Use of an in Vitro Model for the Assessment of Muscle Damage from Intramuscular Injections: in Vitro–in Vivo Correlation and Predictability with Mixed Solvent Systems

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INTRODUCTION

Intramuscular administration is frequently employed in drug therapy for prompt action, when intravenous or oral administration is unsuitable (1–3). Many intramuscular formulations for lipophilic drugs utilize aqueous organic cosolvents, viz., ethanol, propylene glycol, polyethylene glycol, glycerol, and dimethylacetamide, to provide adequate solubility (4). Propylene glycol, ethanol, and polyethylene glycol 400 are among the most commonly used organic cosolvents in injectable formulations, e.g., of hydralazine, lorazepam, phenytoin, digoxin, phenobarbital, pentobarbital, and diazepam (4). However, parenteral administration of the organic cosolvents can cause tissue damage and hemolysis (5–13). The potential of these solvent mixtures to cause skeletal muscle damage (myotoxicity) have not been systematically characterized.

In previous work, we had developed an in vitro technique that measures the release of creatine kinase from an isolated rat muscle model to screen agents for their potential to cause skeletal muscle damage (14). A good rank order correlation was obtained between this in vitro technique and the in vivo myotoxicity of a number of pharmaceutical formulations, as indicated by circulating creatine kinase levels and histological evaluation (which are the commonly utilized indices of skeletal muscle damage both clinically and experimentally) (14). We determine here the myotoxicity of binary mixtures of propylene glycol–water, ethanol–water, and polyethylene glycol 400–water. These in vitro results are then validated with in vivo studies on creatine kinase activity in male New Zealand white rabbits.

The in vitro myotoxicity model was applied to the rational design of intramuscular injection systems. The composition of the solvent system of an intramuscular formulation should cause minimal skeletal muscle damage and patient discomfort, with optimal pharmaceutical (e.g., solubility, stability, and injectability) and biopharmaceutical (e.g., rate and extent of absorption) properties. A series of mixed solvent systems, each possessing equivalent theoretical molar solubility for a model compound (diazepam) was compared to the commercially utilized and quite myotoxic vehicle by evaluating their myotoxicity with the isolated in

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vitro rat muscle model. We intended to determine whether skeletal muscle damage can be reduced by a change in the solvent composition, while maintaining equal solubilization power for the drug, and whether the myotoxicities of each component are additive.

These investigations, to our knowledge, provide the first myotoxicity data for three individual organic cosolvents commonly utilized in intramuscular formulations. These studies also illustrate a rational approach for the design and testing of intramuscular injection solvent systems, accounting for both vehicle myotoxicity and drug solubility.

MATERIALS AND METHODS

Materials

Propylene glycol, ethanol, and polyethylene glycol 400 were obtained from Fisher Scientific Company, Aaper Alcohol and Chemical Company, and Sigma Chemical Company, respectively. Double-distilled water was used in the preparation of the binary and ternary organic cosolvent–water mixtures. All other chemicals were at least reagent grade and obtained from J. T. Baker Chemical Company or Fisher Scientific Company.

Myotoxicity Screening

Skeletal muscle damage was determined using a previously described in vitro isolated rate muscle model (14). Briefly, male Sprague Dawley rats were sacrificed, and the extensor digitorum longus (EDL) muscle isolated and removed. These muscles were injected with 15 µl of the test solution and placed into a 37°C balanced salt solution bubbled with 95% O₂–5% CO₂. The myotoxicity of the injected solutions was evaluated by the cumulative release of creatine kinase (an intracellular cytosolic enzyme) into the incubation medium over a 2-hr period. Creatine kinase activity was measured using the CK reagent (Sigma Chemical Company, St. Louis, Mo.) as described previously. Possible spectrophotometric and kinetic interferences by the test solutions were ruled out in preliminary experiments.

In Vitro Organic Cosolvent Myotoxicity

Binary mixtures of propylene glycol–water, ethanol–water, and polyethylene glycol 400–water (0–100%, v/v, organic cosolvent) were prepared and tested for their in vitro myotoxicity. Four to nine muscle preparations were used at each concentration of the organic cosolvent. In order to use these myotoxicity data in subsequent studies, the data for each individual organic cosolvent (myotoxicity vs molar concentration) were empirically fitted to a sigmoidal curve (propylene glycol–water mixtures and ethanol–water mixtures) or to a straight line (polyethylene glycol 400–water mixtures between 20 and 80%).

In Vivo Organic Cosolvent Myotoxicity

In order to validate the in vitro myotoxicity results, two in vivo studies were carried out. First, the myotoxicity of normal saline, 40% (v/v) propylene glycol, and 40% (v/v) polyethylene glycol 400 was investigated. The skeletal muscle damage caused by the above solvent systems was evaluated using the suggested drug safety guidelines proposed by the Pharmaceutical Manufacturers Association for musculoskeletal irritant effects of drugs (15). Male New Zealand white rabbits (2.0–4.2 kg) were familiarized with the investigators and the experimental surroundings prior to the start of the experiment. The animals were then injected with 1 ml of each test solution in a three-way crossover randomized design, with a minimum of 2 weeks between each phase of the experiment. The injection was made using a 23-gauge 1-in. needle into the midlumbar muscles. The injection sites were randomly rotated to assure that no single muscle area received more than one injection. Blood samples (1 ml) were obtained from the central artery or the marginal vein of the ear at −1.0, −0.75, −0.25, 0.5, 1, 2, 4, 6, 12, 24, 48, and 72 hr after injection. The animals were given access to water from 4 hr on. The samples were stored at −20°C until analyzed (not longer than 4–5 days) and serum creatine kinase activity levels were analyzed using a commercial CK Reagent (Sigma Chemical Company, St. Louis, Mo.). Serum creatine kinase activity was corrected in each animal by subtracting the mean baseline creatine kinase activity (determined from the −1.0, −0.75, and −0.25 hr samples). The area under the corrected serum creatine kinase activity versus time curve between 0 and 72 hr was calculated using the linear trapezoidal rule. The reported values are the mean and standard deviation of five animals.

A second in vivo experiment was later carried out to include the presence of a myotoxic compound in an intramuscular injection solution. Indocyanine green (5 mg/kg) in normal saline or in 40% (v/v) propylene glycol (total injection volume of 0.5 ml/kg) was injected into the midlumbar muscles of male New Zealand white rabbits (two rabbits per treatment). Heparinized blood samples (1.5 ml) were collected at 0, 0.33, 0.66, 1.0, 1.5, 2, 4, 6, 8, 12, 24, 72, 120, 168, and 240 hr after injection. The data were treated as previously described. Plasma concentrations of indocyanine green were examined using high-performance liquid chromatography (16). The in vitro myotoxic potentials of these indocyanine green solutions were determined using the isolated rat muscle model.

Ternary Cosolvent–Water Mixtures

A series of ternary mixtures (viz., propylene glycol–ethanol–water, polyethylene glycol 400–ethanol–water, and polyethylene glycol 400–propylene glycol–water) was selected based on their equivalent ability to solubilize diazepam. The mixtures were chosen by using the linear solubilization relationships of Yalkowsky and associates (17,18). In this theoretical relationship, Eq. (1),

$$\log(S_m/S_w) = f_1\delta_1 + f_2\delta_2$$

the log ratio of drug solubility in the ternary solvent mixture ($S_m$) to that in water alone ($S_w$) is estimated as a linear combination of the product of the volume fraction of each cosolvent ($f_1, f_2$) and the solubilization slope for the drug in the respective cosolvent ($\delta_1, \delta_2$). Assuming that this ideal linear relationship exists for diazepam solubility in these ternary mixtures, a series of solutions having theoretically equivalent diazepam molar solubility to a reference solution was prepared (Table I). The reference solution was 40% (v/v)