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STANDARDIZED PROTOCOLS FOR PLASMA CLEARANCE OF IOHEXOL ARE NOT APPROPRIATE FOR DETERMINATION OF GLOMERULAR FILTRATION RATES IN ANESTHETIZED CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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Abstract: Plasma clearance of iohexol was evaluated in eight anesthetized California sea lions (*Zalophus californianus*), without evidence of renal dysfunction, to determine if the one-compartment model and the sample protocol used in dogs and cats could be applied to this species. Nonlinearity between samples in 75% (6/8) of sea lions voided those results. An additional two anesthetized sea lions were sampled at 5, 30, 45, 60, 120, 180, 240, and 360 min post iohexol injection and semi-logarithmic curves calculated. Plasma iohexol clearance values calculated by one-, two-, and noncompartment models were in poor agreement, suggesting that the standardized protocol described for dogs and cats cannot simply be applied to California sea lions, probably due to the effects of the dive reflex induced during anesthesia.

Key words: Renal dysfunction, renal disease, kidney, iodinated contrast medium, contrast media reaction.

BRIEF COMMUNICATION

Diminished renal function may occur in California sea lions (*Zalophus californianus*) in association with leptospirosis, nephrolithiasis, urogenital neoplasia, septicemia, toxic insult, and age-associated degeneration.[4,9] Evaluating renal parameters in free-ranging sea lions with such diseases, to determine those most likely to survive, is appealing. Blood urea nitrogen (BUN) and serum creatinine levels only increase following loss of approximately 75% of global renal function, and BUN varies depending on a variety of factors. Conversely, creatinine excretion does correlate with glomerular filtration rate (GFR); however, significant elevations are only appreciated in severe renal dysfunction.[5] Determination of renal function via GFR is integral to accurate early prognosis.

Plasma iohexol clearance has been validated as an accurate estimation of GFR in other species by comparison to inulin, exogenous creatinine, and radionuclide plasma clearance.[5,6,8] Neither inulin, nor its clearance test, are currently commercially available; the requirement for urethral or ureteral catheter placement for 24-hr urine collection precludes the use of exogenous creatinine clearance testing, and the application of radiopharmaceutical studies would be challenging. Thus, the aim of this study was to evaluate if pre-existing protocols for evaluating plasma clearance of iohexol protocols, described for dogs and cats as an accurate estimate of GFR, could be directly applied to free-ranging California sea lions without modification.

Eight California sea lions (four male yearlings, two male juveniles, and two adult females) undergoing rehabilitation at The Marine Mammal Center (Sausalito, California, USA) were selected for inclusion in this prospective study. The study inclusion criteria were that all sea lions required anesthesia for radiography, ultrasonography, or wound debridement, and additionally had hematology and serum biochemistry parameters within normal ranges for this species; had negative serum antibody titers against *Leptospira interrogans* serovars *pomona*, *grippotyphosa*, *hardjo*, *bratislava*, *canicola*, and *icterohemorrhagica*; had no clinical signs of renal compromise; were normally hydrated; and were voluntarily feeding.[1,9] Isoflurane (Hospira Inc., Lake Forest, Illinois 60045, USA) was administered at 4% to yearlings, by mask, using manual restraint. Additional safety concerns around handling larger animals (juveniles and adults) existed, and isoflurane was administered following a midazolam (5 mg/ml; Mayne Pharma [USA] Inc., Paramus, New Jersey 07652, USA)–butorphanol (Butorject®, Phoenix Pharmaceuticals

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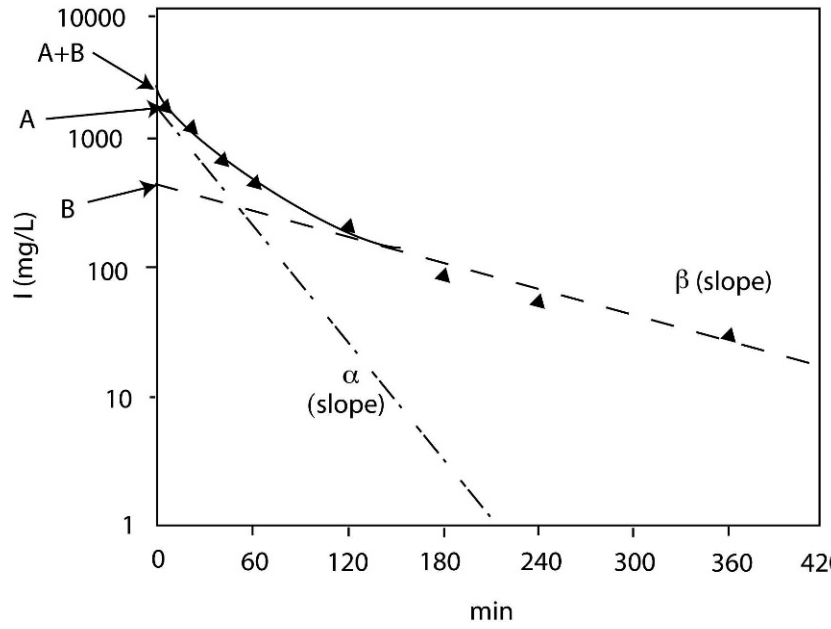

Figure 1. Extensive semi-logarithmic plot of iohexol concentration, I (mg/L), against time in minutes from the first additional adult female sea lion. She was administered iohexol (300 mgI/kg), and blood samples were taken at 5, 30, 45, 60, 120, 180, 240, and 360 min post injection. The plot shows a prolonged α (distribution) phase with significant area under the α slope. **A.** y-intercept for the distribution slope; **B.** y-intercept for the elimination curve.

Inc., St. Joseph, Missouri 64503, USA) combination (each at 0.15 mg/kg i.m.).[10] Following induction, 300 mgI/kg iohexol (Omnipaque®, Amersham Health Inc., Princeton, New Jersey 08540, USA), equating to 1.25 ml iohexol/kg, was administered as a bolus via the subclavian vein using a 20 G, 1.5-in needle, and the exact end injection time was recorded. Other procedures were performed, and sea lions were recovered and re-anesthetized as needed for blood sampling at 2, 3, and 4 hr post iohexol. Blood samples were collected into 2, 6-ml thrombin-activated vacutainer tubes (Vacutainer®, BD Technologies, 21 Davis Drive, Research Triangle Park, North Carolina 27709, USA) using the contralateral subclavian vein. The exact time of each sample collection was recorded. Serum was separated immediately and then shipped on ice to the Diagnostic Center for Population and Animal Health (DCPAH), Toxicology Section, P.O. Box 30076, Lansing, Michigan 48909-7576, USA, for iohexol concentration quantification via high performance liquid chromatography, using the previously described method.[5] Linearity (determined as $R^2 > 0.98$) was calculated from semi-logarithmic plots of terminal plasma iohexol concentrations versus time. Iohexol concentrations were determined from linear data by a one-compartment model[5] in which total-area-under-the-curve (AUC) = C_0/k, (C_0 = plasma iohexol concentration extrapolated at the time zero; k = elimination rate constant [slope] of the decay curve). Clearance (Cl) in ml per min per kg = dose (mg iohexol/kg body weight)/AUC. Two adult female sea lions with refractory seizures necessitating euthanasia, that met the study criteria, were recruited to evaluate causes for nonlinearity, were euthanized 360 min after iohexol administration, and underwent necropsy examination within 1 hr of death. The same method was followed, except blood samples were taken at 5, 30, 45, 60, 120, 180, 240, and 360 min. GFR was calculated from these data using the one-compartment model described above, a two-compartment model [$Cl_{(t)} = Ae^{-\alpha t} + Be^{-\beta t}$], and a noncompartment model (AUC) via the standard trapezoid method.

Samples from six of the initial eight sea lions demonstrated nonlinearity ($R^2 < 0.98$), and GFR could not be estimated. Two samples with linearity ($R^2 > 0.98$) permitted GFR estimation using the one-compartment model, and estimated GFRs were 3.39 and 6.80 ml per min per kg, respectively.[2,5] The two extensive, semi-logarithmic plots demonstrated a prolonged α (distribution) phase compared to other species and was markedly different between the two female sea lions (Figs. 1, 2). Results for the first female's

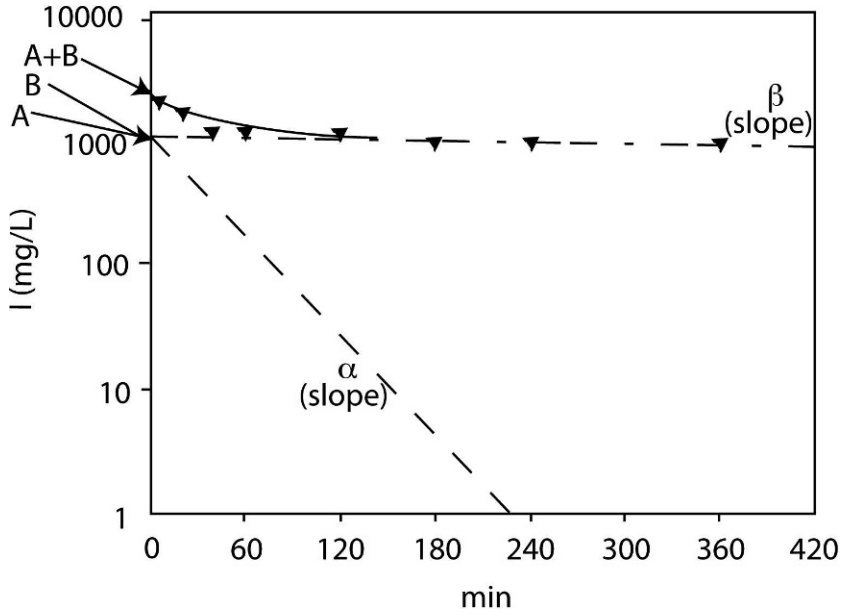

Figure 2. Extensive semi-logarithmic plot of iohexol concentration, I (mg/L), against time in minutes from the second additional adult female sea lion. She was administered iohexol (300 mgI/kg), and blood samples were taken at 5, 30, 45, 60, 120, 180, 240, and 360 min post injection. Note that the shape of the curve is markedly different to the curve shown in Figure 1, with even more area under the α slope, inferring greater protraction of the α (distribution) phase. **A.** y-intercept for the distribution slope; **B.** y-intercept for the elimination curve.

GFRs were 2.84, 4.22, and 2.96 ml per min per kg for one-, two-, and noncompartment models, respectively, when the curve extended to 4 hr post-iohexol injection; and 2.77, 5.16, and 2.82 ml per min per kg, respectively, when the curve extended to 6 hr. For the second female, GFRs were 0.31, 0.32, and 1.1 ml per min per kg, respectively, when the curve extended to 4 hr post-iohexol injection; and 0.17, 0.17, and 0.78 ml per min per kg, respectively, when the curve extended to 6 hr.

Histopathology on the two adult female sea lions confirmed hippocampal atrophy and gliosis consistent with the long-term effects of domoic acid.[12] No significant renal pathology was identified.

One yearling experienced cardiorespiratory arrest during recovery from anesthesia immediately following iohexol administration. He was administered 20 mg doxapram i.m. (Dopram-V®, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA), 0.4 ml 1:1,000 epinephrine s.c. (Phoenix Pharmaceuticals Inc.), 20 mg dexamethasone i.m. (Phoenix Pharmaceuticals Inc.), and 500 ml lactated ringer's solution s.c. (Hospira Inc.) while staff successfully ventilated and performed cardiac massage. No further problems occurred despite additional anesthetics for blood sampling; thus, the reaction was attributed to iohexol.[11]

Iohexol is a water-soluble, nonionic, monomeric iodinated radiographic contrast medium excreted unchanged almost exclusively by glomerular filtration. The pharmacology of iohexol is biphasic: rapid distribution (α slope) and slower elimination (β slope). Normal canine, feline, porcine, and equine iohexol clearance ranges have been established.[2,5,6,8] Despite the bi-exponential disposition of iohexol, a one-compartment model using three terminal elimination phase samples is used to accurately estimate GFR in dogs, cats, and pigs. This ignores the minor contribution of the rapid distribution phase, unlike the more-accurate, multi-sample noncompartment model.[2,5,6,8] However, the one-compartment model only provides an accurate estimate of GFR when there is linearity between samples.[2,5,6] Nonlinearity suggests premature (α phase) sampling, problems with iohexol administration (perivascular injection, inadequate or excessive dose calculation), or blood sampling (collecting from the same site as iohexol administration, or incorrect time of sampling recorded), or distribution of iohexol to multiple compartments from which excretion occurs at varying rates.[2,5,6,8] In these sea lions, no injection problems were noted, sampling was always performed from the contralateral vein to

the injection, and timings were checked by at least two personnel. The nonlinearity exhibited in 6/8 of the original animals rendered the protocol described for dogs and cats inappropriate for estimation of GFR in California sea lions and prompted two additional extensive curves to determine if the error was attributable to the timing of blood sampling post-iohexol injection. In this present study, one-, two-, and noncompartment models were not in agreement within the same animal, or between animals, indicating that individual curves and calculation of the total AUC from a noncompartment model are needed to estimate GFR in sea lions. Other species have demonstrated the need for species-specific modifications.[2,5,6,8] In a study evaluating foal GFRs, the data fit a three-compartment model, and a terminal two-time point sampling protocol was determined sufficient following correlation with exogenous creatinine clearance.[6] In pigs, a correction formula was necessarily developed when using a one-compartment method.[5,6,8]

An extremely low GFR was estimated from the second extensive curve by all methods of calculation. No significant renal disease was identified clinically, or on histopathology, in this sea lion, and the results may be attributable to an anesthesia-induced dive reflex.[10] The dive reflex is well developed in air-breathing aquatic animals,[3,10] causing a reduction in inulin clearance in the Weddell seal by up to 90% during short, forced laboratory dives and naturally protracted voluntary dives.[3] An anesthesia-induced dive reflex may produce similar results.[10] In an ideal situation, nonanesthetized California sea lions would have been preferable to exclude the effects of an anesthesia-induced dive reflex on the linearity of results and GFR, but was not feasible using a free-ranging population. A midazolam-butorphanol combination was selected as a premedication for the larger sea lions due to the limited effects on GFR demonstrated in dogs;[7] however, the specific effects of these drugs on GFR in California sea lions is unknown. As such, these results may have been affected by a direct drug effect in addition to the dive reflex.

In conclusion, the standardized protocol developed for dogs and cats cannot simply be applied to California sea lions to estimate GFR due to nonlinearity between samples in up to 75% of cases. Reductions in GFR when linearity does exist may be due to disease, or to an anesthesia-induced dive reflex. Additionally, severe adverse reactions to iodinated contrast media can occur in sea lions.

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