Predicting Injection Site Muscle Damage II: Evaluation of Extended Release Parenteral Formulations in Animal Models

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Purpose. The goal of this study was to find a resource sparing alternative to the rabbit lesion model (RbLV) for assessing injection site toleration in extended release (ER) intramuscular (IM) formulation screening.

Methods. ER formulations (danofloxacin oily and aqueous suspensions) were evaluated in RbLV, rat and rabbit plasma creatine phosphokinase (CK), and rat foot edema (RFE) models as described in the companion article.

Results. None of the short term models could consistently predict acute and chronic effects of the. For example, RFE predicted little muscle damage from aqueous vehicle (0.03 ± 0.03 g) and 60 mg/ml (0.08 ± 0.03 g) formulation; while $RbLV_{days1-3}$ was marked and greater ($p < 0.05$) for 60 mg/ml (6.0 ± 3.1) than vehicle (2.2 ± 2.9) formulations. Furthermore, $RbLV_{days1-3}$ for vehicle (6.5 ± 7.5) and 60 mg/ml (4.9 ± 4.6) danofloxacin oily formulations were worse ($p < 0.05$) than oil alone (1.4 ± 2.2); an observation not predicted by CK models, since they apparently reflected only the acute muscle damage of formulation components immediately available to surrounding tissue at the time of injection.

Conclusions. The CK models may be useful to screen those ER formulations with unacceptable acute damage due to immediately available components. However, to evaluate potential *delayed* effects from ER formulations, the long-term model RbLV was still recommended.

KEY WORDS: rabbit; rat; lesion; creatine kinase; extended release formulations; sesame oil; danofloxacin.

INTRODUCTION

Several formulation strategies have recently been published to either extend the pharmacokinetic profile and/or reduce the muscle damage of intramuscular (IM) products. These include the use of oils[1], polymers[2] and microspheres[3]. Muscle damage is probably a function of both local tissue concentration and contact-time of drug and/or formulation components. An ideal extended release (ER) formulation would both reduce the local concentration of the responsible component (*s*) below a level that caused significant cell lysis (*i.e.* critical concentration, C_{crit}), and provide the desired pharmacokinetic profile. While in theory a constant infusion could provide such an ideal profile, actual formulations are more variable.

The goal of this study was to find a resource sparing alternative to the rabbit lesion model for assessing injection site tolerance in ER formulation screening. The objectives of the present study were 1) to determine the extent of muscle damage in rabbits following IM injection of a variety of ER formulations, using biochemical (*e.g.* CK) and morphological methods, and 2) compare alternative animal models.

METHODS

Materials

Formulations of danofloxacin were selected since their development represented realistic challenges to the formulation scientist. Danofloxacin aqueous (DAQ) and oily (DOIL) suspension formulations (vehicle, 30 and 60 mg/ml) were made under "clean" conditions with "clean" reagents (Pfizer Central Research, Groton, CT). Since danofloxacin had an aqueous solubility >60 mg/ml at pH 4, <0.5 mg/ml at pH 7.4 and a solubility in sesame oil of <1 mg/ml, the mechanism of extended release (ER) was one of dissolution limited solubility. This ER characteristic was demonstrated *in vitro* (dialysis) and *in vivo* (lower C_{max} and sustained concentrations) for both formulations (W. Boettner, personal communication).

Creatine Kinase (CK) Assay

A commercially available kit (47-UV, Sigma, St. Louis, MO) was used to determine plasma CK concentrations[7].

Animal Models

All protocols were approved by the institutional ACUP Committee, which administered the principles of laboratory animal care as found in NIH #85–23, 1985. Animal models were employed as described in detail elsewhere[7].

Statistics

Mean ± SD was reported throughout. Statistical comparisons were completed as described in detail elsewhere[7].

RESULTS AND DISCUSSION

Rabbit Lesion Volume (RbLV) Model

Since IM injection of saline alone[7] produced lesions which resolved in 1–2 days, only lesions present on days 3–21 were considered remarkable. The histology score attributed to necrosis of myofibers, hemorrhage, granulocytic cell infiltration or mononuclear cell infiltration was averaged for each study day, and then summed over days 3, 7, 14 and 21 (Table I)[7]. $RbLV_{days1-3}$, and $RbLV_{days7-21}$ were calculated as previously described[7]. As shown in Figure 1, DOIL RbLV appeared to be maximum on day 3 for vehicle (11.1 ± 8.4 cm^3), day 7 for 30 mg/ml (7.8 ± 5.4 cm^3) and day 14 for 60 mg/ml (4.8 ± 1.9 cm^3). In contrast, sesame oil alone produced practically no lesion at all. DOIL $RbLV_{days1-3}$ were significantly greater ($p < 0.05$) than sesame oil alone. Unlike immediate release (IR) formulations, where $RbLV_{days7-21}$ was marginal (~0.2 cm^3)[7], DOIL lesions appeared to persist beyond day 3. For example,

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Table 1. Summary of Animal Model Evaluation of the Extended Release Suspension Formulations; Mean ± SD, n = 3–4 For Each Time Point, Except $RbLV_{days1-3}$ (n = 6–12) and $RbLV_{days7-12}$ (n = 9–16)

		AQUEOUS Concentration (mg/ml)			OIL Concentration (mg/ml)			
Model	Parameter	0	30	60	oil	0	30	60
Rat foot edema (RFE)	(g)	0.03 ± 0.03	0.06 ± 0.03	0.08 ± 0.03	0.15 ± 0.4	0.23 ± 0.08#	0.21 ± 0.16#	0.23 ± 0.07#
Rat CK	C_{max} (U/L)[a]	142 ± 122	534 ± 254*	599 ± 345*	178 ± 45	184 ± 96	615 ± 139#,*	802 ± 96#,*
(RtCK)	T_{max} (hr)[b]	4	6	2	1	24	3	2
	AUC_{0-24}(U/L-hr)[c]	428	5,633	4,516	1,245	1,480	7,729	9,118
Rat lesion	Score[d] 4 hr	0.2 ± 0.3	1.2 ± 0.3*	0.5 ± 0.0	0.5 ± 0	0.5 ± 0.0	0.7 ± 0.3	0.8 ± 0.3
(RtHS)	(0–4) 0–24 hr	0.3 ± 0.3	0.9 ± 0.5*	0.3 ± 0.2@	0.3 ± 0.2	0.6 ± 0.7	0.4 ± 0.3	0.9 ± 0.5#,*
Rabbit CK	C_{max} (U/L)	296 ± 91.3	2110 ± 1156*	3279 ± 853*	479 ± 172	303 ± 167	2184 ± 699*,#	7228 ± 2005*,@,#
(RbCK)	T_{max} (hr)	9.5 ± 9.7	20 ± 9	24 ± 0*	17 ± 13	19 ± 10	10 ± 9.0	21 ± 7.3
	AUC_{0-72} ($\times 10^{-3}$) (U/L-hr)	4.71 ± 4.20	91.9 ± 45.0*	124 ± 39.8*	6.51 ± 7.77	5.40 ± 5.16	87.3 ± 25.6*,#	291 ± 80.8*,#,@
Rabbit lesion	Volume Days1–3	2.2 ± 2.9	8.3 ± 8.0*	6.0 ± 3.1*	1.4 ± 2.2	6.5 ± 7.5	6.5 ± 6.8#	4.9 ± 4.6#
(RbLV)	(cm^3) Days7–21	0.0 ± 0.0	1.2 ± 1.7	1.7 ± 1.6	0.1 ± 0.3	1.4 ± 2.2	4.1 ± 4.2[e]	3.5 ± 1.7#,*
(RbHS)	Score[d] Days1–3	1.2 ± 1.0	1.5 ± 0.7	1.8 ± 0.7*	0.4 ± 0.7	1.3 ± 1.0#	1.6 ± 1.0#	1.3 ± 0.9#
	Days7–21	0.0 ± 0.0	0.5 ± 0.6*	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.2
Necrosis[f]	3–21 days	1.5	15.5	16	1	15	15.7	16
Hemorrhage[f]	3–21 days	5	6.3	4.3	1.3	8.6	2.4	4.8
Granuloc[f]	3–21 days	3	5.3	9.3	2	0	5	3.7
Mononuc[f]	3–21 days	0	7.7	10.7	0.7	12.5	12.6	11
CHS[f]	3–21 days	9.5	34.8	40.3	5	36.1	35.7	35.5

Note: # Different from oil (ANOVA, $p < 0.05$); * Different from vehicle (ANOVA, $p < 0.05$); @ Different from 30 mg/ml (ANOVA, $p < 0.05$).

[a] Largest group mean.

[b] Post-injection time of the C_{max} designated group.

[c] AUC of group means.

[d] Hemmorhage score.

[e] Does not include outlier.

[f] Scores for necrosis of myofibers, hemorrhage, granulocytic cell infiltration or mononuclear cell infiltration were averaged, and then summed (CHS) over days 3, 7, 14, and 21.

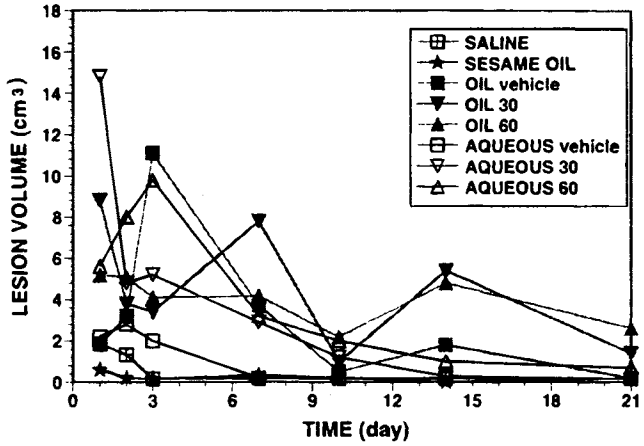

Fig. 1. Average lesion volume (cm^3) 1–21 days post IM injection of 0 (vehicle), 30 and 60 mg/ml danofloxacin oily and aqueous suspension formulation in rabbits (n = 4–8).

for 30 mg/ml DOIL $RbLV_{days7-21}$ (8.2 ± 14 cm^3) was larger ($p < 0.05$) than vehicle. However, when an outlier (which was 38× the average of the remaining RbLV) was excluded, this difference was no longer significant ($0.05 < p < 0.1$, Table I). $RbLV_{days7-21}$ for 60 mg/ml DOIL was greater ($p < 0.05$) than vehicle or oil.

As shown in Figure 2, DAQ produced maximum lesions on day 1 (30 mg/ml: 15.5 ± 10.9 cm^3) and day 3 (vehicle: 2.6 ± 4.2 cm^3, and 60 mg/ml: 9.8 ± 4.0 cm^3). Although DAQ $RbLV_{days1-3}$ was greater ($p < 0.05$) for 30 mg/ml and 60 mg/ml than for vehicle, $RbLV_{days7-21}$ was similar for DAQ and DOIL. Histology scores were generally larger for active formulations than for vehicles, although DOIL vehicle was apparently worse than DAQ vehicle, and oil alone produced the smallest scores. It appeared therefore, subtle differences in the way the two formulations were tolerated by muscle.

Rabbit Hemorrhage Score Model

Except for 30 mg/ml DAQ, the conclusions noted above for $RbLV_{day1-3}$ were in most cases observed for the $RbHS_{day1-3}$. Since both vehicle and 30 mg/ml DAQ $RbHS_{day7-21}$ were similar, this parameter failed to predict the significant RbLV for the latter.

Rabbit CK (RbCK) Model

CK T_{max} following 30 and 60 mg/ml DOIL was 10 and 21 hr, respectively. The prolonged elevation in CK for 60 mg/ml DOIL was consistent with the delayed RbLV maximum stated above. RbKC C_{max} for both active DOIL formulations was greater than ($p < 0.05$) vehicle, and was dose dependent. It was possible that the larger mass of suspended particles in the higher strength formulation contributed to the higher CK C_{max}. CK following sesame oil alone or DOIL vehicle was essentially identical and very low (Figure 3). Similarly, 30 mg/ml DOIL RbCK AUC was larger than ($p < 0.05$) vehicle, and 60 mg/ml DOIL RbCK AUC was much larger ($p < 0.05$) than 30 mg/ml.

RbCK release following DAQ is shown in Figure 2. Compared to vehicle, 60 mg/ml DAQ CK T_{max} was again delayed ($p < 0.05$), and C_{max} was greater ($p < 0.05$). Both 30 and 60 mg/ml DAQ CK C_{max} and AUC were similar.

Gray[1] suggested that formulations that produced RbCK activities <2000–3000 U/L were predictive of human tolerance, while RbCK activities >3000 U/L were not likely to be well-tolerated in human. RbCK C_{max} correctly predicted the 60 mg/ml DOIL and DAQ would be unacceptable, as both formulations produced significant acute lesions ($RbLV_{1-3day}$). DOIL vehicle RbCK was not elevated however, failing to predict the subsequent marked muscle damage $RbLV_{days1-3}$. Although RbLV was typically variable (partly attributed to the small "n"), the present study suggested that certain formulation components may cause muscle damage without the characteristic release of CK. Gray[2] also reported an occasionally low CK activity with formulations that produced a lesion. In these rare instances, the macroscopic exam always weigh more heavily in the final analysis.

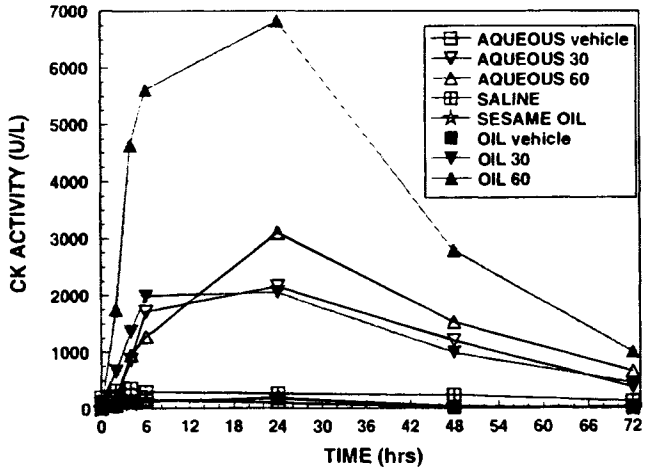

Fig. 2. Average plasma creatine kinase (CK) concentrations from 0–24 hrs post IM injection of oil, 0 (vehicle) 30 and 60 mg/ml danofloxacin oily and aqueous suspension formulation in rabbits (n = 4–8).

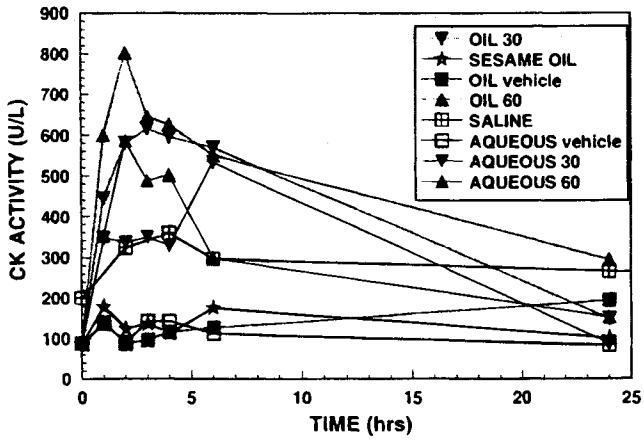

Fig. 3. Average plasma creatine kinase (CK) concentrations from 0–24 hrs post IM injection of oil, 0 (vehicle) 30 and 60 mg/ml danofloxacin oily and aqueous suspension formulation in rats (n = 4–8).

Rat CK Model

CK T_{max} appeared to be earlier in rat than in rabbit (Figures 2 and 3). RtCK release following sesame oil and DOIL vehicle was very low for both formulations. DOIL RtCK C_{max} apparently was dose-dependent, but unlike RbCK, this trend did not achieve statistical significance in the rat.

DAQ RtCK was similar for both strengths (Table I). As was seen for RbCK, very little CK was released in the rat following DAQ vehicle.

In a previous report[7], IM injection of ER formulations that produced RbCK > 3000 U/L *always* produced RtCK > 1000 U/L. Based on the results of that study, a RtCK of 1000 U/L was equated with a RbCK > 3000 U/L (the level at which a formulation is considered unacceptable for humans). As observed in the present work however, ER formulations producing a CK C_{max} > 3000 U/L in the rabbit did not consistently produce a CK C_{max} > 1000 U/L in the rat: 60 mg/ml DOIL RbCK C_{max} was >3000 U/L, while RtCK C_{max} *was not* >1000 U/L, 60 mg/ml DAQ RbCK C_{max} was >3000 U/L, while RtCK C_{max} *was not* >1000 U/L. This inconsistency suggested that either i) rat muscle was less sensitive to damage than rabbit muscle, ii) rat lymphatics cleared irritating formulation components faster than the ER formulation could supply and/or iii) rapid CK clearance in the rat. These differences in formulations and/or models obfuscate a prediction at the "moderate" level of muscle damage.

Rat Hemorrhage Model

Rat hemorrhage scores did not consistently support RbLV findings. This might be due to the smaller injection volume, or limitations of the scoring procedure.

Rat Foot Edema (RFE) Model

RFE was significantly ($p < 0.05$) greater for vehicle, 30 and 60 mg/ml DOIL than sesame oil alone. RFE was very low for all DAQ formulations. RFE correctly predicted an acute vehicle effect ($RbLV_{days1-3}$) for DOIL, yet failed to predict an acute effect for active DAQ formulations. DOIL RFE was also not consistently predictive of *chronic* muscle damage ($RbLV_{days7-21}$). Whereas DOIL vehicle RFE was greater ($p < 0.05$) than sesame oil alone, DOIL vehicle $RbLV_{days7-21}$ was *not* significantly greater than oil alone. Perhaps these inconsistencies could be explained by intrinsic differences between muscle damage after IM injection and the onset of edema after SC injection. The inconsistent predictive capability of RFE therefore rendered it unacceptable as a predictive model for muscle damage of ER formulations.

Comparison of Immediate and Extended Release Formulations

Whereas for immediate release (IR) formulations, attention was usually focused on *acute* muscle damage (*e.g.* 1–3 days post injection), for ER formulations, equally important was the prediction of chronic muscle damage ($RbLV_{days7-21}$).

Compared to an IR 60 mg/ml danofloxacin formulation[7], the ER formulation RbCK T_{max} were twice as extended and C_{max} was one-third to three-fourths as large. Again compared to the IR formulation, the lower RbCK release by DAQ was not predictive of the observed larger chronic lesion. Perhaps DOIL *initially released* less muscle damaging components, but as this release continued, the subsequent *extended exposure* delayed resolution of the muscle lesion. The accurate prediction of *acute* damage was probably due to the presence of immediately available muscle damaging formulation components. For 60 mg/ml formulations, DOIL RbCK was greater than DAQ at every time point (Figures 3 and 4). The larger DOIL RbCK AUC was consistent with the observed chronic muscle damage.

Muscle damage is probably a function of both local tissue concentration and contact-time of drug and/or formulation components. A poorly tolerated IR formulation may make immediately available muscle damaging components that exceed a critical level (C_{crit}). Assuming that the formulation did not interfere with CK activity, then as C_{crit} is reached, cell lysis occurs, and released CK is detected. However, ER formulations by definition release muscle damaging components at a slower rate than IR formulations. At slow rates, dilution and lymphatic clearance of such components, and cellular repair may lessen muscle damage. An ER formulation that allows sufficient amounts of these components to be immediately available to surrounding tissue upon injection would have acute muscle damage predicted by the CK models. If C_{crit} continued to be exceeded, repair might be hampered, resulting in chronic muscle damage (compare lesions for azithromycin[4] and 60 mg/ml DOIL on days 3 and 21). Finally, if C_{crit} was not initially achieved, but achieved 48 to 72 hr later, then the CK models (as presented here) may not have predicted either the acute or chronic muscle damage from the delayed C_{crit}.

Although CK release is commonly thought of as an all-or-nothing phenomenon- the sequelae of events is likely more complicated. Gray suggested that certain lesions may release more CK than others[5]. Brazeau and coworkers[6,7] have reported that for organic cosolvents, the relationship between muscle damage and CK release may in some cases involve an intracellular mechanism of calcium mobilization. The authors recognize that CK release is complex and that additional studies are needed to further determine the relationship between CK and lesions following IM injection of formulations. Perhaps more studies with IR and ER formulations are needed to determine whether the apparent inconsistencies are attributed to formulation or animal model.

In conclusion, none of the short term models (CK, RFE or $RbLV_{days1-3}$) predicted the chronic effects of the ER formulations, since they apparently reflect only the acute muscle damage of such components of the ER formulation that were immediately available to surrounding tissue at the time of injection. As was the case for IR formulations[7], the RtCK model can screen those ER formulations with unacceptable acute damage due to immediately available components. However, to evaluate potential *delayed* effects from ER formulations, the long-term model RbLV was still recommended.

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