limit of Quantification). The amplification primers were targeted to the L2-genome sequence of reovirus, T3D (GenBank number HM159614.1)

Data handling, analysis and reporting

Provantis® 7.0.3.3 (Instem), In-Life module for data collection and reporting system was used in this study. SoftMax® Pro Software, version 5.2 (Molecular Devices) and MxPro QPCR Software, version 3.00 (Strategene) were used for RNA quantification by Ribogreen® (Life Technologies) and

Table 3 Food consumption (g/animal/day) of Control and Test groups of Sprague-Dawley rats

Groups	Days							
	−4 to −1	−4 to 1	−1 to 1	1 to 3	1 to 4	4 to 8	8 to 11	11 to 14
Male- Control								
Mean (g/animal/day)	21.8	NA	NA	NA	NA	NA	NA	NA
Standard Deviation	2.42	NA	NA	NA	NA	NA	NA	NA
N	4	0	NA	0	0	0	0	0
Male- Test								
Mean (g/animal/day)	22.1	22.6	NA	23.4	24.9	27.7	31.7	33.6
Standard Deviation	1.60	2.54	NA	2.01	3.29	4.34	5.87	5.83
N	10	15	NA	5	5	5	5	5
Female- Control								
Mean (g/animal/day)	19.3	NA	NA	NA	NA	NA	NA	NA
Standard Deviation	1.87	NA	NA	NA	NA	NA	NA	NA
N	4	0	0	0	0	0	0	0
Female- Test								
Mean (g/animal/day)	19.5	18.5	21.0	20.4	19.9	20.6	24.1	22.6
Standard Deviation	1.83	2.03	NA	1.75	2.28	2.02	3.64	4.37
N	11	14	1	5	5	5	5	5

NA Not Applicable, *N* Number of animals

RT-qPCR bio-distribution studies, respectively. SAS 9.1 (SAS Institute Inc.) was used for statistical analysis and Microsoft® Office and GraphPad Prism® (GraphPad Software Inc.) for data reporting. Other statistical tools used in this study include Analysis-of variance (ANOVA), Dunnett's T-test and Krushal-Wallis test.

Results

The results from control and test groups from only the highest Reolysin® dose formulation (6.5×10^9 $TCID_{50}$/animal) have been incorporated and discussed further in this study.

Clinical observations, body weight, food consumption and mortality

Clinical observations, body weight, food consumption and mortality were recorded prior to and after a 15-min intravenous infusion of Reolysin® in male and female Sprague-Dawley rats. Male and female Sprague-Dawley rats used in this study showed no Reolysin®-related mortalities and all the animals survived until the scheduled necropsy. The clinical observations (Table 1) related to fur (thin or stained) were considered incidental as it was noticed across groups and are prevalent in this species of laboratory animal. Few instances of dry, scabbed and/or red skin were recorded and frequencies of these observations were also noticed in medium and low dose formulation groups of Reolysin® (data not shown). Thus, it was difficult to find a strong relationship between dose levels and incidence and hence considered secondary for the purpose of this study. Consistent growth patterns were observed in terms of body weight (Table 2) and food consumption (Table 3). Minor fluctuations in the control and test groups (high, medium and low dose formulations) were considered to be a reflection of the normal inter-animal variation for animals of this age and species (data not shown).

Bio-distribution of Reolysin® in Sprague-Dawley rats using RT-qPCR

The levels of the Reovirus RNA were evaluated in different tissues/organs (blood, bone marrow, brain, heart, tail-infusion site, kidney, large intestine, liver, lungs with bronchi, ovaries, prostate, small intestine, spleen, stomach, testes and uterus) of the male and female Sprague-Dawley rats as applicable by RT-qPCR. The schematic representation of the average viral RNA (Fig. 1, represented as number of copies/μg RNA)

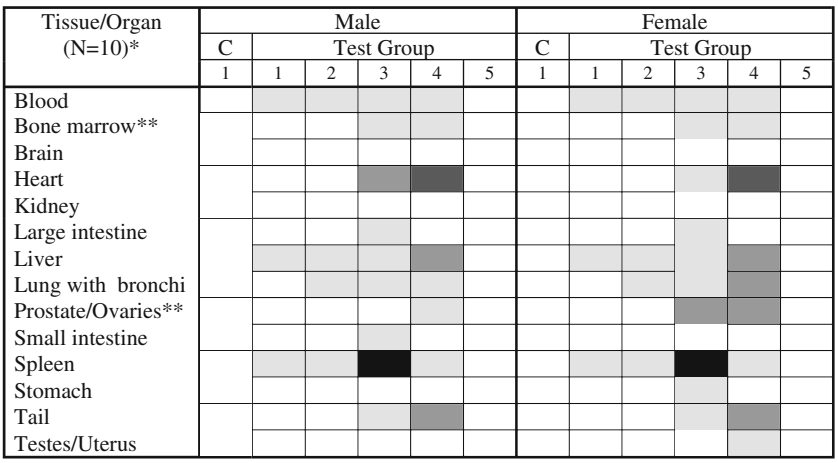

Fig. 1 A schematic representation of the virus transcript levels (average) after RT-qPCR in different tissues/organs of Sprague-Dawley rats

Means of copies/μg RNA were calculated by attributing a value of "0" when no copies were detected and half the LLOQ value (62.5copies/μg RNA) for BLOQ samples.

*: N=10 rats per tissue (5 males and 5 females independently analyzed in triplicate)
**: Bone marrow and Ovaries analyzed in independent duplicates
BLOQ: Below Lower Limit of Quantification (LLOQ; 125copies/μg RNA)
C: Control group
1: 8.5 min post-infusion
2: 2 hours post-infusion
3: 24 hours post-infusion
4: 72 hours post-infusion
5: 336hr (Day 15) post-infusion

showed a distinct bio-distribution pattern in the tissues/organs tested following infusion with Reolysin® (6.5×10^9 $TCID_{50}$/animal, high dose formulation). Figures 2, 3, 4, and 5 are a graphical representation (the trend line connects mean values) of the viral RNA levels (copies/μg RNA) for all the tissues/organs tested at various time points (8.5 min, 2 h, 24 h, 72 h and 336 h). In general, the RNA levels in male and female rats remained high at 24 h and 72 h post-infusion time points (Fig. 1). Mostly all the samples at 336 h (Day 15) post-infusion showed no signal or were BLOQ and were comparable to the control group (Fig. 1). In blood samples, copies of RNA were detected as early as 8.5 min and remained measurable until 72 h post-infusion (Figs. 1 and 3). The spleen tissue in both male and female Sprague-Dawley rats started showing detectable levels of viral RNA at 8.5 min (125–9,999 copies/μg RNA) and recorded more than 100,000 copies/μg RNA at 24 h post-infusion (Figs. 1 and 3). In heart, an average of 89,000 copies/μg RNA and 71,000 copies/μg RNA (at 72 h time point) was noticed for male and female Sprague-Dawley rats, respectively (Figs. 1 and 3). The RT-qPCR results from testes/uterus samples for 24–72 h time points were observed to be low (Figs. 1 and 2). A similar pattern of bio-distribution results were observed for the kidney, stomach and small intestine in both rat genders (Figs. 1, 4 and 5). The results from the prostate were comparable to the control group, however in the ovaries, the data was in the range of 10,000–49,999 copies/μg RNA at 24–72 h post-infusion (Fig. 1). The copies/μg RNA for the ovaries approached 40,000 (Fig. 2) at 24 h and continued to drop for the next two time points (72 and 336 h). The lung samples for the test groups of male and female rats showed detectable levels (about 5,000–15,000 copies/μg RNA) of viral RNA at 24–72 h post infusion (Figs. 1 and 3). The RT-qPCR results of Sprague-Dawley rat test groups for liver and tissue from infusion site (tail) showed an increasing trend until 72 h and

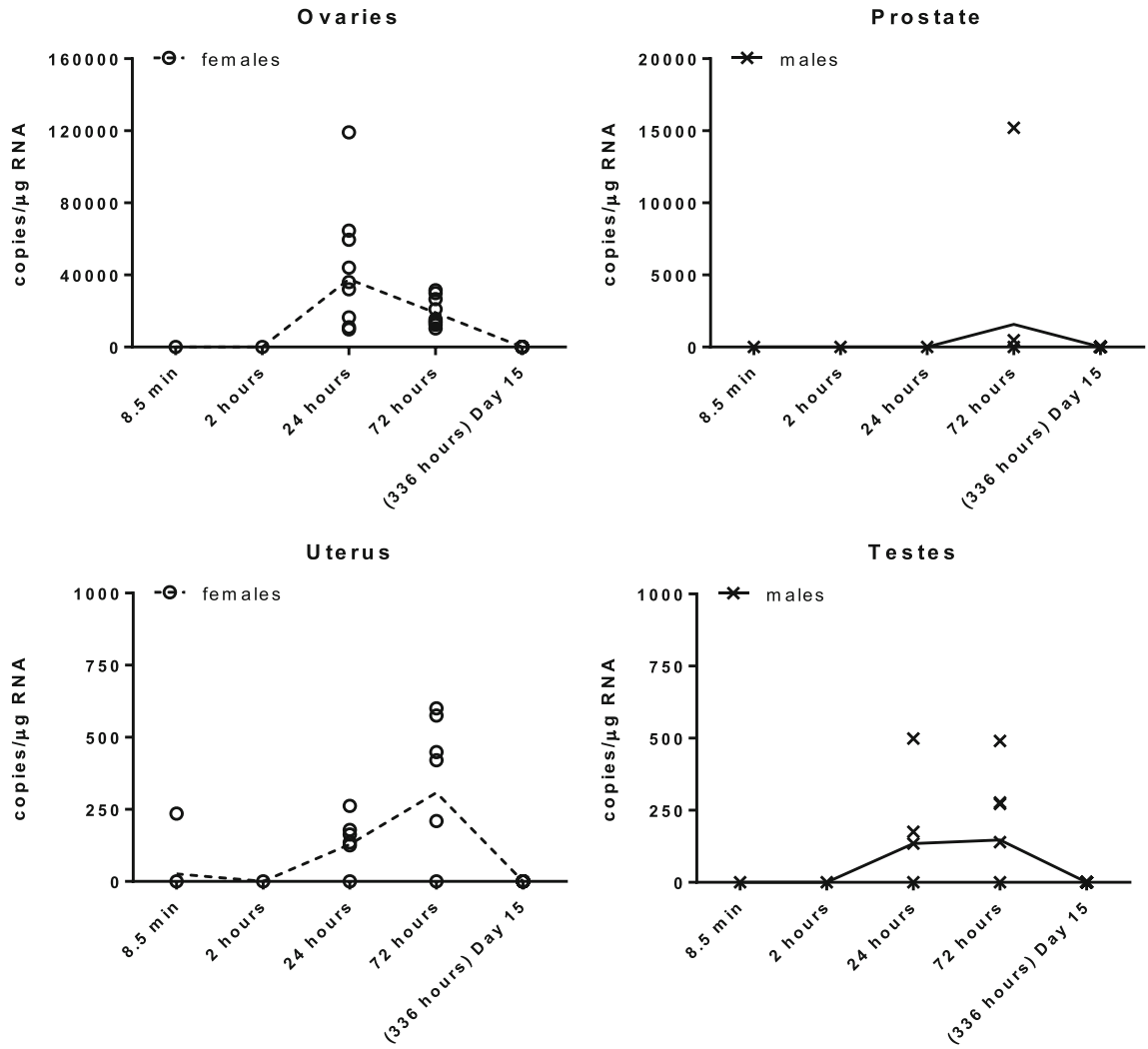

Fig. 2 RT-qPCR results comparing the virus RNA levels (copies/μg RNA) in reproductive tissues (Ovaries, Prostate, Uterus and Testes) of male and female Sprague-Dawley rats at five time points. The trend line connects the mean value for each time point ($N=15$)

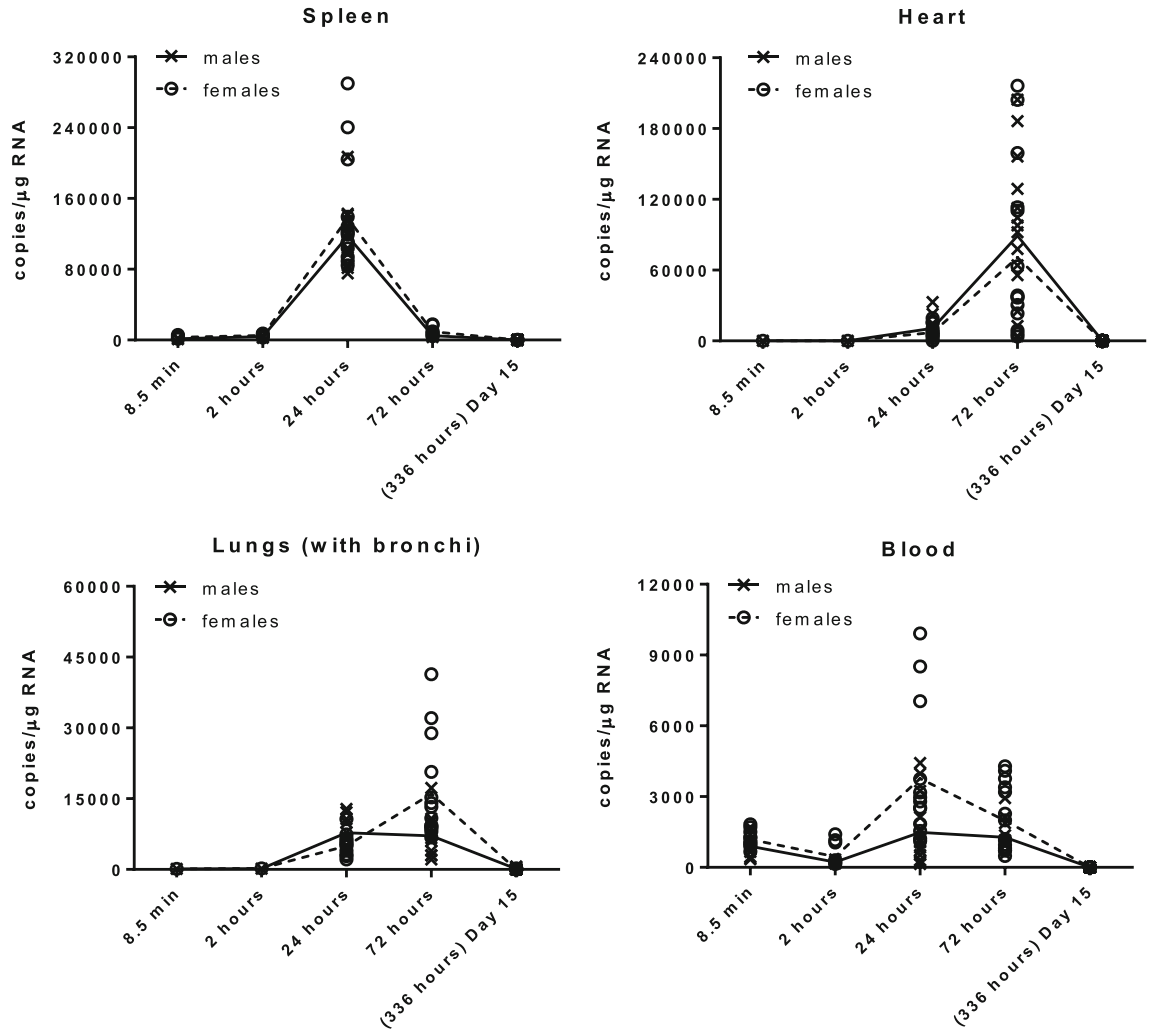

Fig. 3 RT-qPCR results comparing the virus RNA levels (copies/µg RNA) in respiratory/lymphoid tissues (Spleen, Heart, Lungs with bronchi and Blood) of male and female Sprague-Dawley rats at five time points. The trend line connects the mean value for each time point (N=15)

reduced thereafter (Figs. 1, 4 and 5). The increase in the detectable level of RNA copies (between 24 and 72 h) at the infusion site is considered to be an off-target effect. However, in bone marrow samples, the average levels were below 2,000 copies/µg RNA at 24 h time point (Fig. 5). Unquantifiable levels of reovirus RNA was observed in brain tissue (Figs. 1 and 5).

When comparing RT-qPCR results from the respiratory/lymphoid tissues (spleen, heart, lungs and blood), the spleen recorded the highest copies of mean viral RNA followed by heart, lungs and blood (Fig. 3). In the case of digestive tissues (Fig. 4), stomach samples had the lowest levels (less than 300 copies/µg RNA) and the greatest levels were observed in the liver (approximately 10,000–25,000 copies/µg RNA). However, in reproductive tissues (Fig. 2), the mean levels of viral RNA for prostate (less than 3,000 copies/µg RNA), uterus and testes (less than 300 copies/µg RNA) were evidently much lower when compared to samples from ovaries (approximately, 40,000 copies/µg RNA).

Discussion

The purpose of this study was to determine the bio-distribution of Reolysin® in healthy Sprague-Dawley rats and demonstrate in vivo viral clearance through RT-qPCR. By using this technique it was possible to rapidly quantify the amount of reovirus transcript distributed in various tissues/organs and trace viral clearance over a treatment period of 336 h. A single 15-min intravenous infusion of Reolysin® was administered to male and female rats at three different dose levels; a high dose of 6.5×10^9 $TCID_{50}$/animal, a mid dose of 6.5×10^8 $TCID_{50}$/animal, and a low dose of 6.5×10^7 $TCID_{50}$/animal. The dosages were based on the equivalent human clinical dose (3.0×10^{10} $TCID_{50}$) and average Sprague-Dawley rat body weight of 225 g. The sacrifice time points for this study were at 8.5 min, 2 h, 24 h, 72 h and 336 h (Day 15) post Reolysin® administration. The levels of reovirus transcripts obtained from RT-qPCR may not necessarily imply infectious virus particles [15].

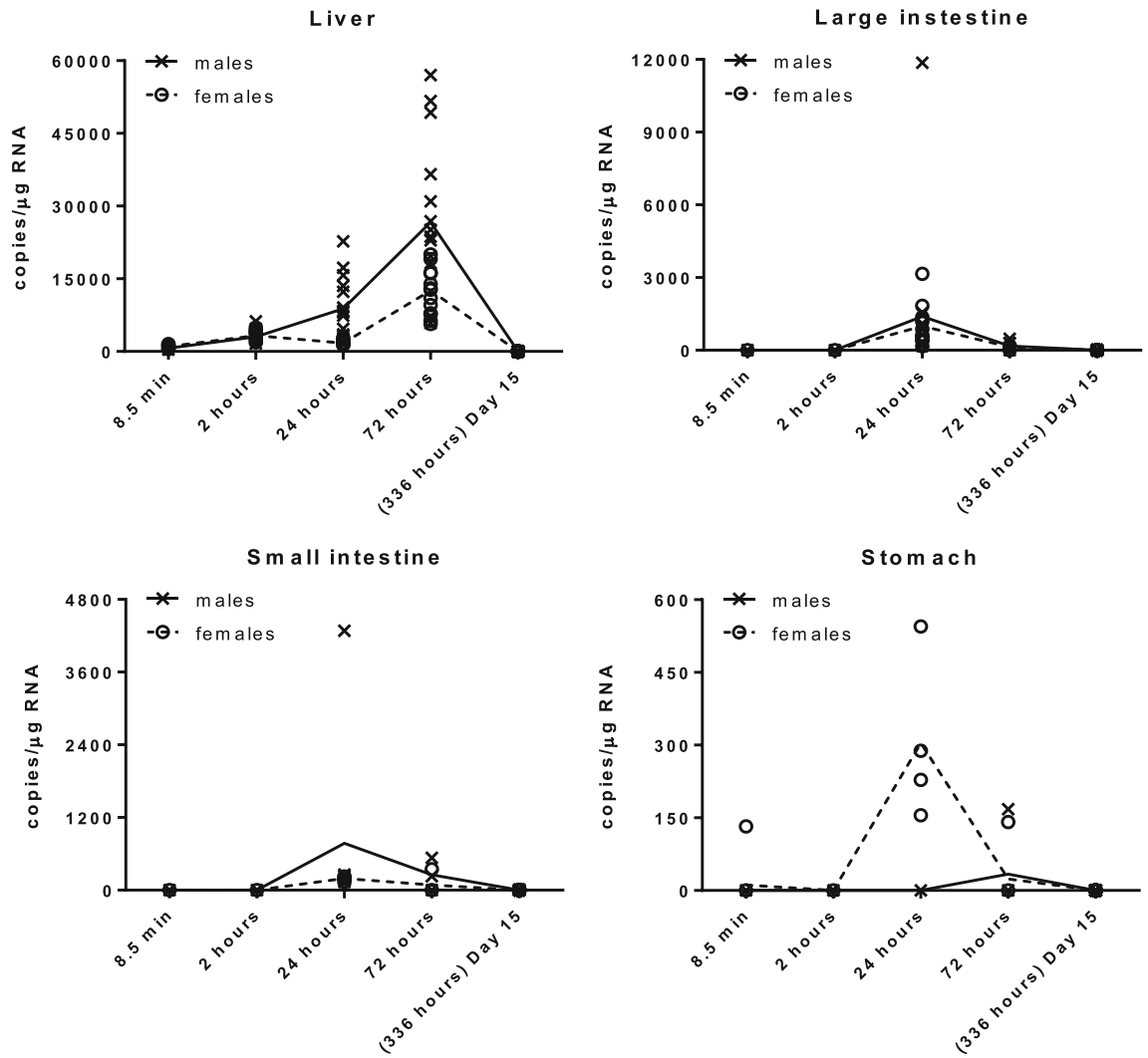

Fig. 4 RT-qPCR results comparing the virus RNA levels (copies/µg RNA) in digestive tissues (Liver, Large intestine, Small intestine and Stomach) of male and female Sprague-Dawley rats at five time points. The trend line connects the mean value for each time point ($N=15$)

Clinical observations related to fur and skin suggests there were no adverse side-effects of Reolysin® treatment and were considered incidental as these were shared across groups and are predominant in laboratory animals. There were also tail findings that were considered procedure related. As the frequency of these observations were similar in both the medium and low dose groups, it was difficult to demonstrate a strong association between the dose levels and their occurrence or severity. These outcomes support the use of Reolysin® as there were no Reolysin®-related mortalities, effects on body weights, or food consumption in the Sprague-Dawley rats. The minor differences that were observed are considered to be normal inter-animal variations in this species.

The systemic spread of reovirus RNA was measured in the respiratory/lymphoid tissues (spleen, heart, lungs with bronchi, and blood), with the spleen accumulating the highest levels of reovirus genome at the 24 h time point (100, 000 and 130, 000 copies/per µg RNA in male and female rats, respectively) (Fig. 3). It was evident that after only 8.5 min, detectable levels of reovirus RNA (more than 900 copies per µg RNA) were found in the spleen, and these levels peaked at 24 h post-infusion. The high amounts of reovirus RNA detected in this organ are not uncommon and are indicative of its role in viral clearance and non-specific uptake of oncolytic viruses [13, 16, 17]. Similarly, accumulation of reovirus genome copies in the heart at 72 h post-infusion in both male and female rats ranged from 60, 000 to 100,000 copies per µg RNA. This was anticipated given the nature of the intravenous systemic administration of Reolysin® which leads to the transport of reovirus through the haematogenous route [18, 19]. The pathogenesis of reovirus in lungs of Sprague-Dawley rats were previously reported [20] and 5,000 and 15,000 copies/µg RNA of reovirus were observed at 72 h post-infusion. Of the respiratory/lymphoid tissues, blood was the only one that had detectable levels of reovirus RNA at the first time point through to the 72 h time point. Reovirus RNA continued to be observed in the blood as it was distributed to

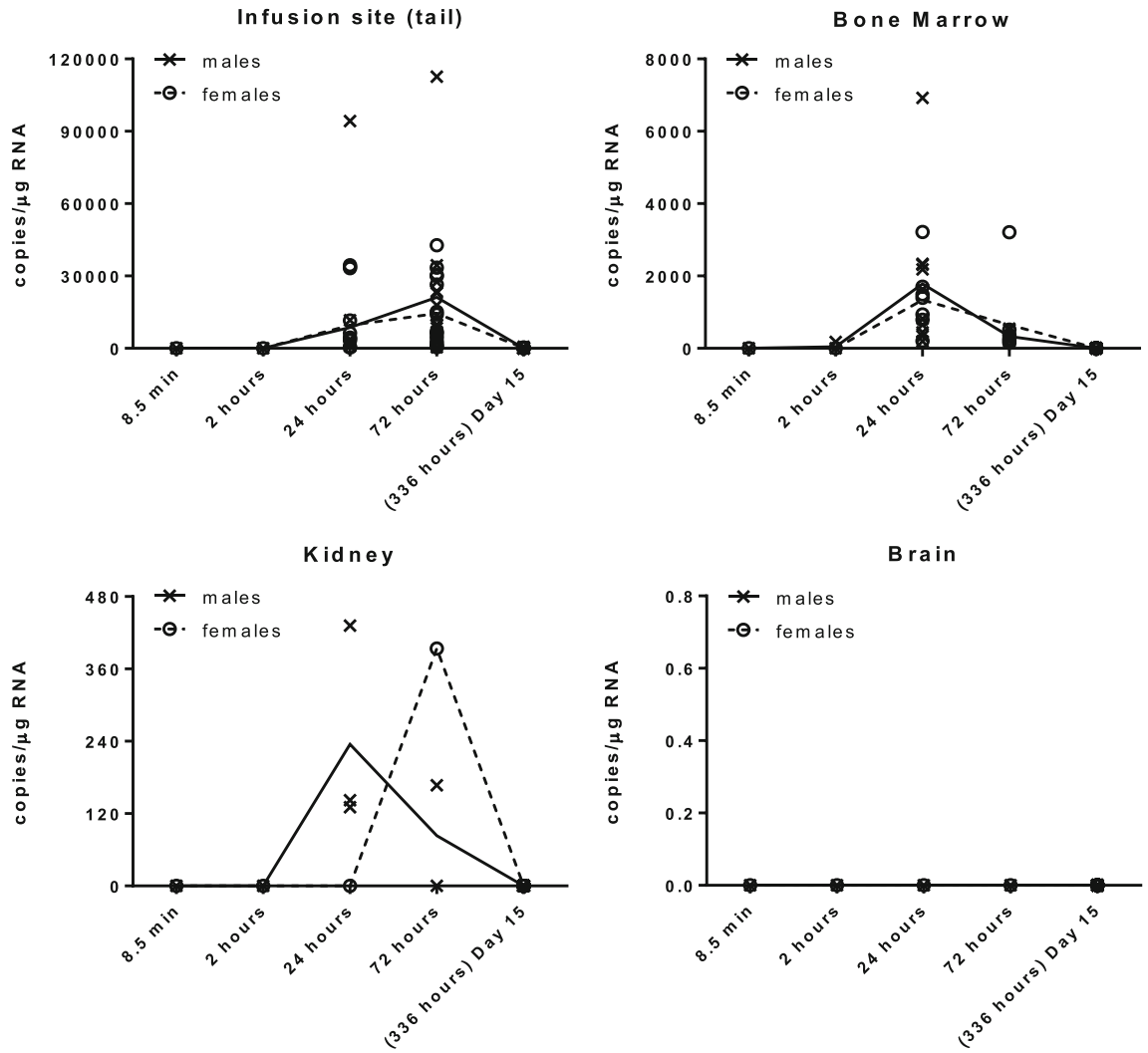

Fig. 5 RT-qPCR results comparing the virus RNA levels (copies/µg RNA) in other tissues (Infusion site-tail, Bone marrow, Kidney and Brain) of male and female Sprague-Dawley rats at five time points. The trend line connects the mean value for each time point ($N=15$, for bone marrow $N=10$)

organs involved in non-specific uptake and/or viral clearance. Importantly, after 336 h post infusion, the majority of the reovirus genome copies were unquantifiable in any of the respiratory/lymphoid tissues. This is true for other tissue/organs also, thereby suggesting substantial to complete clearance of the virus in 14 days.

The pattern of reovirus RNA distribution in the digestive tissues, specifically in the liver, suggests the route for reovirus clearance [16, 17]. Stomach, small and large intestine accumulated much lower levels of the reovirus genome when compared to liver. Noticeably after the 336 h time point reovirus RNA reaching the LLOQ (Lower Limit of Quantification) was observed in these digestive tissues. Interestingly, the single 15-min intravenous infusion of Reolysin® in Sprague-Dawley rats resulted in unquantifiable levels of reovirus RNA in brain tissue. Of all the organs examined in this bio-distribution study, it was possible to distinguish spleen, heart, tail (the site of infusion), liver, lung and ovaries from the rest, as these had higher traffic of reovirus RNA than other organs.

Consistent with the earlier viral clearance study [13], the data from this bio-distribution study suggests that Reolysin® is quickly cleared from the bloodstream and concentrates predominantly in the spleen. This is followed by the heart, ovaries, tail (infusion site), liver and lungs. Despite differences in the methodologies used in the viral clearance study [13] and this bio-distribution study, the routes of reovirus clearance for the two methods remain similar. This bio-distribution study quantified the amount of viral transcript accumulation in various tissues/organs, whereas the Verdin studies [13, 14] measured the levels of radiolabelled reovirus (serotypes 1 and 3) protein in different tissues. Viral transcription and translation kinetics and frequencies differ [21], therefore direct comparisons between the reovirus detection times cannot be made as discrepancies will exist due to the difference in what was being measured; however, the identification and comparison

of the organs involved in reovirus clearance can be made between the two studies. Substantial copies of the reovirus RNA were distributed to the ovaries of healthy Sprague-Dawley rats at 24 h post infection and were subsequently cleared at later time points.

An earlier study [22] demonstrated the unique ability of reovirus (Type 3 Dearing) to effectively treat ovarian cancers, including localized and metastatic tumors. Additionally, the findings showed that reovirus exerted cytopathic effects in ovarian cancer cell lines in vitro and in vivo, whereas an ex vivo ovarian tumor model indicated that reovirus infection was confined to tumor cells thereby sparing non-transformed healthy cells [22]. A more recent study [23] further demonstrated the ability of Reovirus Type 3 Dearing to effectively kill local and distal ovarian cancer cells. Not only did the reovirus enhance immune recognition of cancer cells, it also modulated the frequencies of suppressive immune cells, thereby playing a key role in the development of antitumor immunity. In animal studies, reovirus prevents the development of peritoneal carcinomatosis or was shown to alleviate the severity of already developed peritoneal carcinomatosis, thereby significantly prolonging survival [23]. In a human Phase I/II trial, reovirus was detected specifically in tumor cells of patients diagnosed with ovarian and primary peritoneal cancers [24].

Conclusions

The bio-distribution landscape of Reolysin® (16 organs/tissues) and traces of the reovirus transcripts were examined using RT-qPCR over a treatment regime lasting 336 h (Day 15). The findings indicate preferential accumulation/uptake of reovirus RNA in the spleen, heart, ovaries, tail (infusion site), liver and lungs (with bronchi). Unquantifiable RT-qPCR results at the last time point (336 h) suggested substantial to complete clearance of the reovirus genome from the test groups. This data is relevant in the design of future clinical studies utilizing Reolysin®.

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