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Predictors of Subcutaneous Injection Site Reactions to Sustained-Release Buprenorphine in Rhesus Macaques (*Macaca mulatta*)

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Subcutaneous injection site reactions to sustained-release buprenorphine hydrochloride (Buprenorphine SR) in macaques have been reported in only a single case report. In the current study, we evaluated the incidence rate and predictors of buprenorphine SR reactions in the subcutaneous tissue of rhesus macaques (M*acaca mulatta*) based on retrospective review of macaque buprenorphine SR injection records. Potentially predictive variables were identified with logistic regression modeling and were evaluated using model selection based on Akaike information criterion. Record review revealed subcutaneous tissue reactions occurred in 52 (3%) of 1559 injections and were noted between 4 and 311 d after injection. Model selection showed that body weight and MHC allele Mamu-B*29 were the best predictors of subcutaneous reactions. Based on these results, we recommend consideration of potential risk factors prior to the administration of buprenorphine SR to a rhesus macaque. In addition, the authors advise that using the highest concentration of buprenorphine SR available may reduce injection site reaction rates due to the injection of less copolymer.

Abbreviations: BCS, Body Condition Score; Buprenorphine SR, sustained-release buprenorphine hydrochloride; ONPRC, Oregon National Primate Research Center; PRIMe, Primate Records and Information Management electronic medical records

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Buprenorphine plays a pivotal role in providing analgesia to macaques. Veterinarians commonly use buprenorphine to manage acute and chronic painful conditions, such as postoperative pain, trauma, gastrointestinal disease, dystocia, endometriosis, and neoplasia including gastrointestinal adenocarcinomas. Subcutaneous administration of sustained-release buprenorphine hydrochloride (Buprenorphine SR, ZooPharm pharmacy subsidiary of Wildlife Pharmaceuticals, Windsor, CO) delivers buprenorphine within one hour of administration and provides 5 d at plasma concentrations believed to be analgesic.[23]

Adverse drug reactions in the subcutaneous tissue after buprenorphine SR administration were reported in rhesus macaques, Northern elephant seals, dogs, and Göttingen minipigs.[21,24,31] In these studies, microscopic examination of lesions revealed pyogranulomatous inflammatory reaction surrounding a central clear or gray material consistent with injection site reactions. Reactions appeared in elephant seals between 1 and 7 d after administration. Reactions in dogs were detected 10 or more days after drug administration, and reactions in pigs appeared 1 wk after injection. Reports of acute dermal necrosis in rodents occurring within 24 h after administration have been attributed to improper injection technique.[13] However, the pathogenesis of the delayed subcutaneous reactions to buprenorphine SR was not determined in macaques, seals, dogs, and pigs.

Buprenorphine SR is buprenorphine hydrochloride in a proprietary biodegradable polymer drug delivery system that is also referred to as a sustained-release matrix. The matrix is DL-lactide-*co*-caprolactone copolymers [poly(DLLA-*co*-CL)] with N-methyl-2-pyrrolidone (NMP) as a solvent.[7,13] Poly(DLLA-*co*-CL) is a 50/50 molar ratio of DL-lactide to ε-caprolactone polymers.[13] The sustained-release matrix is hydrophobic, water-insoluble, and precipitates in body fluids to form a gel depot for sustained release of a drug.[38,39] Buprenorphine is released by erosion of the polymer material by either hydrolysis or immune cell degradation.[38,40] Copolymer poly(DLLA-*co*-CL) remains in the body subcutaneously for up to 2 y, where it elicits a foreign body reaction resulting in the eventual degradation of the polymers by multinucleated giant cells.[10,20]

Over the first year of buprenorphine SR use at the Oregon National Primate Research Center (ONPRC), injection site reactions were identified after buprenorphine SR (3 mg/mL concentration) injection in rhesus macaques (Figure 1). Nineteen buprenorphine reaction sites were biopsied. Histopathology showed pyogranulomatous inflammation surrounding optically clear foci, which were interpreted as a potential drug vehicle within the subcutaneous tissue and deep dermis (Figure 2). We hypothesized that shared factors among cases might be useful for predicting the development of these reactions in rhesus macaques.

Foreign-body reactions begin when immune cells internalize antigens and then process and present them via MHC glycoproteins. Macaques have MHC class I and II glycoproteins that interact with T-cells. The genes that code for these MHC glycoproteins are extremely variable as a consequence of differing alleles. This repertoire of alleles controls T-cell responses, which are required to initiate cell-mediated hypersensitivity. The T-cell response may be directed against pathogens or exogenous materials such as injected drugs and their vehicle. MHC allele-associated

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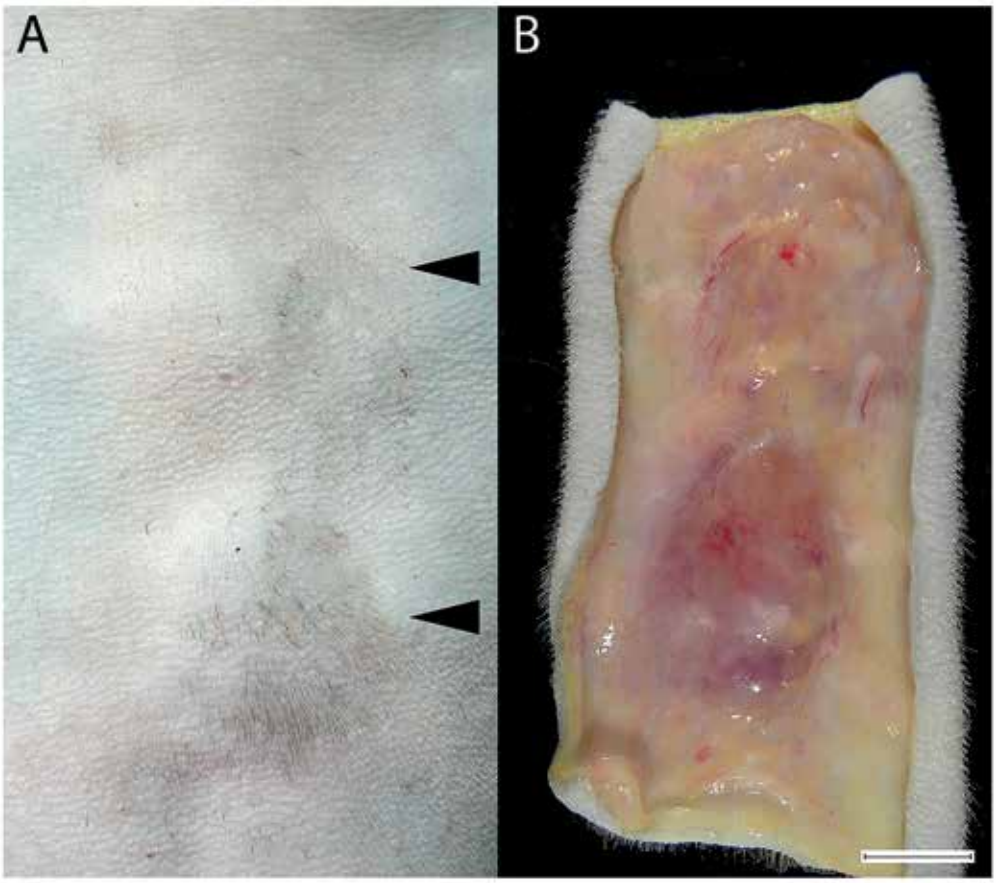

Figure 1. Five year old male indoor-housed rhesus macaque with 2 buprenorphine SR reactions (marked with black arrows). Reactions were found at a project study end point necropsy. Patient had received injections 20 and 49 d prior to presentation. Both lesions are typical of buprenorphine SR intact healthy skin and subcutaneous swelling from underlying cellulitis. Scale represents 1 cm.

adverse reactions to drugs are not well understood, nor well documented. In one investigation, Mauritian cynomolgus macaques developed T-lymphocyte mediated delayed immune responses to drugs that were MHC allele-associated.[35] The MHC alleles of humans are also associated with delayed hypersensitivity immune reactions to drugs in certain cases.[29] We aimed to perform a retrospective study to identify specific MHC alleles and demographic factors that could predict the development of buprenorphine SR injection site reactions.

Our primary hypothesis was that certain MHC types would predict buprenorphine SR reactions. Although 150 MHC alleles were known in macaques administered buprenorphine SR at the ONPRC, case-control analysis was only possible for the alleles Mamu-A1*01, Mamu-A1*02, Mamu-A1*08, Mamu-A1*11, Mamu-B*01, Mamu-B*08, Mamu-B*17, Mamu-B*29, Mamu-DPB*106, Mamu-DRB*10401/06/11, and Mamu-DRB*w201. We also investigated other potential predictors of reactions, including the persons performing the injection, the presence of traumatic wounds at the time of injection, sex, social rank, body weight, housing environment, age, body condition score (BCS), and alopecia score.

Materials and Methods

Subjects. Subject animals receiving buprenorphine SR were Indian-origin or hybrid Chinese-Indian origin rhesus macaques (*Macaca mulatta*) housed at the Oregon National Primate Research center (ONPRC), an AAALAC International accredited facility. All animals were assigned to ONPRC breeding colonies and managed under the IACUC-approved protocol of the Oregon Health and Science University West Campus. Housing and care were in accordance with standards established by the US Federal Animal Welfare Act[1] and the Guide for Care and Use of Laboratory Animals.[16] Data were collected from Primate Records and Information Management (PRIMe), an electronic medical records system that uses case classifiers, Systematic Nomenclature of Medicine (SNOMED) codes, and free text to capture routine husbandry practices and veterinary medical care.

Risk factor analyses. Medical records were reviewed for the administration of buprenorphine SR to identify animals that had received an injection, the incidence of subcutaneous reactions at the site of injection, and potential predictors of injection site reactions, including MHC alleles and demographic factors. The volume of buprenorphine SR injected subcutaneously ranged from 0.1 mL to 1.4 mL and the dose was 0.2 mg/kg for both 3 mg/mL and 10 mg/mL concentration formulations.

Demographic information included binary variables for housing environment (indoor or outdoor) and the presence of a traumatic wound at the time of injection (yes or no), social rank (dominant animal in the group or not), and sex (male or female). The person performing an injection was obtained from the medical record and anonymized. Additional quantitative demographic information was obtained, including the number of previous buprenorphine SR injections, BCS, alopecia score, body weight, and age. BCS and alopecia scoring were performed regularly by trained veterinary staff.[14,30] Dominant animals in social groups were identified in medical records.

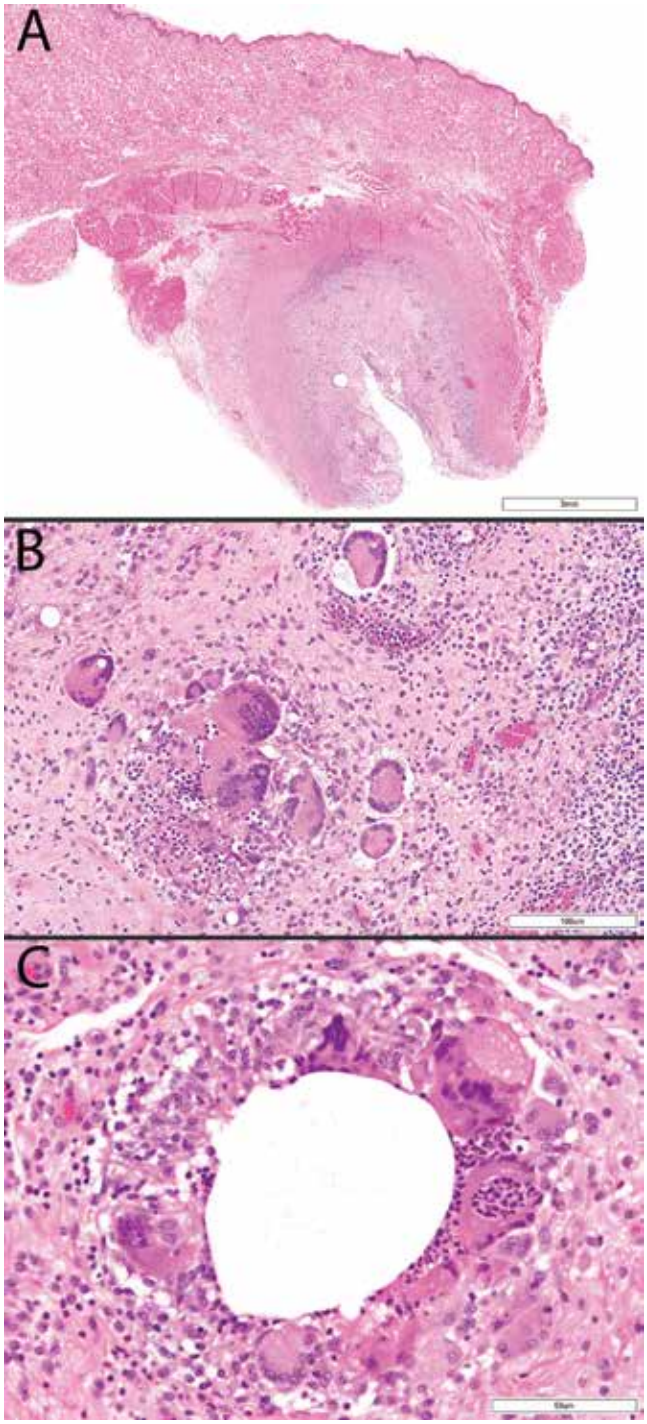

Figure 2. Histopathology of subcutaneous adverse reactions following buprenorphine SR. Macroscopic section of skin with underlying cellulitis (A panel). 40× (B panel) and 100× (C panel) magnification detail pyogranulomatous reaction and multinucleated giant cells surrounding variably sized optically clear foci that sometimes contain small amounts of wispy pale eosinophilic material.

MHC Typing of Expressed Alleles. MHC typing of both class I and class II alleles was performed using blood samples collected routinely from monkeys at semiannual physical exams that were performed as part of normal colony management. Prior to 2014, samples were sent to the Watkins service laboratory for MHC class I and class II allele testing. Watkins service laboratory provided PCR tests with sequence-specific primers for panels of MHC class I and II alleles, as described in previously published methods.[17] After 2014, the ONPRC genetics core set up inhouse MHC class I testing; however, MHC class II allele typing was still conducted at the Watkins service laboratory. ONPRC inhouse testing used Illumina sequencing of MHC cDNA amplicons as the method to rapidly determine MHC class I transcript profiles in macaques.[33] Finalized MHC alleles were uploaded into the PRIMe database. A subset of the rhesus macaque population at ONPRC have MHC allele data available in the PRIMe medical record system. MHC class I alleles analyzed in the study are Mamu-A1*01, Mamu-A1*02, Mamu-A1*08, Mamu-A1*11, Mamu-B*01, Mamu-B*08, Mamu-B*17, and Mamu-B*29. MHC class II alleles in the study are Mamu-DPB*106, Mamu- DRB*10401/06/11, and Mamu-DRB*w201.

Subcutaneous Reactions to Buprenorphine SR. Analyses were limited to injections of 3 mg/mL buprenorphine SR in which at least 311 d of postinjection monitoring were available. We used 311 d of monitoring after injection for our analysis because this was the longest period between injection and clinical presentation for a subcutaneous reaction. Injections with insufficient follow-up period due to an animal death were excluded from the study. These injection events were removed from analyses because evaluation for buprenorphine SR reactions had not routinely been investigated in initial animal deaths.

An incidence rate of site reactions to an injection of 10 mg/mL buprenorphine SR was calculated for injections that were monitored for at least 72 d after the injection. A 72 d follow-up period was used for the 10 mg/mL formulation because at the time of publication, the duration of monitoring after injection of the concentrated formulation was insufficient for use of 311 d. However, 72 d was the average time period between injection and clinical presentation for injection site reactions. Because the incidence rate would be subject to misclassification error from reactions that occurred after the available monitoring period, we calculated the comparison rate for 3 mg/mL buprenorphine SR with a 72 d follow up period to compare injection site reaction rates for the 2 formulations.

Subcutaneous reactions in response to buprenorphine SR were defined as subcutaneous swelling greater than 0.5 cm in diameter at the injection site of buprenorphine SR. Reactions of acute dermal necrosis, swelling less than or equal to 0.5 cm in diameter, and erythema were not included in our definition of buprenorphine SR reactions.

Statistical analysis. Potential risk factors were evaluated in a 2-step analysis using the first univariate analysis to screen variables for consideration, and the second multivariate analysis to evaluate screened variables as predictors of reactions. The univariate analysis provided scientific support for variables included in the multivariate analysis and was not used to determine significant associations. Univariate logistic regression with a binary outcome for the presence or absence of buprenorphine SR reactions was done using R statistical program. A relaxed P value < 0.2 was used to indicate variables to evaluate in multivariate analysis. To check for multicollinearity between variables, we examined the variable inflation factors (VIF), which we calculated using the VIF function in the usdm package in R.[22,28] Variables with VIF > 3 indicate a strong linear relationship with another variable.[42]

Multivariate generalized mixed-effect models were made using the lme4 package in R statistical program.[4,28] Model selection was performed through comparison of multiple models, starting with the simplest model including intercept and a random effect for each animal. We compared the simplest model and all models with one variable difference using relative Akaike information criterion (AICc). R package AICcmodavg

calculated AICc values for all models.[19,28] Forward-direction model selection evaluated all models by adding one variable in a step-by-step fashion. At each step, models were ranked by AICc difference where the lowest AICc indicated the best fit model.[6] Steps continued adding the most important variables until no additional covariates could be added. Models within 2 AICc units from the minimal AICc were considered essentially equal fit models, and both models were kept in consideration as plausible models in the same stepwise fashion.[5] Models within 2 AICc of the minimal AICc that had extra parameters were not considered plausible models.[2] The model with the lowest AICc, as well as any models within 2 AICc of this model with the lowest number of parameters were selected from the plausible models as final models.

Results

Buprenorphine SR Reactions. Overall, 52 reactions (3%) were identified from 1559 buprenorphine SR injections given to rhesus macaques between May 24, 2018, and May 29, 2019. The reactions were noted on average 72 d after injection (range 4 to 311 d). Macaques that experienced reactions typically did not receive buprenorphine SR again; however, 2 macaques had 2 reactions after 2 injections. The first rhesus macaque received 2 injections of buprenorphine SR 29 d apart that were found at postmortem exam (Figure 1). The other rhesus macaque developed a reaction noted at 43 d after their first injection. Then 52 d later, a second injection was given followed by a second reaction noted 35 d after their second injection.

Nineteen (37%) of the 52 reactions were biopsied based on clinical veterinary discretion, and the microscopic features of all 19 biopsies confirmed the clinical impression of a subcutaneous injection site reaction to buprenorphine SR (Figure 2; Table 1). Microscopic examination reported that 18 (95%) of 19 biopsies had pyogranulomatous reaction surrounding unidentified clear material. Histopathology reports also described multinucleated giant cells present in biopsy samples in 18 (95%) of 19 biopsies. Special histochemical stains excluded bacterial or fungal pathogenesis of lesions. Six (12%) of the 52 subcutaneous reactions were cultured at veterinary discretion. Two (33%) had no growth, 3 (50%) had a few colonies of bacterial growth consistent with skin contamination, and 1 had *Escherichia coli* growth (17%).

Injections of 3 mg/mL buprenorphine SR given to Japanese macaques (*Macaca fuscata*) and cynomolgus macaques (*Macaca fascicularis*) were not included in our retrospective study. Subcutaneous reactions occurred in 2 (4%) of 50 injections given to Japanese macaques, and no reactions were noted after 168 buprenorphine SR injections in cynomolgus macaques.

Univariate Risk Factor Analysis. The relationship study between reactions and buprenorphine SR injections was initially restricted with a univariate analysis with P values < 0.2 indicating significance and justifying inclusion in subsequent multivariate analysis. Injections of buprenorphine SR given on presentation for a traumatic wound were associated with reactions ($P = 0.087$; Table 2). Age and weight were associated with reactions (Table 2). The mean age of macaques with reactions was 7.1 y (± 0.03 SE) and 5.5 y (± 0.01 SE) for those without reactions ($P = 0.002$). Mean weight of macaques with reactions was 7.6 kg (± 0.06 SE) and 6.6 kg (± 0.07 SE) for those without reactions ($P = 0.003$). No particular individual administering the injection was associated with reactions ($n = 31$, $P = 1.0$ for all injectors).

Mamu-B*01 ($P = 0.095$), Mamu-B*17 ($P = 0.085$), Mamu-B*29 ($P = 0.106$) alleles were associated with buprenorphine SR reactions (Table 3). VIF values were 6.75 for Mamu-B*17 and Mamu-B*29 allele variables, suggesting collinearity of these 2 variables. Previous studies have indicated Mamu-B*17 and Mamu-B*29 MHC alleles are closely linked and often occur together as macaque serological haplotype B17.[11,36] Despite this link in serological testing, our inhouse cDNA testing for allele expression found 25 macaques in our dataset expressed the Mamu-B*29 allele but not the B17 allele. For this reason, we included both Mamu-B*17 and Mamu-B*29 alleles in the multivariate analysis.

Multivariate Risk Factor Analysis. Independent predictors of reactions were identified using generalized mixed-effects modeling with hierarchical model selection in R computational software. The presence or absence of a reaction within 311 d of injection was set to a binomial distribution, with individual included as a random effect to account for subject level variability in the model.

The variables of age, Mamu-B*01, Mamu-B*17, and trauma at time of injection were eliminated during the model selection process. The model selection process concluded with one final model having 2 predictors of reactions, body weight and MHC allele Mamu-B*29 (Table 4). All reported β coefficients are conditional values, meaning they are determined with all other coefficients held constant. In the final model, occurrences of reactions increased multiplicatively by weight. The predicted occurrence of reactions increased by 1.3 times for every kilogram of weight. The odds of a reaction occurring also increased by 2.1 times with the Mamu-B*29 allele.

Formulation reaction rate comparison. Of the 471 injections of 10 mg/mL buprenorphine SR administered to rhesus macaques between January 7, 2020 and July 27, 2020, 2 reactions (0.4%) were noted. The reactions were noted 7 and 39 d after injection. For comparison, during a 72 d monitoring period after injection with 3 mg/mL buprenorphine SR injections, 34 reactions (2%) were noted from 1818 injections. These 72 d monitoring rates were reported for comparison between the 3 and the 10 mg/mL concentrations, but these rates use an insufficient monitoring period to note all reactions postinjection and are not as accurate as incidence rates reported earlier in our study.

Discussion

We report injection site reactions to subcutaneous administration of buprenorphine SR at a rate of 3% in our rhesus macaques. Reactions were characterized by pyogranulomatous inflammation, centered on optically clear space rarely containing amphophilic material. This small subset of macaques receiving buprenorphine SR was used to investigate potential predictive factors for injection site reactions.

The optically clear space surrounded by inflammation at the reaction site observed by histology analysis remains unidentified. While the drug buprenorphine and the solvent NMP are metabolized relatively quickly, the copolymer component remains in the tissue for up to 2 y, inciting a pyogranulomatous immune response consistent with that seen in histology reports in our study.[10,20,39] These injection site reactions were also reported in buprenorphine pK studies and case reports of buprenorphine SR in which pyogranulomatous inflammation was diagnosed in the subcutaneous tissue at the injection site.[21,24,31] Pathogenesis of the inflammatory response is unclear, but may include either a delayed hypersensitivity reaction or a heightened foreign body reaction to the drug vehicle.

This retrospective study in rhesus macaques demonstrated several predictive factors for the development of a subcutaneous pyogranulomatous reaction to buprenorphine SR, with MHC

Table 1. Summaries from 19 buprenorphine SR reaction biopsies with patient signalment and histologic findings of subcutaneous reaction.

Sex	Age (years)	Weight (kg)	Housing	Days postinjection to reaction	Focal cellulitis	Pyogranulomatous reaction surrounding clear material	Multinucleated giant cells	Neutrophils
Male	8.6	11.4	Outdoor	7	+	+	+	+
Male	4.0	6.6	Indoor	11	+	+	+	-
Male	5.9	8.0	Indoor	11	+	+	+	+
Male	5.6	8.9	Indoor	20	+	+	+	+
Male	7.2	9.5	Indoor	27	+	+	+	+
Male	5.8	8.8	Outdoor	29	+	+	+	+
Male	4.5	8.9	Outdoor	43	+	-	+	-
Female	5.8	7.1	Indoor	56	+	+	+	+
Male	5.3	8.1	Indoor	69	+	+	+	+
Male	4.7	8.3	Outdoor	71	+	+	+	+
Female	5	7.2	Outdoor	93	+	+	-	+
Female	14.6	7.9	Outdoor	121	+	+	+	+
Female	4.3	5.1	Outdoor	124	-	+	+	+
Female	2.6	4.2	Outdoor	135	+	+	+	-
Female	6.8	7.2	Outdoor	148	-	+	+	+
Female	5.5	7.0	Outdoor	156	+	+	+	+
Female	3.8	6.5	Outdoor	161	+	+	+	+
Female	1.9	3.0	Outdoor	234	+	+	+	+
Female	2.3	2.8	Outdoor	311	+	+	+	+

Table 2. Univariate analysis of binary demographic factors for buprenorphine SR injections with reaction and those with no reaction. Generalized logistic regression was used to calculate *P* values to evaluate relevant risk factors for inclusion in multivariate analysis. Variables with *P* values less than 0.2 were considered for the multivariate analysis and indicated by an asterisk.

Variable Continuous variables	Reaction (n = 52) Mean ± SE	No reaction (n = 1507) Mean ± SE	*P* value
Age, years	7.1 ± 0.03	5.5 ± 0.01	0.002*
Weight, kg	7.6 ± 0.06	6.6 ± 0.07	0.003*
Body condition score	3.0 ± 0.07	2.9 ± 0.01	0.304
Alopecia score	1.4 ± 0.07	1.2 ± 0.03	0.370
Injection number	1.8 ± 0.07	1.6 ± 0.03	0.434
Binary variables	n (%)	n (%)	
Outdoor housing	24 (46.2)	795 (50.3)	0.350
Trauma at time of injection	15 (28.8)	615 (38.9)	0.087*
High social rank	2 (3.8)	98 (6.2)	0.448
Female	29 (55.8)	833 (52.7)	0.944

allele Mamu-B*29 being the strongest predictive factor in our final model. MHC alleles Mamu-B*01 and Mamu-B*17 showed an association with reactions in the univariate analysis, but these MHC alleles were eliminated as predictors of injection site reactions during model selection in the multivariate analysis. A complete set of MHC alleles in our case animals would better inform our study, as 19 of 52 cases (37%) had limited or no MHC allele testing available for analysis. We used injection data from rhesus macaques in our analysis of other risk factors as well. Body weight was a predictive factor in the final model with a multiplicative effect per kilogram, which potentially makes the variable a stronger predictor than Mamu-B*29. A positive body weight predictor of reactions suggests a dose-related response to the drug. The drug's recommended dose in macaques is 0.2 mg/kg, so heavier animals require more buprenorphine and, thus, receive more of the sustained release matrix.[23]

A dose-related adverse drug response to copolymers may be mitigated by using more concentrated formulations of buprenorphine SR. More concentrated formulations of buprenorphine SR have less copolymer per milliliter of drug. For example, the 10 mg/mL buprenorphine SR has about 3 times less copolymer per milliliter than the 3 mg/mL buprenorphine SR. Since this study was completed, ONPRC has changed all buprenorphine SR to the most highly concentrated version available commercially, 10 mg/mL, to reduce the amount of copolymer injected subcutaneously. We followed up on this change and reported comparative reaction rates between 3 mg/mL and 10 mg/mL buprenorphine SR. We found the 10 mg/mL buprenorphine SR was 5 times less likely to result in an injection site reaction when we followed injections for a minimum 72 d after injection.

In our study, we found an MHC allele was predictive of injection site reactions; however, the actual gene or combination of genes associated with reactions are yet to be determined. Establishing a direct correlation between a disease and an MHC gene is particularly difficult. MHC-associated conditions are nearly

Table 3. Univariate analysis of MHC class I and class II allele frequencies for buprenorphine SR injections with reaction and those with no reaction.

	Reaction		No reaction			
Allele	MHC Pos (%)	MHC Neg	MHC Pos (%)	MHC Neg	Not Tested	*P* value
Mamu-A1*01	8 (21.1)	29	242 (21.9)	863	403	0.966
Mamu-A1*02	7 (18.9)	29	267 (25.6)	777	464	0.406
Mamu-A1*08	11 (29.7)	25	238 (23.0)	795	475	0.299
Mamu-A1*11	3 (8.1)	33	93 (9.0)	939	476	0.887
Mamu-B*01	16 (43.2)	20	321 (31.1)	711	476	0.095*
Mamu-B*08	6 (15.8)	31	173 (15.6)	934	401	0.925
Mamu-B*17	10 (26.3)	27	179 (16.2)	929	400	0.085*
Mamu-B*29	10 (27.8)	25	182 (17.7)	846	480	0.106*
Mamu-DPB*106	0 (0.0)	6	31 (27.9)	80	1396	0.993
Mamu-DRB*10401/06/11	2 (33.3)	4	38 (33.9)	74	1395	0.976
Mamu-DRB*w201	3 (50)	3	46 (41.4)	65	1396	0.680

Table 4. Variables incorporated in the final model for buprenorphine SR reactions: Reaction approximately weight, Mamu-B*29. Variables with positive β coefficients positively predict reactions.

Variable	β estimate (SE)	Lower 95% CI	Upper 95% CI	Odds ratio
Weight	0.24 (0.096)	0.053	0.431	1.3 per kilogram
Mamu-B*29	0.74 (0.511)	−0.261	1.742	2.1

all multifactorial, and linkage disequilibrium of many MHC alleles with MHC and non-MHC genes prevents identification of a single gene-locus.[15] Despite the difficulty, the first MHC allele-associated condition in macaques was described in 1997.[3] Rhesus macaques with the Mamu-B*01 allele were resistant to collagen-induced arthritis, and investigators using monkeys began to request MHC type testing to preselect subjects that were Mamu-B*01 negative.[9,32] MHC alleles have also been associated with delayed drug-induced hypersensitivity reactions in the Mauritian-origin cynomolgus macaque.[35]

Multiple models show different mechanisms to explain MHC-associated, immune-mediated response to an otherwise nonimmunogenic drug. The first model, the hapten hypothesis, explains that a small molecule drug binds with a self-protein and, together, they become antigenically stimulating to immune system cells.[8,27] Another, the hapten-independent model, posits that a drug directly binds immune cells or MHC glycoproteins by mimicking a shared epitope with the natural ligand of the immune cell or MHC glycoprotein.[26,37] The last, the altered repertoire model, states a similar mechanism where the drug binds to an immune cell or MHC glycoprotein outside of the amino acid binding area, and then alters the function of a receptor to bind self-ligands and inappropriately activate the immune system.[25]

In our institution, performing buprenorphine SR injections was restricted to veterinary staff members with specific training in its administration. Injection techniques were based on published recommendations and informal technical bulletins posted on ZooPharm's portal website, Wildlife Pharmaceuticals's pharmacy subsidiary.[13,41] Our study reports a 3% overall reaction rate despite precise injection technique training. In addition, our evaluation found no association between the individual injecting the drug and the incidence of adverse reactions.

Retrospective analysis study design is limited in that subcutaneous reactions may not be clinically detected in every case, particularly if the reaction is minor. Subcutaneous reactions have been reported to self-resolve in certain cases.[31] In addition, lesions may have been missed at death because the histologic evaluation of the scruff of macaques is not a routine practice for every animal death or terminal study end point. The histology findings in this report are limited to those cases from which biopsies were collected at veterinary discretion. Our results suggest that a study should be performed in which sequential histologic changes can be documented with time after injection, regardless of the clinical appearance of a reaction. This information may provide a better understanding of the mechanisms that mediate potential adverse drug reactions to the active pharmaceutical ingredient and reactions to copolymers that remain in subcutaneous tissue well beyond the buprenorphine HCl. Effects on research outcomes that use immune system measurements should also be investigated.

Buprenorphine SR is a valuable drug in primate medicine. In 97% of injections of our macaques, no clinically detectable injection site reactions occurred. However, we accumulated enough cases over a year to conduct a retrospective study that showed reactions with predictable risk factors such as MHC alleles and body weight. The risk factors for reactions in this study are important because they indicate that reactions may not be idiosyncratic or idiopathic, and that the pathogenesis should be investigated to either minimize or avoid the use of buprenorphine SR in certain animals.

Alternatives to 3 mg/mL buprenorphine SR should be used when choosing a pain-relieving drug for larger macaques and for macaques known to express the MHC allele Mamu-B*29. The copolymer poly(DLLA-CL) has a resorption or breakdown rate of 6 mo to 2 y in subcutaneous tissue where the adverse drug reactions occurred.[10,12,20,34] Simbadol (Zoetis, Parsippany, NJ), an FDA-approved highly concentrated buprenorphine solution, does not contain copolymers and delivers buprenorphine for 48 to 72 h in macaques.[18] At the time of publication, no subcutaneous tissue adverse reactions have been reported after Simbadol injection.

A clinical trial of the use of buprenorphine SR in rhesus macaques and 2 y of follow up time with histopathology is needed to investigate adverse reactions. Such a trial would be important to determine the safety of this drug. In the meantime, we reported only 0.4% of injections with 10 mg/mL buprenorphine SR lead to injection site reactions. Because the 10 mg/mL formulation had a lower reaction rate than the 3 mg/mL

formulation, using the highest concentration of buprenorphine SR available may reduce the number of injection site reactions.

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