Predicting Injection Site Muscle Damage I: Evaluation of Immediate Release Parenteral Formulations in Animal Models

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Purpose. The current animal model generally accepted by the pharmaceutical industry and the FDA for assessment of muscle damage following intramuscular injection (IM) is the rabbit lesion volume model (RbLV). However, this model is resource intensive. The goal of this study was to find a resource sparing alternative to the rabbit lesion model for assessing injection site toleration in IM formulation screening.

Methods. Short term animal model alternatives to RbLV for evaluating IM formulations were examined. In addition to RbLV, myeloperoxidase (MPO), p-nitrophenyl N-acetyl-β-glucosaminide (NAβG) and/or plasma creatine phosphokinase (CK) activities were determined in rabbits (Rb) and rats (Rt) after injection of formulations (digoxin, azithromycin and danofloxacin). The edema from these formulations 24 hr after subcutaneous injection into the rat footpad (RFE) was also determined.

Results. MPO and NAβG were not considered very useful as biochemical predictors of muscle damage for these formulations. Histology generally correlated with RbLV values. Compared to saline, RbLV was marked for all formulations within 1–3 days of injection. After day 3, lesions quickly resolved, and no significant differences were found. For these formulations, all CK animal models and RFE were generally predictive of RbLV. A formulation with RtCK > 1000 U/L or RbCK > 3000 U/L, was predicted to be poorly, tolerated.

Conclusions. Due to ease, number of animals, time and intrinsic mechanism, we concluded that for most formulations, 2 and 4 hr RtCK data alone should be reasonably predictive of muscle damage.

KEY WORDS: rabbit; rat; lesion; creatine kinase; saline; digoxin; azithromycin; danofloxacin.

INTRODUCTION

For intramuscular (IM) therapy, toleration of the injected formulation is often an issue. To aid the formulation scientist in achieving the least irritating formulation, a variety of *in vivo* and *in vitro* screening tools are available. The current animal model generally accepted by the pharmaceutical industry and the FDA for assessment of muscle damage following IM is the rabbit lesion volume model (RbLV). In addition, commonly reported animal models are the rabbit creatine kinase (CK) models (1,2,3) rat lesion and CK models (4), and the rat paw lick model (for the assessment of pain (5)). However, a thorough study of animal models, using the same agents, in the same lab, is lacking. Laboratory and assay variability make it difficult to compare between studies.

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The goal of this study was to find a resource sparing accurate methodology for assessing injection site tolerance in 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 formulations, using biochemical, macroscopic and microscopic methods, and 2) compare alternative animal models.

Creatine kinase (CK), aspartate aminotransferase (ASAT) and lactate dehydrogenase (LDH) are cytosolic enzymes that are released from muscle cells in response to injury (6,7). Following IM digoxin, ASAT and LDH activities were not, while CK activity was significantly elevated in humans (6). Since CK had also been correlated with acute muscle damage in humans, swine and rabbits (8) and in rats (4), its activity was determined in this study. Saline (negative control) was used to compensate for any effect from handling and injection. Digoxin was used as a positive control, since it had been reported to cause significant muscle damage (9) and CK increase (7) in rabbits. In addition to CK, tissue myeloperoxidase (indicator of neutrophils, commonly associated with tissue necrosis (10)), and tissue N-acetyl-β-glucosaminidase (indicator of monocytes, commonly associated with tissue inflammation (11)) were examined as potential biochemical predictors of muscle damage. The macroscopic and microscopic damage observed in the rabbit lesion model was then compared with rabbit plasma CK, and rat lesion and plasma CK after IM injection of the same formulations. The rat foot edema model (RFE) was internally used both to screen anti-inflammatory compounds, and predicting whether a compound might be tolerated after parenteral administration. Therefore, the inclusion of this model in the evaluation provided a useful reference.

METHODS

Materials

Normal saline, 0.25 mg/ml digoxin (Lanoxin® lot 2T2341: 40% propylene glycol, 10% ethanol, 0.3% Na_2PO_4, 0.08% citric acid), Pfizer Central Research formulations of azithromycin (2 mM citric acid, pH adjusted to 6.6 with NaOH) and danofloxacin (1.6% lactic acid, 0.25% phenol, pH adjusted to 4 with NaOH) were used. Formulations of azithromycin and danofloxacin were selected since their development represented realistic challenges to the formulation scientist. Formulations were either purchased sterile, or rendered sterile prior to injection by filtration through a 0.2μ membrane. Reagent grade common buffers and salts, analytical grade chemicals used in the assays and compendia grade of other formulation components were used.

Creatine Kinase (CK) Assay

A commercially available kit (47-UV, Sigma, St. Louis, MO was used to determine total activity of all CK isoenzymes (12). Quality control standards were used in the assay, which was linear over the range used in this study (5–50 U/L). Samples were stable at room temperature for 24 hr, but were always assayed on the day of the experiment. Interference between compounds and the CK assay (13) was ruled out for digoxin, azithromycin and danofloxacin (data not shown).

Myeloperoxidase (MPO) Assay

A modified form of the methods of Miller *et al.* (14) was used. Approximately 100 mg of tissue was homogenized, frozen and thawed. As specified by Miller *et al.* (14), this freeze—thaw cycle was repeated and the sample was centrifuged. An aliquot of the supernatant was incubated with KH_2PO_4, dimethylformamide, *o*-dianisidine dihydrochloride and H_2O_2, at 37°C for 15 min., and then quenched with glycine. Absorbance against known standards at 450 nm was determined on a Perkin Elmer Lambda 3 spectrophotometer for 2 min. One unit of MPO activity was defined as that required to degrade 1 μmol of hydrogen peroxide/min at 37°C.

N-acetyl-β-glucosaminidase (NAβG) Assay

A modified form of the methods of Pettipher *et al.* (11) was used. Fifty to 100 mg of tissue was thawed, homogenized, and then centrifuged. An aliquot of the supernatant was incubated with 1 mg/ml p-nitrophenyl N-acetyl-β-glucosaminide in 0.1 M citrate/phosphate buffer (pH 4.5), at 37°C for 60 min., and then quenched with 1 M glycine/NaOH (pH 10). Absorbance against known standards at 405 nm was determined on a Perkin Elmer Lambda 3 spectrophotometer for 2 min. One unit of NAβG activity was defined as that required to liberate 1 μg phenol from the substrate per hour at 37°C.

Animal Models

Animals were acclimated for several days (rats, Charles River) or weeks (rabbits, Hazleton Research Products) prior to study. All protocols were approved by the institutional ACUP Committee, which administered the principles of laboratory animal care as found in NIH #85-23, 1985.

Rabbit Lesion Model

The rabbit lesion model as previously described (2,3,15) was followed. New Zealand White rabbits (2.7–2.9 kg) of either sex (3–8 per time point, per formulation) were injected approximately 0.6 cm deep into the sacrospinalis muscle with 1.0 ml of the formulation of interest, using 23-gauge sterile needles. Groups of animals were euthanitized at 3, 7, 14 and 21 days or 1, 2 and 11 days post injection. Lesions were subjectively scored (15) for hemorrhage (RbHS, reddening) as follows: 0 (no grossly detectable coloration), 1 (slightly red), 2 (mild), 3 (moderate), 4 (black). Scoring was an average of the independent evaluation of two unblinded investigators. These scores were virtually identical for all samples. The total volume (RbLV) of abnormal appearing tissue (necrotic, hemorrhagic, *etc.*) was measured as L-W-D (length [anterior to posterior]-width [midline to side]-depth [top to bottom of lesioned muscle]) at the point of maximum involvement. Samples were fixed in 10% formalin, trimmed sections were dehydrated in graded alcohols, embedded in sliced, paraffin, sectioned at a thickness of 6 um, placed on glass slides, stained with hematoxylin and eosin,and coverslipped. Microscopic changes in skeletal muscle from animals injected with formulations containing 0 (vehicle), 25 or 60 mg/ml danofloxacin and 100 mg/ml azithromycin were quantified by subjective evaluation of the following criteria which were scored on a scale of 1 to 5: necrosis of myofibers, hemorrhage, granulocytic cell infiltration and mononuclear cell infiltration. The score for each sample was based upon the most severe changes present but did not reflect relative size of lesion. These scores were averaged across animals for each day and index, and then summed across days 3, 7, 14 and 21 to give a cumulative histology score (CHS).

Rabbit CK Model (RbCK)

A different group of rabbits was surgically cannulated at the jugular vein with a vascular access port (Model SLA, Access Technologies, Skokie, IL). Ports were flushed with heparinized saline (500 U/ml) weekly, and CK activity was determined. Usually within one week following aseptic surgery, CK activity was determined. Usually within one week following aseptic surgery, CK activity was back to baseline values (~100 U/L). For each study, 4–6 cannulated rabbits were similarly injected with the formulation. Blood was collected from the ports just before, and 2, 4, 6, 24, 48 and 72 hr after IM injection of the test formulation, centrifuged, and plasma CK activity immediately determined.

Rat CK and Lesion Models

Separate groups (n = 4–6) of Sprague Dawley male rats (0.25–0.35 kg) were briefly anesthetized with methoxyflourane, shaved, and injected with 0.5 ml of the test formulation approximately 0.4 cm deep into the gluteus medius using 23-gauge sterile needles. Blood was collected from animals by cardiac puncture at 1, 2, 3, 4, 6 or 24 hr post injection, centrifuged, and plasma separated for immediate CK determination. Animals were immediately euthanitized and the site of injection was scored for hemorrhage.

Rat Foot Edema Model

In this model, the edema observed in the foot pad following subcutaneous (SC) injection of the highly irritating protein carrageenan was compared in the presence or absence of a potential anti-inflammatory compound. Rats were physically restrained, the right hind foot pad injected with 0.1 ml of the formulation, and the left foot pad served as control (no injection). The animals were euthanitized 24 hr later, the hind feet were amputated and weighed.

Statistics

Mean ± SD was reported throughout. Differences in formulations were investigated for the parameters RFE, RtCK C_{max} (group with largest average CK activity) RbCK C_{max} (first occurrence of maximal CK activity), RbCK T_{max} (time of C_{max}), RbCK $AUC_{0-.72}$ (linear trapezoidal rule), $RbLV_{Day3}$, $RbLV_{1-3day}$ (average of values for days 1–3), $RbLV_{7-21day}$ (average of values for days 7–21). One-way and 2-way ANOVA were completed with the GLM procedure of SAS statistical software (SAS Institute Inc., Cary, NC). Hemorrhage scores and pathological evaluations were not statistically evaluated, but were included for their comparative value. Least squares means differences were considered significant at p values <0.05.

RESULTS AND DISCUSSION

Myeloperoxidase (MYO) Assay

At 24 hr., MYO activities were higher for tissue injected with digoxin than with saline (1.32 and 0.44 U/100 mg, respec-

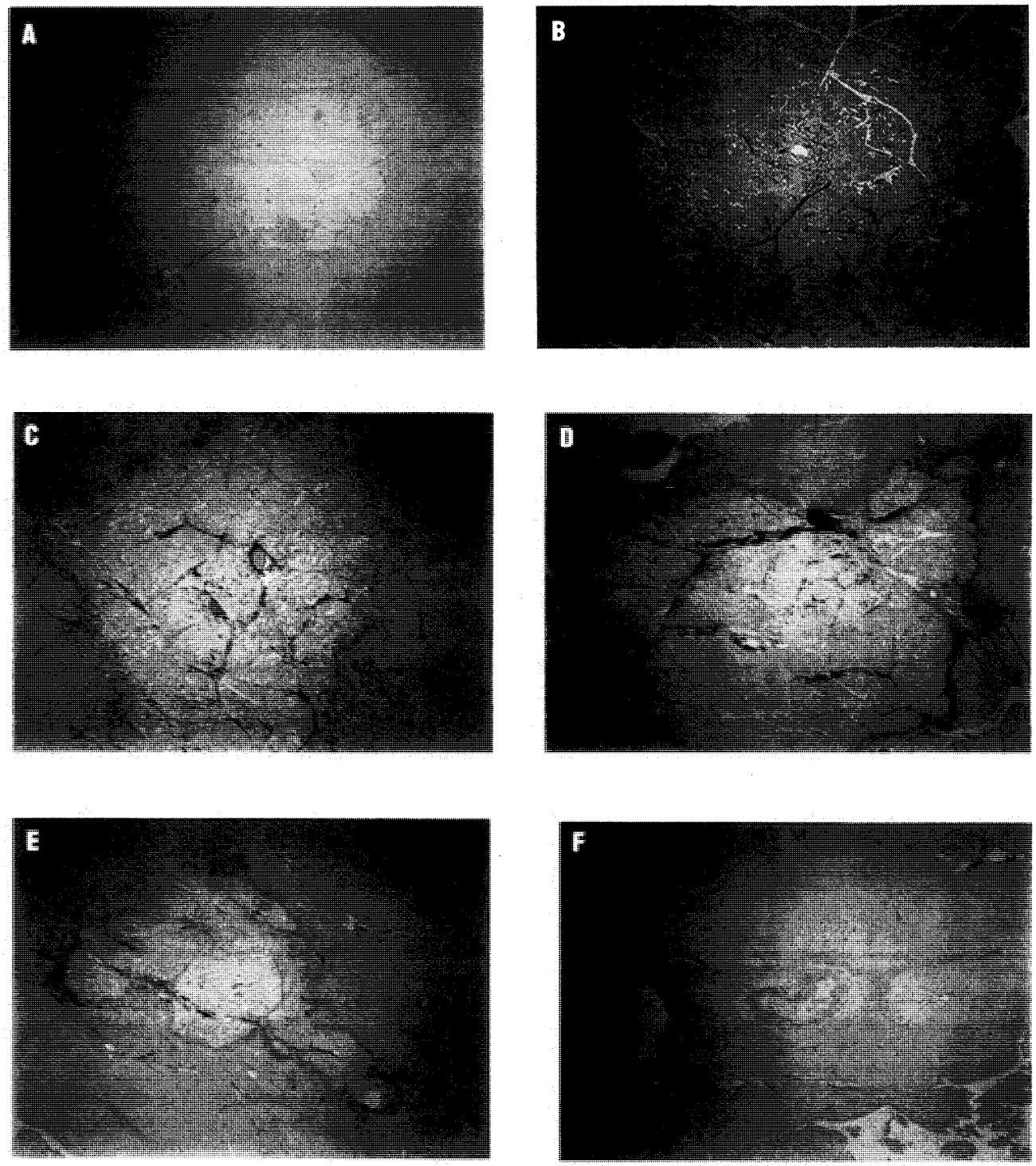

Fig. 1. Representative micrographs (15×) of tissues from rabbits administered saline or digoxin. A: control needle stick, B-F: digoxin on days 2, 3, 7, 14, and 21.

tively), but were essentially identical after 72 hr. Although MYO correlated with lesion volumes ($r^2 = 0.78$) and CK reported in these same animals, MYO was an order of magnitude less than reported in the literature for ileitis (14). Microscopic examination of these tissues also confirmed a paucity of neutrophils (Figure 1A–F). This may be due to the sterile nature of the formulations and/or that the cytotoxic nature of digoxin. While assay of tissues exposed to other formulations were not completed, initial results suggested that the MYO tissue assay may not be a resource or time sparing biochemical predictor of toleration.

N-acetyl-β-glucosaminidase Assay

NAβG activities in lesions formed by injection of danofloxacin were not correlated with RbLV ($r^2 = 0.11$). Although these formulations apparently stimulated the migration

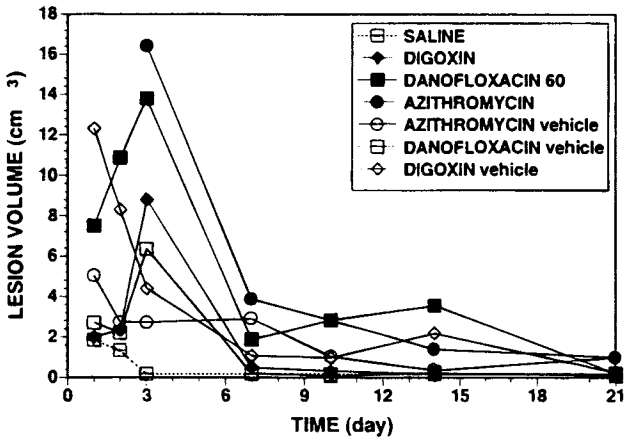

Fig. 2. Lesion volume (cm^3) 1–21 days post IM injection of saline (0.9%), digoxin (0.25 mg/ml), azithromycin (100 mg/ml) danofloxacin (60 mg/ml) and vehicles in rabbits.

of monocytes to the injection site (9 of the 14 samples assayed scored 3–4 for mononuclear cell infiltration), NAβG and mononuclear scores did not correlate very well ($r^2 = 0.29$). Interestingly, correlation with histological scoring of necrosis, hemorrhage, mineralization or granulocytes was even worse (data not shown). The NAβG tissue assay may therefore not be a useful biochemical predictor of toleration for these formulations.

Rabbit Lesion Volume Model

Injection of saline alone produced minor lesions which resolved in 1–2 days. As qualitatively shown in Figure 1, and quantitatively shown in Figure 2, digoxin, azithromycin and danofloxacin produced maximum lesion volumes on day 3. Others (16,22) have also reported marked muscle lesion 3 days after injection of formulations. Also in agreement with others (8,17, digoxin caused significantly ($p < 0.01$) more muscle damage than saline ($RbLV_{Day3}$: 8.8 ± 6.4 and 0.10 ± 0.17 cm^3, respectively). While digoxin vehicle produced a larger lesion than saline on Day1 ($p < 0.01$) and Day2 ($p < 0.05$), this difference was not significant ($p = 0.07$) on Day3 (4.4 ± 1.1 cm^3). Since both digoxin and vehicle have been reported (18,19) to cause similar muscle damage, the failure of vehicle to achieve statistical significance on Day3 was likely due to the small "n". Compared to saline, $RbLV_{Day3}$ after azithromycin (16.4 ± 13.7 cm^3) and danofloxacin (60 mg/ml) formulation (13.8 ± 5.1 cm^3) were larger ($p < 0.01$). Azithromycin and danofloxacin (60 mg/ml) formulation were also more damaging ($p < 0.05$) than their vehicles (2.7 ± 0.8 cm^3 and 6.4 ± 7.7 cm^3, respectively). The large variability observed for azithromycin (5× difference in range) and danofloxacin (3×) was probably a consequence of the severe irritation these formulations caused. Similar large variability (4×) was reported (20) for clopenthixol and chlorpromazine.

Because marked pain on injection (POI) was observed in the "Day 3, 7, 14 and 21 group" following administration of azithromycin, completion of the "Day 1, 2 and 10 group" did not seem warranted. The azithromycin vehicle apparently did not cause any discomfort. The issue of pain and its relevance to muscle damage is controversial (21). Comereski (5) ranked pain (measured by the rat paw lick model) with muscle damage (gross pathology and histopathology in the rabbit lesion model) for a series of cephalosporins. However, Svendsen (1) implied that pain resulting from injection injuries of nerves through faulty technique may not be related to muscle damage. It could not be determined from these experiments why only azithromycin caused POI, while $RbLV_{Day3}$ was significant for all three formulations. Interestingly, IV azithromycin (when diluted) was well tolerated in humans (D. Luke, Pfizer, personal communication).

As shown in Figure 2, muscle damage after formulations was dramatically reduced after day 3. In agreement with the literature (17), digoxin vehicle $RbLV_{1-3day}$ was more damaging ($p < 0.01$) than saline. While the $RbLV_{1-3day}$ for the 25 mg/ml danofloxacin formulation was not different than vehicle, 60 mg/ml formulation $RbLV_{1-3day}$ was greater than vehicle ($p < 0.01$) and the 25 mg/ml formulation ($p < 0.05$). The histology scores ranked with RbLV for vehicle, 25 and 60 mg/ml danofloxacin formulations (Table 1).

Rabbit Hemorrhage Score Model

Except for 60 mg/ml danofloxacin, the conclusions noted above for lesion volumes were in most cases observed for hemorrhage scores (Table I). However, since the hemorrhage score for danofloxacin (60 mg/ml) was not different from vehicle, this model failed to predict the muscle damage for this formulation.

Rabbit CK (RbCK) Model

RbCK activities following injection of saline and test formulations are shown in Figure 3; RbCK C_{max}, T_{max} and AUC_{0-72} are listed in Table I. Consistent with the literature (8), RbCK T_{max} ranged from 6 to 24 hr, returning to background activities by 72 hr. The RbCK C_{max} after saline was comparable to reported (7) values of ~350 U/L. Digoxin produced a CK C_{max} that was 25-fold greater than saline in rabbits. In addition to elevated CK release in rabbits (7), digoxin has caused a significant release of CK in rat muscle (18), and humans (17) and severe pain upon injection in patients (22). Consistent with *in vitro* models (18), digoxin vehicle also caused significant RbCK release. Gray (3) suggested that formulations that produced RbCK activities <3000 U/L were predictive of human tolerance, while RbCK activities >3000 U/L were not likely to be well-tolerated in man. Using this definition, none of the active formulations, or the digoxin vehicle would be acceptable. IM administration of azithromycin or danofloxacin vehicles produced low RbCK levels, consistent with the small lesions observed in the rabbit. The values of RbCK AUC paralleled those of C_{max} (Table I), and did not provide any additional discrimination between formulations.

Rat CK Model

CK activities in samples collected by cardiac puncture (eg. 2 hr: 158 ± 76.7 U/L) were similar to those obtained by other methods shown to have no effect on CK activities (4). The ease of the single-time-point-per-animal method (i.e. no catheterization) outweighed the slight fluctuation in average CK activity (Figure 4). Compared to the rabbit, less CK was more rapidly released following injection of formulations in the rat (compare Figures 3 and 4). RtCK C_{max} for the formulations and digoxin

Table 1. Summary of Animal Model Evaluation of Various IM Formulations (mean ± SD, n = 3–8 for each Time Point, See Text for Details)[a]

			Digoxin		azithromycin		danofloxacin		
Model	Parameter	Saline	vehicle	0.25 mg/ml	vehicle	100 mg/ml	vehicle	25 mg/ml	60 mg/ml
Rat foot	RFE (g)	<0.05	0.11 ± 0.06$^{@@}$	0.24 ± 0.07$^{@@,*}$	0.04 ± 0.02	0.31 ± 0.11$^{**,@@}$	0.02 ± 0.03	0.09 ± 0.06$^{@}$	0.14 ± 0.03$^{*,@@}$
Rat CK	C_{max}(U/L)	403 ± 159	1014 ± $^{@@}$	1311 ± 315$^{@@}$	834 ± 225	1327 ± 504**	179 ± 88.0	1764 ± 455**	2614 ± 485**
(RtCK)	T_{max}(hr)	4	1	2	1	2	1	4	4
Rat lesion	4 hr	0.3 ± 0.3	2.5 ± 0.5	2.5 ± 0.6	0.0 ± 0.0	2.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.3
(RtHS)	(0–4)								
Rabbit CK	C_{max}	406 ± 90	3269 ± 611$^{@@}$	5528 ± 1218$^{@@,**}$	532 ± 189	4544 ± 665**	1317 ± 623	3549 ± 1662**	9251 ± 2215**
(RbCK)	(U/L)								
	T_{max}(hr)	14.5 ± 19.3	6.0 ± 00	6.5 ± 1.9	10 ± 9.0	15 ± 10	20 ± 18	4.5 ± 1.7	10 ± 8.1
	AUC_{0-72} ($\times 10^{-3}$)	3.74 ± 2.66	111 ± 21.2$^{@@}$	214 ± 36.2$^{@@,**}$	14.2 ± 5.1	154 ± 16.2**	41.6 ± 22.2	132 ± 72.0**	261 ± 75.5**
(RbLV)	1–3day	0.4 ± 0.7	7.9 ± 6.2$^{@@}$	9.24 ± 5.4$^{@@}$	3.3 ± 2.1	NC[b]	3.8 ± 4.5	5.7 ± 5.7	11.5 ± 6.3$^{**,@,\#}$
(cm^3)	7–21day	0.0 ± 0.0	1.2 ± 1.3	2.0 ± 1.0	1.1 ± 1.3	NC	0.2 ± .2	1.0 ± 1.5	2.1 ± 1.9
(RbHS)	1–3day	1.4 ± 1.0	2.0 ± 0.5$^{@}$	2.0 ± 0.8$^{@}$	2.2 ± 0.6	NC	1.5 ± 0.8	2.4 ± 0.8**	2.1 ± 0.9
(0–4)	7–21day	0.04 ± 0.1	0.5 ± 0.6	0.2 ± 0.4	0.7 ± 0.9	NC	0.3 ± 0.4	0.4 ± 0.8	0.8 ± 0.6
Necrosis[c]	3–21 days	NC	NC	NC	NC	5	4.7	7.7	10.3
Hemorrhage[c]	3–21 days	NC	NC	NC	NC	2	2.7	4	4.3
Granuloc[c]	3–21 days	NC	NC	NC	NC	8	1	1.5	2
Mononuc[c]	3–21 days	NC	NC	NC	NC	6.3	5.7	8.5	9.2
CHS[c]	3–21 days	NC	NC	NC	NC	21.3	14.1	21.7	25.8

[a] Different from 25 mg/ml (#) saline (@), vehicle (*): one symbol $p < 0.05$, two symbols $p < 0.01$, ANOVA.

[b] NC: not completed (see text).

[c] 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.

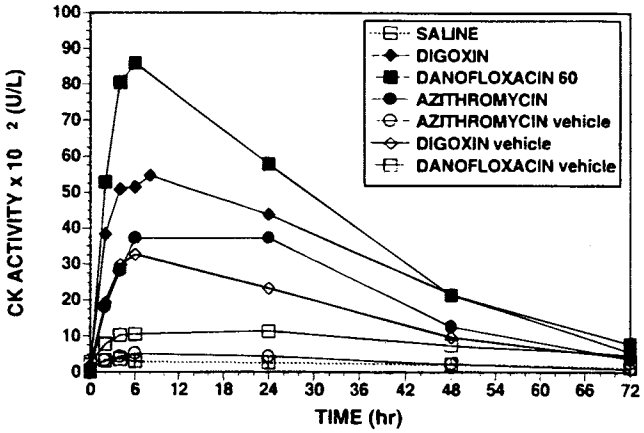

Fig. 3. Plasma creatine kinase (CK) activity 0–72 hr post IM injection of saline (0.9%), digoxin (0.25 mg/ml), azithromycin (100 mg/ml) danofloxacin (60 mg/ml) and vehicles in rabbits.

vehicle was between 1000 and 3000 U/L. On average, the rat response in this study was one-third of the rabbit response (Table I). This difference may in part reflect a less sensitive rat muscle and/or a more rapid clearance of CK in the rat (23,24). Baseline CK levels were generally reached in 24 hr for rats and in 72 hr for rabbits. Meltzer *et al.* (7) also reported that the increase in serum CK activity after IM injection of Thorazine® was much less in rats than in rabbits, and lasted only about 24 hours. Surber (4) reported "very severe" macroscopic damage in the rat after IM imipramine and navaminsulfon-Na; of the formulations tested, only these two produced 2 hr rat CK activities in the 1000 U/L range. Therefore, a formulation resulting in a RtCK C_{max} > 1000 U/L would, as one producing a RbCK C_{max} >3000 U/L, not be well-tolerated in humans (3). Although differences in muscle damage may be more difficult to predict with the RtCK model, these observations support earlier less complete studies (4,25) that suggested the RtCK model may be predictive of muscle damage.

While the surrogate parameter RtCK AUC_{0-24} correlated with RtCK C_{max}, it held no advantage over C_{max}, and was more resource intensive (data not shown).

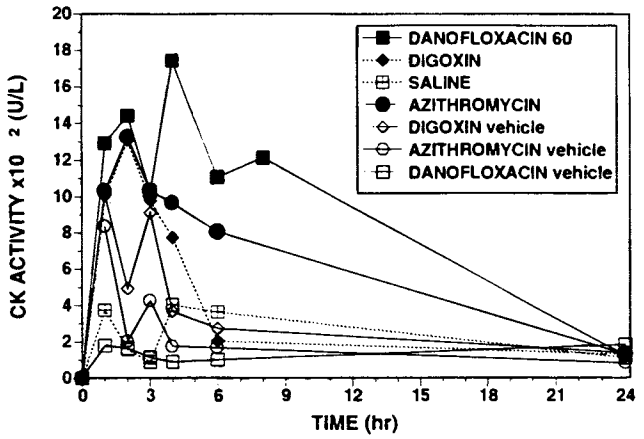

Fig. 4. Plasma creatine kinase (CK) activity 0–24 hr post IM injection of saline (0.9%), digoxin (0.25 mg/ml), azithromycin (100 mg/ml) danofloxacin (60 mg/ml) and vehicles in rats.

Rat Hemorrhage Model

The rat hemorrhage scores at 4 hr ($RtHS_{4hr}$) are included in Table I, for qualitative comparisons only. Although $RtHS_{4hr}$ also appeared to correlate with the $RtHS_{24hr}$ values (4) (data not shown), these parameters were not consistently predictive of RbLV.

Rat Foot Edema (RFE) Model

RFE was negligible after subcutaneous (SC) injection of saline (Table I). RFE was greater ($p < 0.05$) than the respective vehicle for digoxin, azithromycin and danofloxacin (60 mg/ml). The ability for RFE to discriminate formulations was generally good. This was unexpected, since the mechanism of edema following SC injection was likely different from that of muscle damage following IM injection. It remains to be shown whether the RFE model could be predictive of muscle damage for a wide number of drug classes and formulations. It is possible that the intrinsic mechanisms and ensuing measurements from the two models could differ in subtle, unexpected ways.

Conclusion

An important criteria for a model is its ability to determine whether a formulation is damaging. RbCK C_{max}, RtCK C_{max}, and RFE were equally predictive of $RbLV_{1-3day}$. However, the RbCK model was as resource intensive as the RbLV model, and the mechanism of action in the RFE model may be fundamentally different than that in the RbLV model. Therefore, the RtCK C_{max} model apparently held some advantage over the other models for this subset of formulations. Brazeau and coworkers (19,26) 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 elucidate the relationship between CK and lesions following IM injection of formulations. Nevertheless, we conclude that for most immediate release formulations, 2 and 4 hr RtCK data alone should be reasonably predictive of muscle damage.

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REFERENCES

1. Svendsen, O: *Studies of Tissue Injuries Caused by Intramuscular Injection of Drugs and Vehicles*, Thesis, Royal Veterinary and Agricultural University, Copenhagen, 1988.
2. Gray, J. E.: In: Sustained and Controlled Release Delivery Systems, JR Robinson (ed.), Marcel Dekker, NY, 1978, pp. 351–410.
3. Gray, J. E.: *Fund Appl Tox* **1** 290–292, 1981.
4. Surber C. and H. Sucker: *Pharm Res* **4**:490–494, 1987.
5. Comereski C. R., P. D. Williams, C. L. Bregman and G. H. Hottendorf: *Fund Appl Tox* **6** 335–338, 1986.

6. Anderson, K. E. and Damsgaard, T.: *Acta med. scand.* **199**:317–319, 1976.
7. Meltzer H. Y., S. Mrozak, M. Boyer: *Amer. J. Med. Sci.* **259**:42–48, 1970.
8. Steiness E., F. Rasmussen, O. Svendsen and P. Nielsen: *Acta Pharmacol et Toxicol* **42**:357–364, 1978.
9. Svendsen O., F. Højelse and R. E. Bagdon: *Acta Pharmacol et Toxicol* **56**:183–190, 1985.
10. Lefkowitz D. L., K. Mills, D. Morgan and S. S. Lefkowitz: *Proc. Soc. Exp. Biol. Med.* **199**:204–210, 1992.
11. Pettipher E. R., B. Henderson, S. Moncada and G. A. Higgs: *Br. J. Pharmacol.* **95**:169–76, 1988.
12. Szasz G., W. Gruber and E. Bernt: *Clin. Chem.* **22** 650–656, 1976.
13. Brazeau G. A. and H.-L. Fung: *Biochem J* **257** 619–621, 1989.
14. Miller M. J. S., H. Sadowska-Krowicka, S. Chotinaruemol, J. L. Kakkis and D. A. Clark: *J. Pharmacol. Exp. Ther.* **261** 11–16, 1993.
15. Shintani S., M. Yamazaki, M. Nakamura and I. NakayamaL: *Tax Appl Pharmacol* **11** 293–301, 1967.
16. Oshida S., K. Degawa, Y. Takahashi and S. Akaishi: *Tnhoku J exp Med* **127** 301–316, 1979.
17. Steiness E., O. Svendsen and F. Rasmussen: *Clin Pharmacol Ther* **16** 430–434, 1974.
18. Brazeau G. A. and H.-L. Fung: *Pharm Res* **6** 167–170, 1989.
19. Brazeau, G. A. and H.-L. Fung: *J Pharm Sci* **79** 393–397, 1990.
20. Svendsen, O.: *Local muscle damage and oily vehicles: Acta pharmacol et. toxicol* **52** 298–304, 1983.
21. Brazeau, G., Gatlin, L. A., Jackson, M., Sutton, S. C., Gupta, P. K.: *Symposium on The Evaluation of Pain, Irritation and Tissue Damage with Parenteral Formulations*. AAPS Tenth Annual Meeting, Miami Beach, FL, 5–9 Nov., 1995.
22. Greenblatt J., D. W. Duhme, M. Penna, T. Greiner and H. Gold: *N Eng. J Med* **288** 651–654, 1973.
23. Meltzer, H.: *Biochem. Pharmacol.* **20** 1739–1748, 1971.
24. Warnock, D. G. and G. L. Ellman: *Science* **164** 726, 1969.
25. Paget and H McG Scott: *Brit J Pharmacol* **12** 427–433, 1957.
26. Brazeau, G. A., S. S. Watts and L. S. Mathews: *J Parent Sci Tech*, **46** 25–30, 1992.