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Marije Risselada
Purdue University, mrissela@purdue.edu

Robyn McCain
Purdue University, rmccain@purdue.edu

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AJVR

Platinum measurements in Dulbecco's phosphate-buffered saline are lower than in plasma and can be sampled with ultrafiltration probes in vitro

Marije Risselada, DVM, PhD, DECVS, DACVS-SA[1]*, and Robyn McCain, BS, RLATg[2]

[1]Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN
[2]Center for Clinical Translational Research, College of Veterinary Medicine, Purdue University, West Lafayette, IN

*Corresponding author: Dr. Risselada (mrissela@purdue.edu)

OBJECTIVE
To compare the influence of fluid on carboplatin elution, and assess the feasibility of ultrafiltration (UF) probe sampling

SAMPLE
20 samples of 5 mg carboplatin in 1.0 mL 30% poloxamer 407 eluting in Dulbecco's phosphate-buffered saline (DPBS) or canine plasma and 6 samples of UF probe sampling in 0.01 mg/mL carboplatin in DPBS or plasma.

METHODS
Carboplatin-gel specimens in dialysis tubing (12- to 14-kDa pores) were placed in 100 mL of DPBS or canine plasma (37 °C and 600 rpm stirring) in a nonlidded and lidded experiment. Samples were collected in decreasing frequency for 96 hours. The 0.01-mg/mL carboplatin solutions in DPBS and plasma were sampled 6 times by UF probe (30-kDa pores) or direct aspiration. Platinum was measured using inductively coupled plasma mass spectrometry.

RESULTS
High fluid evaporation was noted in the nonlidded but not the lidded experiment. A burst release was seen in plasma (first 2 hours) and DPBS (first 5 hours) with the highest hourly increase in the first hour in both DPBS (6,040 ppb/h) and plasma (2,612 ppb/h), with no further increase after the first 22 hours. Platinum content in the specimens was higher at 96 hours than the surrounding fluid. Higher platinum concentrations were measured by both direct and UF probe sampling in DPBS than in plasma.

CLINICAL RELEVANCE
Platinum concentrations measured in DPBS were higher than in plasma, but elution patterns were similar. Ultrafiltration probes can be used to sample platinum in vitro and could be used in vivo to measure local unbound Pt tissue concentrations in local chemotherapy delivery.

Keywords: elution, in vitro, chemotherapy, plasma, surgical oncology

Local tumor control with wide margins might not always be feasible due to biological behavior, tumor size, or location,[1] and local adjunct chemotherapy might be an additional avenue to consider. Chemotherapy as a local treatment for gross tumors or microscopic residual disease in the tumor bed has been investigated for this purpose.[2–5] Delivery can occur via a carrier agent allowing sustained slow release and providing a high local dose over a prolonged period. Carriers that provide a high local dose and increased exposure of the local tissue while limiting rapid systemic absorption and systemic toxicity are favored.[2,4,6,7] Sustained release formulations of carboplatin have been described for this purpose, and most research has focused on in vitro elution.[8–12] In vitro elution carboplatin studies have mostly been performed in PBS including release from poloxamer 407[8] and release into a gelatin block to mimic tissue.[13] Elution studies in plasma might more closely resemble an in vivo or clinical environment with potential interactions between platinum (Pt) and proteins. Carboplatin binds to proteins (40% to 50%) in vivo although this was not replicated in vitro,[14] and use of inductively coupled plasma mass spectrometry (ICP-MS) to measure Pt in plasma has been validated[15] and used previously.[16,17]

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Determining the duration and tissue concentrations and their duration to then tailor intended treatment concentrations on IC_{50} data would necessitate in vivo measurements. Repeat sampling of tissue concentration has been described in 1 feline study.[18]

Sampling probes might allow to obtain repeat samples of drug released into surrounding tissues from sustained release compounds and be a less invasive alternative than obtaining tissue biopsies. Microdialysis catheters have been used for sampling Pt in vivo in anesthetized mice,[19] but their use in awake rats was unsuccessful due to catheter failure.[20] Ultrafiltration probes have been used successfully in awake dogs[21] to sample carprofen distribution in vivo but have not been described to sample Pt. Their accuracy in sampling Pt has not been compared to directly obtained samples by pipetting nor has their use to sample Pt in the presence of proteins been validated before investigating their use in vivo. The ability to monitor the amount of Pt over time in the wound bed and investigate the properties of sustained-release compounds in vivo would be the ultimate goal.

In this study, we aimed to (1) determine and compare the influence of elution fluid on rate, pattern, and completeness of carboplatin elution in vitro by measuring Pt; and (2) compare ultrafiltration (UF) probe sampling with direct samples. We hypothesized that (1) measured Pt content in plasma was less than in Dulbecco's phosphate-buffered saline (DPBS) after carboplatin elution but that (2) UF sampling would provide similar results as direct sampling.

Methods

Ultrafiltration probe sampling

Two specimens were prepared containing 10 mg/mL of carboplatin (0.3 mL, 0.3 mg; 10 mg/mL carboplatin solution; Accord Healthcare) total in 29.7 mL of either DPBS (Gibco, Thermofisher Scientific) or canine plasma (IGCNPLAK2E; 500 mL canine plasma; lot 41273; Innovative Research) for a fluid concentration of 0.01 mg/mL of carboplatin. The intended Pt concentration was 52,400 ppb with a 5% accepted bioequivalence range (49,780 to 55,020 ppb).[12] Canine Ultrafiltration Probes (BASi Instruments) were assembled per manufacturer instructions,[22] and additional sealing of any connecting points was performed with glue (3-g tube; The Gorilla Glue Company). Probes were tested for patency and sampling using DPBS before use. Vacutainers without additives (3.0-mL vacuette; Greiner Bio-One) were used to collect the probe samples and additional negative pressure was created by removing 15 mL of air from the tube to increase sampling speed. The initial probe sample of the specimen was discarded to avoid dilution of the sample with DPBS. The solutions were agitated before starting the sampling but not in between each sampling. DPBS probe samples and all direct samples were taken at 0, 5, 10, 15, 30, and 60 minutes. The probe sampling the carboplatin in plasma was allowed to collect over 0 to 4 minutes (t = 0 sample), 5 to 9 minutes (t = 5 sample), 10 to 14 minutes (t = 10 sample), 15 to 29 minutes (t = 15 sample), 30 to 59 minutes (t = 30 sample), and 60 to 65 minutes (t = 60 sample) due to the low flow rate of the probe. Samples of stock carboplatin, DPBS, and plasma were taken before the start of the study. All samples were stored at −78 °C until batch analysis.

Nonlidded elution

A 10 multiposition hotplate with magnetic stir bar was used (RT 10; IKA Magnetic Stirrers) at 37 °C and 600 rpm and placed in the middle of a fume hood (Safeaire; Hamilton Industries) per the instructions of the Institutional Biosafety Officer (submission of a written protocol and approval by the Institutional Biosafety Committee not required). Three 150-mL crystallizing dishes (Synthware glass) were filled with 100 mL DPBS without calcium chloride or magnesium chloride added (Gibco, Thermofisher Scientific) and 3 contained canine plasma (IGCNPLAK2E; 500 mL canine plasma; lot 42002; Innovative Research). The 3 dishes with DPBS were placed on the row closest to the hood opening, and the ones with plasma were placed further in the hood. All fluids were prewarmed on the hot plate for 1.5 hours before starting the elution study. Temperature and humidity in the hood were recorded at each sampling time point (temperature and humidity gauge; Antonki). All handling was performed double gloved and a protective gown was worn.

Six carboplatin-gel elution specimens were prepared. Each contained 5 mg carboplatin (9.51 mg Pt) (0.5 mL of a 10-mg/mL carboplatin solution; Accord Healthcare) in 1.0 mL 30% poloxamer 407 (Pluronic F-127; Sigma-Aldrich). The specimens were prepared in individual 3.5-mL syringes < 2 hours before use and stored refrigerated and shielded from light. Six precut 10-cm lengths of a 1-inch dialysis tube with 12- to 14-kDa pores (Carolina Biological Supply Co) were placed in distilled water for 1 hour. The distal end was folded in half along the long axis and then folded over for a length between 5 and 10 mm. Two alternating large stainless-steel clips (Hemoclips Teleflex) were placed to close that end. The free end was gently digitally manipulated and rolled to separate the near and far sides and loosen the tube. Immediately before instilling the carboplatin gel, the free end of the tube was moistened in distilled water and the tube was gently fed over the tip of the 3.5-mL syringe until the 1-mL mark. The carboplatin gel was slowly injected into the tube and allowed to move to the distal part of the tube by gravity. The free end of the tube was folded over and closed with hemoclips in a similar fashion to their application at the distal end. All 6 specimens were prepared in a sampling order of DPBS (labeled A, B, and C) and then plasma (labeled A, B, and C) and placed on a sheet of aluminum foil to avoid the tube membrane sticking to the surface. Specimens were placed in the prewarmed DPBS or plasma within 5 minutes after assembly, and the t = 0 sample was taken immediately

after. Additional samples (0.15 mL) were taken over 96 hours with a decrease in frequency (1, 2, 3, 4, 5, 6, 8, 10, 13, 17, 22, 27, 34, 42, 48, 58, 66, 72, and 96 hours). A sample (by needle aspiration) of the fluid contained within the dialysis tube was taken at 96 hours. The dishes were covered by aluminum foil always folded over the edges except when sampling. The 3 dishes containing DPBS were lined up in front, and the 3 dishes containing plasma along the back, with the stirrer plate lengthwise aligned with the hood opening.

Lidded elution

All specimens (D, E, and H) were prepared, and fluids (DPBS; canine plasma; lot 42355) were prewarmed in a similar fashion as for the dish elution study, including placement within the hood and positions on the plate. Twelve-ounce straight-sided glass jars with screw-on caps (S-22916P-W; ULine) were used for this study. Sampling times were identical to the nonlidded elution study.

Sample and data analysis

The quantity of Pt in each sample was determined via ICP-MS (Perkin Elmer NexION 300D) as previously described.[19] The short-term precision was less than 3% relative standard deviation (RSD), and the long-term stability was 4% RSD over 4 hours. Isotope-ratio precision was less than 0.08% RSD. DPBS samples were diluted in 2% HNO_3 and plasma samples were digested overnight at 70 °C in a 1:1 mixture of 70% HNO_3 and H_2O_2 and analyzed using ICP-MS to determine the concentration of Pt within each sample. The Pt detection limit was 0.07 ng/mL, with the quantification limit at 0.2 ng/mL, and all samples below this limit were recorded as 0 ng/mL. Platinum concentrations are expressed in parts per billion, with 1 ppb = 1 ng/mL. Hourly increase in Pt was calculated as the difference between the measured values at 2 subsequent time points divided by the hours between time points (expressed as ppb/h). A log transformation of the calculated hourly increase was performed in Graph Pad Prism 10.0.0 (GraphPad Software) for additional kinetic elution analysis.[23] The repeat sampling values will be reported as individual results and a mean ± SD (RSD) in an observational manner without statistical analysis. The RSD was calculated by dividing the SD by the mean and will be expressed as a percentage. The elution data are expressed graphically as mean ± SD of the 3 elution specimens.

Results

Ultrafiltration probe sampling

Ultrafiltration probes were able to provide samples containing Pt. Results between time points and method of sampling varied **(Table 1)**. One measured Pt value in the first DPBS sampling series ("first experiment") was ~1.5 times the calculated concentration and this part of the experiment was repeated ("second experiment") to rule out any constitution or dilution errors. The remainder of the first experiment (plasma) and the repeat experiment (DPBS) were within a 5% bioequivalence margin[12] and used for comparisons and further analyses. Both UF probe and pipette sampling underestimated the plasma Pt concentration, but reported DPBS Pt concentrations in the second experiment were close to the anticipated value. The RSD for probe sampling was relatively higher than for pipette sampling for both DPBS and plasma and relatively greater for plasma than DPBS samples (Table 1).

Nonlidded elution

The carboplatin gel was liquid at the time of specimen preparation. No leakage was observed during installation or on the aluminum foil before placement. Temperature and humidity in the hood ranged from 65.6 to 66.7 °C and 30% to 38% during

Table 1—Repeat sampling of 0.1 mg/mL carboplatin (52,400 ppb) in DPBS or plasma expressed as Pt in parts per billion.

Repeat sampling of Pt (ppb)	DPBS				Plasma	
	First experiment		Second experiment		Only experiment	
Time (min)	UF probe	pipette	UF probe	pipette	UF probe	pipette
0	10,929.36	48,631.00	42,926.20	32,596.09	10,670.93	9,809.38
5	17,473.44	49,556.99	35,250.82	41,400.79	7,074.61	6,751.51
10	39,320.80	47,398.88	54,346.19	43,129.39	6,896.44	12,137.03
15	41,657.25	43,548.20	54,171.41	46,317.65	20,952.93	12,023.12
30	32,595.57	81,813.46*	45,181.19	53,643.58	37,981.62	9,921.46
60	43,754.05	53,349.78	33,654.50	43,078.42	13,575.75	25,323.27
Mean ±SD (RSD)	30,955.08 ± 13,666.09 (44%)	48,496.97 ± 3,549.20 (26%)**	44,255.05 ± 8,902.10 (20%)	43,360.98 ± 6,845.91 (16%)	16,192.06 ± 11,868.57 (73%)	12,660.97 ± 6,504.97 (51%)

Direct samples were taken using a pipettor at the time point. Ultrafiltration (UF) probe samples were taken by attaching a vacutainer to the probe for 4 minutes at the start time.

DPBS = Dulbecco's phosphate-buffered saline. Pt = Platinum. RSD = Relative standard deviation.

*Measured value higher than the actual concentration and outside a 5% bioequivalence range (11) (49,780 to 55,020 ppb); the experiment in DPBS was repeated in a second session for both sampling methods. **For completeness, the mean ± SD (RSD) of the first experiment with the outlier data point removed is provided separately.

the elution study. At 48 hours, 1 of the specimens in DPBS was no longer fully submerged nor fully filled. The specimen was removed and sampled, and the fluid amount in the dish was measured (11.0 mL, specimen C) and sampled. At 58 hours, the remaining 2 specimens in DPBS were removed due to similar elution fluid decreases (34.0 mL DBPS remained for specimen A and 31.0 mL for specimen B). The 3 plasma elution specimens remained in the study until 96 hours and had 62.0 mL, 35.0 mL, and 48.0 mL of plasma left for specimens A, B, and C. Only endpoint Pt values were reported for this experiment **(Table 2)** and were not used for elution and release analysis.

Lidded elution

Hood temperature and humidity ranged from 63.2 to 67.9°C and 30% to 64%. Residual volumes at 96 hours were 86.5 mL, 84.5 mL, and 85.5 mL of DPBS and 86.0 mL, 86.5 mL, and 85.5 mL of plasma, with a loss/hour between 0.14 and 0.16 mL including sampling and fluid that accumulated within the dialysis tube (Table 2). All dialysis tubes were fully filled at the end of the experiment and did not leak when manipulated with gentle pressure. All tubes contained more Pt than the surrounding fluid at 96 hours **(Figure 1)**. The elution curves had a similar shape for both plasma and DPBS with elution occurring for

Table 2—Platinum at elution endpoints expressed as parts per billion.

DPBS				Plasma			
Time (h)	**Fluid volume (mL)**	**[Pt] in fluid (ppb)**	**[Pt] in specimen (ppb)**	**Time (h)**	**Fluid volume (mL)**	**[Pt] in fluid (ppb)**	**[Pt] in specimen (ppb)**
Nonlidded experiment*				Nonlidded experiment*			
58*	34.0*	7,336.83*	96,966.27*	96*	62.0*	14,991.59*	3.52*
58*	31.0*	7,965.11*	27,495.43*	96*	35.0*	25,654.64*	1.49*
48*	11.0*	13,429.26*	191,962.83*	96*	48.0*	21,103.50*	1.04*
Lidded experiment				Lidded experiment			
96	86.5	9,182.05	40,749.36	96	86.0	5,214.04	35,794.99
96	84.5	9,479.03	34,883.89	96	86.5	4,319.46	31,354.52
96	85.5	11,327.34	38,031.56	96	85.5	4,464.09	20,171.92

Each tube contained 1,746,000 ppb Pt at the start of the experiment. Both the nonlidded and the lidded experiment endpoint data are shown: end time (hours), end volume (mL), and Pt concentration of the fluid and in the remaining sample.

*Data are reported for completeness only. For the lidded experiment, the carboplatin had not fully eluted into plasma at the 96-hour time point. All dialysis tubes were fully filled with fluid and did not leak upon manipulation. This fluid is not counted toward the residual elution fluid volume.

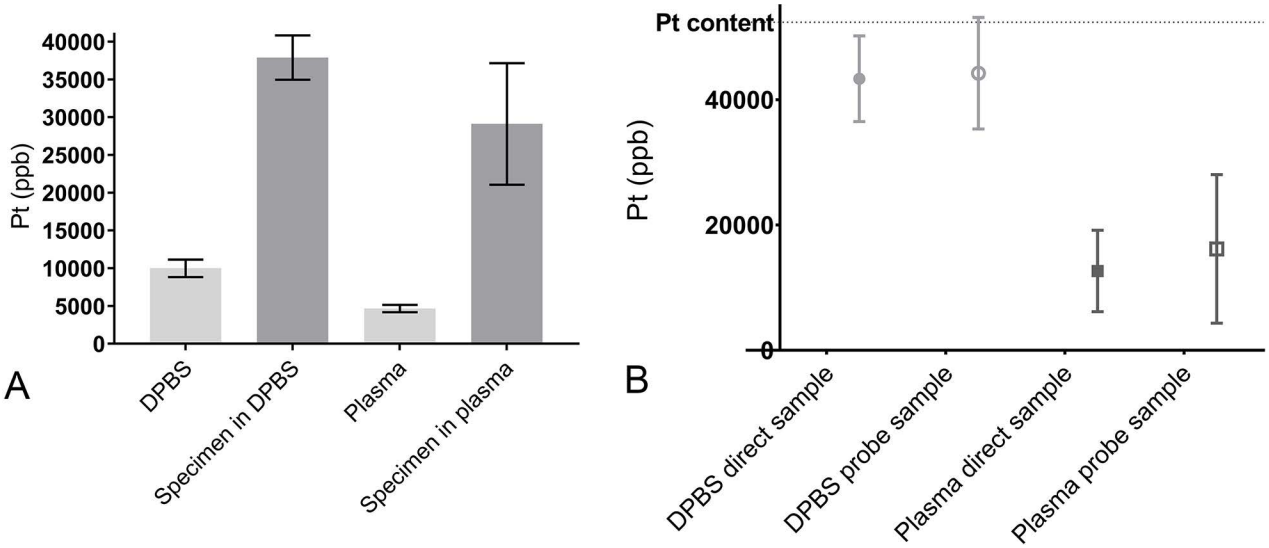

Figure 1—A—Mean ± SD of measured platinum (Pt) at 96 hours expressed as parts per billion. Each tube contained 1,746,000 ppb Pt at the start of the experiment. The carboplatin had not fully eluted into plasma at the 96-hour time point. B—Mean ± SD of 6 repeat samples (dotted line indicates the calculated Pt concentration of 52,400 ppb) of plasma sampling and the second Dulbecco's phosphate-buffered saline (DPBS) sampling experiment.

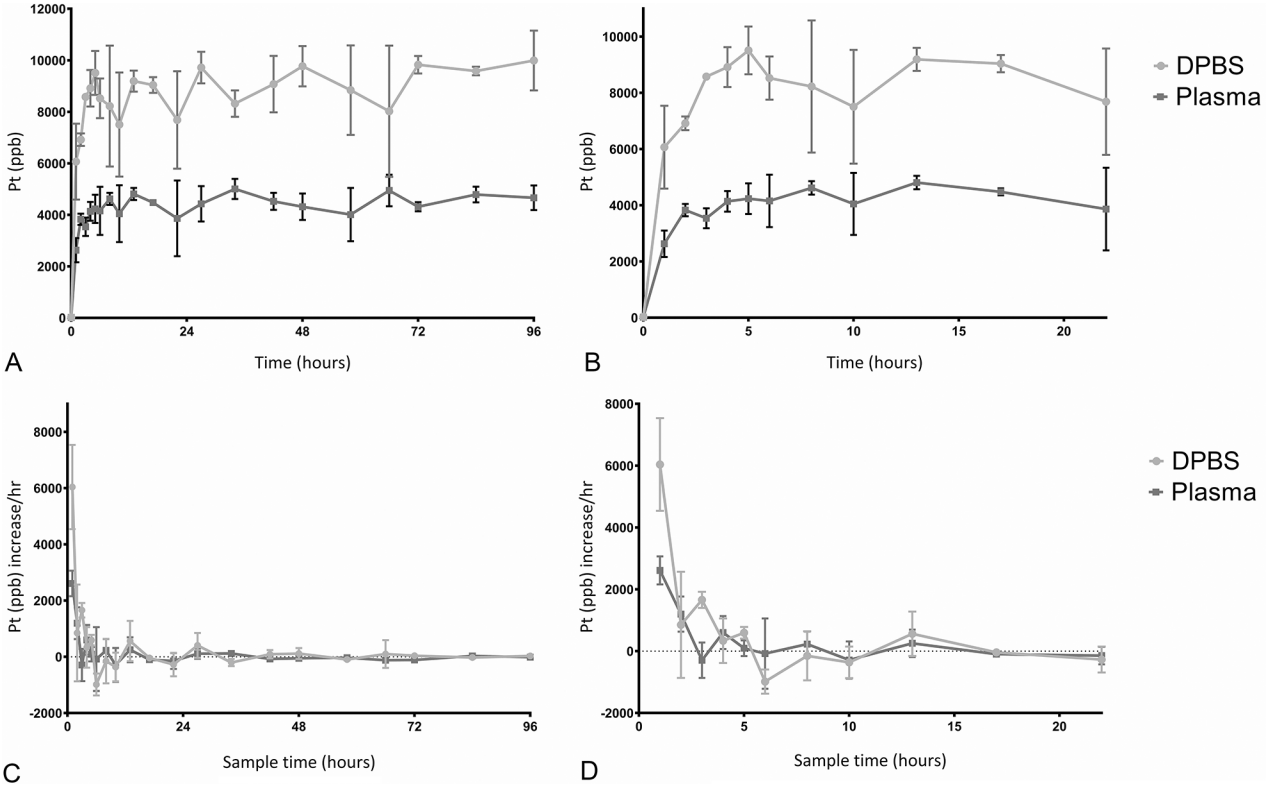

Figure 2—A and B—Elution of Pt into either DPBS or plasma, expressed as parts per billion over 96 hours (A) or the first 22 hours (B). The elution of Pt into DBPS tapered at 3 hours but continued up to 5 hours depicting by the flattening of the curve, whereas increase of Pt in plasma tapered at 2 hours, with the curve flattening thereafter. C and D—Hourly increase of Pt in the fluid over the full experiment (C) or the first 22 hours (D) as parts per billion per hour. The highest hourly increase was in the first hour for both DPBS (6,040 ppb/h) and plasma (2,612 ppb/h). The graphs indicating the plasma elution data are in dark gray and DPBS in lighter gray.

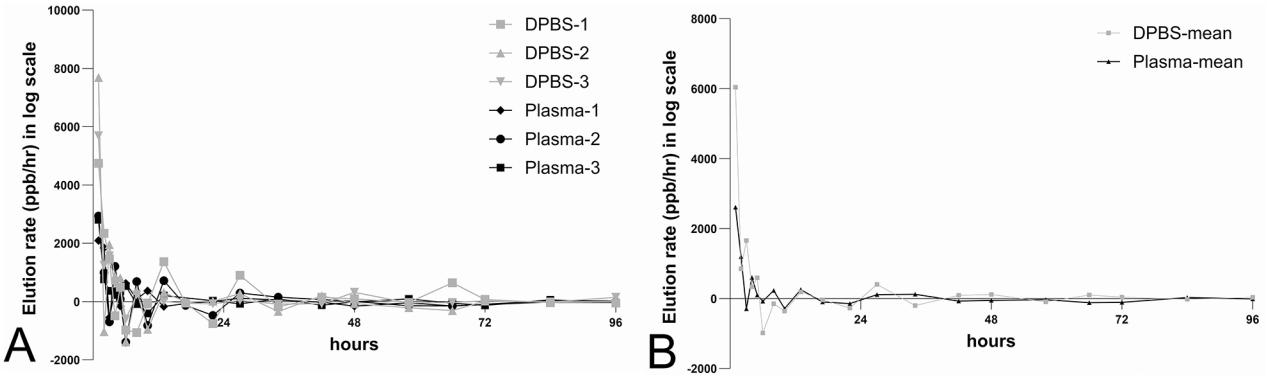

Figure 3—A and B—Log transform representation of hourly Pt into either DPBS or plasma, expressed as parts per billion per hour of all specimens (A) or the mean for either DPBS or plasma (B). The highest hourly increase was in the first hour for both DPBS (6,040 ppb/h) and plasma (2,612 ppb/h). The graphs indicating the plasma elution data are in black and DPBS in lighter gray.

5 hours with curve flattening thereafter. A high initial release was seen for both, with the highest hourly Pt increase occurring in the first hour **(Figures 2 and 3)**: 6,040 ppb/h for DPBS and 2,612 ppb/h for plasma.

Discussion

Sampling of Pt via UF probe was possible. However, measured Pt in samples obtained via UF probe had more variation than those obtained via

direct pipette sampling. Platinum in DPBS samples more closely approximated the expected Pt content than those from plasma. Similarly, measured Pt concentration during the elution study was higher in DPBS than in plasma, although both curves were similar in shape, and elution timing and rate followed a similar pattern.

The concentration of Pt measured in the plasma samples obtained both by UF probe and pipette sampling was lower than the expected concentration. This might be explained by the binding of the Pt to proteins in plasma, although in vitro binding prior was not proven to be similar to in vivo binding, and nitric acid has been shown to unbind Pt from proteins.[24] However, protein binding of Pt might inhibit the bound Pt from being sampled by the UF probe as the membrane is permeable for molecules up to 30-kDa molecular weight, with canine albumin having a reported molecular weight of 66 to 69 kDa.[25] While UF probe sampling was possible, it represents the ultrafiltrate of the fluid and might therefore reflect the unbound fraction of Pt. The discrepancy between the measured Pt in DPBS versus plasma could have been caused by incomplete mixing or separation of larger molecular weight proteins, as the specimens were not constantly agitated during the 60-minute repeat sampling experiment. In addition, it might be possible that there was a variation in the amount of Pt added to each fluid. Duplication of the repeat sampling study could be beneficial as variation between fluids might occur. However, given that each fluid was sampled several times for each method, we felt that the single fluid model could be used to show intrasampling variation between the 2 sampling methods.

A 1:2 carboplatin:poloxamer 407 volume ratio mix was used as the elution specimen in this study, compared to a 1:1 volume ratio in a prior elution study.[8] The 1:2 ratio was chosen to ensure full gelatination even though in prior studies[8,16,26] a 1:1 ratio did fully gel. However, a companion silver elution study[27] was performed with an identical ratio and volumes, and using identical poloxamer ratios might allow direct comparison of elution between different compounds mixed with poloxamer. Some variables were different in this study compared to a prior study[8] using a dialysis membrane (100 mL vs 500 mL of elution fluid), which could impact results. The fluid volume was chosen due to the cost of canine plasma ($2,400/500 mL). A 1:5 amikacin:poloxamer specimen was assessed in an elution study[28] with a different methodology, and fluid concentration was found to peak at 48 hours. Individual specimens (12 mL) in this study were directly exposed to PBS (1 mL), and the fluid was not stirred or agitated. In our study, a dialysis tube was used with 12- to 14-kDa pores, which would allow Pt to move freely but would prohibit proteins from moving into the tube and binding with Pt. However, the unbound Pt could still bind to proteins outside of the dialysis tube, which potentially could explain the lower Pt recovered from the plasma samples in the elution study. In addition to this, EDTA was used as an anticoagulant for plasma, and EDTA has known chelator capabilities as well as being used clinically to remove and excrete plasma toxins.[29] The effect of EDTA on measured Pt in vitro and this study is unknown, as ICP-MS has been used to measure pre- and postchelation therapy metals in urine in people.[30] In addition, nitric acid digestion has been described as a method to free chelated Pt.[31] Measured values should therefore include bound and chelated Pt.

Evaporative fluid losses were higher than expected during the initial nonlidded elution experiment. This might be due to placement in a ducted hood for personnel safety, with the glass shield closed in between sampling and continuous airflow. Evaporative fluid losses were higher in DPBS than plasma, with several specimens no longer submerged in the fluid at various time points during the experiment. Adding fluid was considered but would have influenced the outcome, and stopping the individual specimen elution at that time point was chosen. Given the variation in time points and duration of sampling, we opted to repeat the experiment with screw-on lids. However, we did measure the endpoint concentrations in both the specimen and fluid and reported the findings to illustrate the influence of evaporative fluid loss on elution experiments. The concentrations in both the fluid and in the earlier removed specimens were higher, indicative of evaporative fluid loss in both the specimen and surrounding fluid, as well as incomplete elution of the specimen. The location of the project in a ducted hood for personnel safety coupled with low humidity within the hood might have led to a more rapid evaporation than any prior experiments. Environmental influences on any future elution studies should be kept in mind to minimize their effect by using a fully closed container if larger evaporative fluid losses are expected. The difference between DPBS and plasma could be due to the nature of the fluid itself, although positioning within the hood (DPBS closer to the opening of the hood) and therefore airflow could play a role as well.

Additional limitations to this study are the limited number of specimens that were assessed in the elution study with only 1 sample at each time point. We used a small volume (100 mL) for elution for cost reasons, and high losses due to sampling might have a more pronounced effect compared to when a larger maintained volume would be used. We opted to keep the sampling volume as low as possible and to not repeat sampling in this part of the experiment due to fear of creating more of a gradient by removing additional sampling volume. A larger gradient might also have increased the concentration difference and influenced the speed of carboplatin release and migration across the dialysis membrane. Duplication of the repeat sampling study could be beneficial as variation between fluids might occur. However, given that each fluid was sampled several times for each method, we felt that the single fluid model could be used to show intrasampling variation between the 2 sampling methods.

The results in both plasma direct sampling and the elution experiment might be a truer reflection of

in vivo environment than DPBS. Consideration could be given to using plasma for in vitro experiments involving compounds with known protein binding properties, especially when looking at therapeutic properties and availability or for a more direct comparison to in vivo circumstances. However, if the experimental intent is to observe elution and release properties from various carriers, then PBS or DPBS could still be a valuable and cheaper alternative, as the patterns were similar between DPBS and plasma, and DPBS can be used in vitro to investigate patterns. Sampling via UF probe could be a valid in vivo option to measure tissue unbound Pt concentrations after local carboplatin delivery.

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Author contributions: MR—Study design, methodology troubleshooting, data collection, writing; RM—methodology troubleshooting, data collection, approve of final draft.

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