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Structured Abstract

Objective:

To assess the effect of transfusion using a syringe and microaggregate filter on short-term survival and circulating half-life of autologous feline RBCs.

Design:

Prospective, internally controlled, observational study.

Setting:

A University Teaching Hospital

Animals:

Six apparently healthy, owned pet cats.

Interventions:

Blood collection by jugular venipuncture. Transfusion with labeled, autologous, fresh red blood cells.

Measurements and Main Results:

Anticoagulated whole blood (35 ml/cat) was collected in two equal aliquots. RBCs were washed and labeled at two different biotin densities, before suspension in autologous plasma. Labeled RBCs were then transfused using two methods, gravity flow and pump delivery using a 20ml syringe and 18 μm microaggregate filter.

Whole blood samples were collected from each cat at 2 hr intervals for 12 hours following completion of the transfusions. Additional samples were collected at weekly intervals up to 6 weeks to assess circulating half-life of the transfused cells. Cell survival was assessed via flow cytometry. The proportion of transfused cells remaining in each

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of the two populations was measured. Quantitative changes in the two populations over time were assessed by two-way repeated measures ANOVA.

Biotinylated RBCs were readily detected in all cats over the 6-week sampling period. There was a significant decrease in both populations of labeled cells over the 6-week period ($p<0.01$), as expected. There was no difference in probability that the RBCs would survive up to 12 hours immediately following transfusion, and no significant difference in survival between the two groups over 6 weeks. The average half-life of all labeled cells was approximately 23 days.

Conclusions:

We conclude that, in contrast to findings from dogs, transfusion of autologous feline RBCs using a syringe + aggregate filter method does not significantly impact short- or long-term survival of the transfused cells.

Keywords: mechanical perfusion, blood administration, cats

Abbreviations: RBC, red blood cell; CPDA-1, citrate phosphate dextrose adenine; PBS, phosphate buffered saline; EDTA, ethylenediaminetetracetic acid;

Introduction

It has previously been reported that administration of autologous canine red

blood cells (RBCs) via mechanical pump mechanisms, particularly syringe and

aggregate filter methods, is associated with a high risk of early loss of transfused RBCs

in dogs.[1] In the previously reported study the greatest effect on short-term survival of

transfused RBCs was seen when cells were administered via a syringe pump and an

aggregate filter (Hemo-Nate™). This method is most commonly used for the

administration of small volumes of transfusate, and is particularly commonly used when

transfusing cats and very small dogs. Feline RBCs are markedly smaller than canine

RBCs (MCV of felines is generally reported as 40-55 fl, vs. 55-65 in the dog), while

showing a greater increase in stiffness under hypoxic conditions.[2, 3] Given the

hemorhelogical differences between canine and feline RBCs, and the markedly lower

gross transfusion rates (i.e. total ml/min delivered) in the cat in comparison to the

medium to large breed dogs used in the previously published study of canine RBC

survival following transfusion, the applicability of the previously reported study to feline

transfusion medicine may be questioned. The objective of the study reported here was

to carry out transfusion studies of autologous feline red blood cells, using essentially the

same biotin-labeling and flow cytometry method previously used in the dog, to assess

the effect of transfusion technique on short-term survival and circulating half-life of

autologous RBCs in the cat.

Materials and Methods

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Subjects

Six privately owned domestic cats were volunteered by staff, students and faculty of the teaching hospital for participation in this study. Cats were required to have a minimum body weight of 4.5 kg and had no concurrent medical issues. All cats were housed with their owners, except for one overnight stay following blood collection and transfusion. All cats were in good health and up to date on recommended vaccinations, based on owner-reported histories and physical examination. Animal care and use was overseen by our Institutional Animal Care and Use Committee.

Red Blood Cell Labeling

Red blood cell collection and labeling was performed essentially as previously described,[1] with some minor modifications detailed below. Briefly; whole blood (35 ml/cat) was collected by jugular venipuncture using standard aseptic blood draw techniques. Blood was collected using 18 ga 'butterfly' catheters[a] and 2 x 20 ml syringes[b] each containing 2.5 ml CPDA-1[c]., giving a final ratio of CPDA-1:whole blood of 1:7. Anticoagulated whole blood (2 x 20 ml aliquots/cat) was transferred to sterile 50ml polyethylene centrifuge tubes and centrifuged at 2000 x g relative centrifugal force for 10 minutes. The plasma layer was aspirated and saved under refrigeration in sterile 50 mL polyethylene tubes, being brought back to room temperature (approximately 25°C) before reconstitution. Saved plasma was eventually used to reconstitute red blood cells (RBCs) after biotin labeling. The RBCs were washed 3 times with a sterile filtered[d] phosphate-buffered saline (PBS, pH 7.4[e]) wash buffer containing 11.1 mmol glucose. Washed RBCs were then suspended in sufficient wash buffer to yield a 25% suspension

of RBCs. Cells in each aliquot were labeled using biotin-X-NHS,[f] prepared as a stock solution at a concentration of 2 mg/mL in PBS following initial suspension in dimethylsulfoxide.[g] The biotinylation buffer was adjusted to pH 5.0 with concentrated HCl[h] just prior to dissolution. Cells were biotinylated with either 25 or 150 μg biotin/ml of RBC suspension, these concentrations having previously been found to yield two distinct peaks on flow cytometry in preliminary experiments (data not shown). Cats were randomly allocated to have either 'low' or 'high' biotin densities applied to the cells that were to be transfused via syringe pump.

Cells were biotinylated for 30 minutes with continuous gentle agitation, the biotinylation reaction was then terminated by addition of a five-fold volume of wash buffer, and the cells were washed four times in the PBS wash buffer. Following the last wash step the cells were suspended in autologous plasma and transferred to either a 20 ml syringe or 50 ml blood storage bag.[i] Labeled cells were stored overnight at 4˚C.

Transfusion Techniques

The day following blood collection and labeling all cats were transfused with their own, labeled RBCs. Intravenous catheters (22 ga)[j] were placed in a cephalic vein, then each cat was transfused in random order using either a syringe pump[k] + microaggregate filter[l] or via a standard blood-giving set (10 drops/ml)[m] using gravity. Blood was transfused at 2 ml/kg/hr via syringe pump setting, while administration rates were regulated via drop counting and adjustment for the gravity-administered cells. Gravity administration rates achieved were variable, with most cats receiving the

gravity-administered cells over a period of approximately 60 minutes (an overall rate of approximately 4ml/kg/hr for the typical 4.5 kg cat in this study). Cats were visually monitored continuously during all transfusions, blood pressure was not monitored. No apparent distress was noted from any cat at any time during transfusion.

Post-Transfusion Sampling

Blood samples (1ml) were collected immediately after completion of the transfusions, and then at two-hour intervals until 12 hours after transfusion. Samples were collected by jugular venipuncture using standard aseptic technique and preserved in EDTA-containing microtubes.[n] Whole blood was stored overnight at $4°C$ before processing for flow cytometry the following day.

Further blood samples (1ml) were collected from each cat at seven-day intervals for a total of 6 samples. All samples from the weekly collections were processed for flow cytometry on the day of collection.

Detection of Labeled Red Blood Cells

From each sample, 5 μL of whole blood was transferred to a microcentrifuge tube and red cells were washed twice using the previously described PBS-based wash buffer. The supernatant was removed and the RBCs suspended with 100μL of PBS wash buffer, 4.0μL of streptavidin-phycoerythrin[o] (1mg/mL stock solution) was added and the cells were agitated for 30 minutes at 37 °C. The final working dilution of streptavidin-phycoerythrin was 1:25. Streptavidin-phycoerythrin conjugate was used in

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this study rather than streptavidin-fluorescein as in the original technique described in dogs,[1] as preliminary experiments showed that the streptavidin-phycoerythrin conjugate gave better separation of labeled peaks from the autofluorescence of unlabeled feline RBCs.

Following conjugate incubation the cells were removed from the agitator and the reaction terminated by addition of 1000μl of phosphate buffer wash, the cells were then washed twice in the PBS-based wash buffer. The supernatant was removed, 1000 μL of phosphate buffer was added, and the cells were transferred to 5 mL tubes to which an additional 1000μL of PBS was added before analysis.

Biotin-labeled cells were analyzed using flow cytometry.[p] Five hundred thousand cells were evaluated per sample and number vs fluorescence plotted on a $\log_{10}$ scale. Two gates were assigned to quantify the 2 separate population peaks, to allow quantification of each population over time. These gates were applied consistently to all populations throughout the sampling time (Figure 1a,b).

Statistical Analyses

Data were analyzed using an open source statistical programming environment.[4] Red cell population data were analyzed using a general linear model to model expected variables (time and transfusion method) with the cats as a blocking factor, followed by 2-way analysis of variance with time and transfusion method as explanatory variables. Post-hoc analyses were carried out with Tukey's Honest Significant Difference Test. The R-language modules used for analysis were lm, anova and TukeyHSD. Data for red

cell survival in the immediate twelve hours post-transfusion were analyzed separately from the data from weekly sampling. For all analyses a P value of <0.05 was considered significant. Data from 5 cats were analyzed in the gravity administration group, and six cats in the syringe+filter group, due to failure to identify the gravity administered population in one cat beyond one week.

Results

All cats admitted to the study were neutered males, three were domestic short hairs and three were domestic medium hair breeds. The median age of the cats was 5 years (range 3-9 years).

Labeled RBCs were readily detectable in all samples from all cats. Quantitative recovery of cells was consistent for both transfusion techniques, with each labeled group containing an average of 10.63% (SD 1.63%, range 7.8-12.6%) of the red blood cells counted across all cats in the first post-transfusion sample. Assuming a blood volume of 40ml/kg/cat, the quantitative recovery of labeled red cells was close to expectations (i.e. 17.5 ml blood collected in each population represents 8.75% of the blood volume). One cat showed loss of the RBC population delivered by gravity at one week post-transfusion, all other cats had readily identifiable, distinct populations from both transfusion methods at all time points. There was no significant effect of transfusion method on short-term (Figure 2) or long-term (Figure 3) survival of transfused cells. The mean half-life of all transfused cells was 23 days.

Discussion

The data presented here suggest that, in marked contrast to previously reported findings in dogs,[1] the use of a syringe + microaggregate filter system to administer autologous red blood cell transfusions to cats is not associated with an increased risk of either short-term (<12 hrs) or long-term (up to 6 weeks) accelerated loss of transfused cells. There was no difference in survival attributable to transfusion method in this study (Figures 2 and 3). The overall average half-life of all transfused cells in this study was 23 days. The observed half-life in this study was slightly lower than, but generally consistent with, previously published values of 29-39 days for allogenic and autologous feline RBC transfusions.[5] Preparation of the cells and labeling for this study required substantial in vitro manipulation, which may account for the slightly lower half-life observed.

The influence of transfusion technique on transfused cell survival has been investigated in human medicine by a variety of groups, using several different methods for transfusion, however in most cases these have been in vitro studies that investigate immediate changes in RBC parameters such as osmotic fragility rather than in vivo survival of the cells. Studies of neonatal transfusion techniques have the greatest applicability to this study, as these low volume, low rate transfusions are most similar to the transfusion of feline patients. In one study comparing three separate methods of transfusion (syringe pump without aggregate filter, peristaltic pump and a novel "shuttle" pump mechanism), all three techniques were found to result in significant hemolysis of

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stored human red blood cells, and increased plasma hemoglobin content in the transfusate.[6] The previously cited study used stored human packed RBCs of varying ages, and substantial hemolysis was detected in the RBC units even before transfusion. The duration of storage, and the development of storage lesions in the stored packed RBCs may have increased the susceptibility of these cells to hemolysis.[6] In the study reported here the feline RBCs were stored for a more limited period (overnight, a maximum of 12 hours for any cat), and thus the cells used in the current study were less likely to have suffered the development of significant storage lesions. Interestingly, however, at least some workers have reported a decrease in osmotic fragility in RBCs after delivery through mechanical administration and filtration systems.[6, 7] These authors hypothesized that the more "fragile" RBCs resulting from the development of storage lesions were destroyed during the mechanical delivery process, leaving only those with a lower susceptibility to osmotic damage in the final transfusate. The authors are unaware of any data in the literature regarding the development of storage lesions in feline RBCs. The study reported here used autologous RBCs with short storage times, a situation that is quite different to a situation where the patient receives cells from a donor cat that may have been stored prior to administration. The potential impact of mechanical administration systems on survival of stored, allogenic feline RBCs following transfusion is unknown at this time, but is an area of significant interest.

The transfusion rates used in this study, 2ml/kg/hr for the syringe+pump system and higher, more variable, rates from the gravity supplied systems, were chosen to replicate as closely as possible the technique previously used in dogs,[1] and represent

the highest initial administration rates for transfusions under our hospital's standard

operating procedures. Some authors have suggested starting feline transfusions at

lower rates, as low as 0.25ml/kg/hr for the first 30 to 60 minutes.[8] Interestingly, lower

flow rates induce greater damage to human red blood cells, while needle gauge, tubing

length and tubing diameter had no effect.[9] If these findings hold true for feline red blood

cells, the rate used in this study would be expected to increase the likelihood of red

blood cell damage during transfusion, yet we found no evidence of RBC damage or

altered RBC half-life that could be attributed to transfusion technique.

One cat in the study reported here showed early loss of RBC's in the population

administered via gravity flow, with this population having been lost at one week post-

transfusion. We saw no indications of abnormalities or differences with the transfusion

of this cat, and all reagents used on this cat were also used in other cats.

Given the finding of significant and rapid loss of transfused RBCs in dogs

following administration using the microaggregate filter, the overall aim of the study

reported here was to attempt to replicate this finding in feline patients. The dramatic

difference in effect of the microaggregate filter on feline RBC survival, while reassuring

from a standpoint of clinical utility, was a somewhat unexpected finding. While the use

of the microaggregate filter as a post-syringe, inline filter is common in feline transfusion

practice and specifically recommended by some authors,[8] this is actually contrary to the

manufacturers recommendations that cellular material be aspirated through the filter into

a syringe, then administered after the filter is removed.

Given the lack of difference in red cell survival and half-life with differing administration techniques in this study, the common practice of using these filters inline does not appear to be contraindicated in feline patients. In the case of dogs, however, our opinion remains that this technique should be avoided even in the very small patient.

Figure Captions

Figure 1(a,b) – Representative plot of $\log_{10}$ fluorescence (X axis) vs cell count from the same feline subject's whole blood samples one week (a) and 6 weeks (b) after transfusion with two populations of autologous red blood cells biotin-labeled at two distinct densities. The gates C and D encompass the low and high biotin density populations, respectively.

Figure 2 – Labeled red blood cell survival in cats (relative to 100% at the first sample) in the immediate 12 hours following transfusion using two methods, syringe pump + microaggregate filter (n=6) and gravity delivery (n=6). Lines show a local least squares regression line, while ribbons show the 95% confidence interval of the regression lines. There was no significant effect of transfusion technique on red blood cell survival.

Figure 3 - Labeled red blood cell survival in cats (relative to 100% at the first sample) over a 6 week period following transfusion using two methods, syringe pump + microaggregate filter (n=6) and gravity delivery (n=5). Lines show a local least squares regression line, while ribbons show the 95% confidence interval of the regression lines. There was no significant effect of transfusion technique on red blood cell survival, the mean half-life of the labeled cells is 23 days.

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Footnotes:

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[a] Terumo Surflor® Winged Infusion Set, Terumo Corp, Tokyo, Japan
[b] Monoject 20 ml Luer-slip syringes, Covidien, Mansfield, MA
[c] Baxter Healthcare, Deerfield, IL
[d] 0.2 μm, 47 mm filter, Pall Life Sciences, Ann Arbor, MI
[e] BuPH Phosphate Buffered Saline Packs, Thermo Fischer Scientific, Rockford, IL
[f] Calbiochem/EMD Chemicals, Gibbstown, NJ
[g] Burdick & Jackson, Muskegon, MI
[h] Calbiochem/EMD Chemicals, Gibbstown, NJ
[i] Animal Blood Bank, Dixon, CA
[j] BD Insyte-W, Becton-Dickinson, Franklin Lakes, NJ
[k] AS50, Baxter Healthcare, Deerfield, IL
[l] Hemo-Nate filter, Utah Medical Products, Midvale, UT
[m] INTERLINK Straight type blood set, Baxter Healthcare, Deerfield, IL
[n] BD Microtainer Tubes with EDTA, Becton-Dickinson, Franklin Lakes, NJ
[o] Streptavidin B-Phycoerythrin conjugate, Molecular Probes, Grand Island, NY
[p] Becton-Coulter FC500 Flow Cytometer, Beckman-Coulter Inc, Miami, FL