Latentiation of Dihydrostreptomycin by Pamoate Formation

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Abstract □ Dihydrostreptomycin was formulated as its pamoate salt as part of a search for a long-acting product. In dogs, an intramuscular injection of a cottonseed oil suspension of dihydrostreptomycin pamoate was absorbed more slowly than equivalent doses of dihydrostreptomycin sulfate suspended in cottonseed oil or dissolved in water. Pharmacokinetic studies suggest that plasma data can be described by the one-compartment open model, and that both salts are equally available.

Keyphrases □ Latentiation, dihydrostreptomycin—by pamoate formation □ Pamoate formation—role in dihydrostreptomycin latentiation □ Dihydrostreptomycin pamoic acid salt—synthesis

Dihydrostreptomycin, first reported in 1946 (1), is made by chemical reduction of streptomycin. Both antibacterial agents possess similar activity against Gram-negative and a few Gram-positive bacteria. For example, they are used to treat bovine actinomycosis, calf pneumonia, newborn foal septicemia, horse cystitis, dog acute nephritis, infectious feline panleukopenia, turkey infectious sinsitis, *etc.* (2). But these drugs must be administered often to ensure high blood levels because they are rapidly removed from the blood. For example, after intramuscular injection, dihydrostreptomycin serum concentrations are reduced to about half in several instances, as follows: man, 3 hr. (3); rabbit, 1.5 hr. (4); cow, 3 hr. (5); chicken, 2 hr. (6, 7); goat, 1–2 hr. (8); and cat, 3 hr. (3).

The objective of the present study was to make a long-acting dihydrostreptomycin injectable by making the drug slowly available from the injection site, thus delaying its transit to the bloodstream. Several examples of delayed transport have been reported. Zini (9) reported that dihydrostreptomycin in pectin–procaine solution markedly prolonged significant serum levels after injection in man, but Hammond (5) tested the solution in cattle and found no prolonged serum levels. Malék *et al.* (10) showed that, on injection, antibiotic salts of high molecular weight acids were transported differently and provided more prolonged blood levels than antibiotic salts of inorganic acids. Several potentially useful dihydrostreptomycin salts are known (11–24); they include naphthalenesulfonate (11), chaulmoograte (14), cholate (15), caprylate (17), and laurylsulfonate (20), but the unknown pamoic acid salt[1] was made for this study.

EXPERIMENTAL

Chemical Synthesis—Sodium pamoate (64.8 g.—0.3 equivalent) was dissolved in 600 ml. of distilled water and filtered. This solution was added with mechanical stirring to a solution of dihydrostreptomycin sulfate (73.2 g.—0.3 equivalent) in 400 ml. of distilled water. The gummy precipitate of dihydrostreptomycin pamoate was collected and washed several times with distilled water. It was then dried at 60° in a vacuum oven for 12 hr. to yield 91.5 g. (78%) of tan solid that decomposed at about 230°. In the analysis of pamoic acid, a theoretical value of 50% was calculated.

[1] Pamoic acid is 4,4′-methylenebis(3-hydroxy-2-naphthoic acid).

Table I—Values of k_a, k_e, and Areas Obtained from Data on Administration of Dihydrostreptomycin to Dogs

Salt	Dose,[a] mg./kg.	$10^5\ k_a$, sec.$^{-1}$	$t_{1/2a}$, hr.	$10^4\ k_e$, sec.$^{-1}$	$t_{1/2e}$, hr.	Area,[b] %
Sulfate[c]	4	58.3	0.33	1.76	1.09	100
Sulfate[d]	4	36.8	0.52	1.76	1.09	100
Pamoate[d]	4	5.56	3.46	1.76	1.09	100

[a] Calculated as dihydrostreptomycin base. [b] Based on comparison to the actual area of the dihydrostreptomycin sulfate formulation in water. [c] Dosed in water. [d] Dosed in oil suspension.

The values were found to be 50.5% by UV and 49.6% by nonaqueous titration.

Formulation Studies—A 10% suspension of dihydrostreptomycin pamoate was readily prepared by rotating 6 g. of dihydrostreptomycin pamoate (passed through a 100-mesh screen) in 15 ml. of cottonseed oil[2] in a bottle containing glass beads for 6 hr. The suspension was poured out, and the beads were washed several times with cottonseed oil to make a final 60 ml. of suspension. A 10% suspension of dihydrostreptomycin sulfate in cottonseed oil was prepared in the same way. Dihydrostreptomycin pamoate could not be formulated as an aqueous suspension because it formed a gum.

Plasma Microbiological Assays—Dihydrostreptomycin plasma levels were determined by the *Bacillus subtilis* cylinder plate assay (25) with human serum as the diluent.

Dosing Dogs—Crossover studies were done with three male dogs. First, they were injected intramuscularly with 4 mg./kg. of dihydrostreptomycin (as sulfate) in water and bled at 0, 0.5, 1, 3, 4, and 5 hr. After a rest period of 1 week, the dogs were dosed with 4 mg./kg. of dihydrostreptomycin (as pamoate) in cottonseed oil and bled at 0, 1, 3, 4, 5, 6, 8, 10, 12, and 26 hr. Finally, they were dosed with 4 mg./kg. of dihydrostreptomycin (as sulfate) in cottonseed oil and bled at 1, 3, 4, 5, and 6 hr.

RESULTS AND DISCUSSION

Three injectable dosage formulations of dihydrostreptomycin were compared in dogs. For these studies, the performances of the formulations were judged by their ability to delay absorption. Figure 1 shows that dihydrostreptomycin absorption from the pamoate formulation is slower and more prolonged than from the sulfate formulations. For example, dihydrostreptomycin pamoate in cottonseed oil provides dihydrostreptomycin levels above 3 mcg./ml. for about 7 hr., while dihydrostreptomycin sulfate in water and in cottonseed oil provides plasma levels above 3 mcg./ml. for about 4 and 4.5 hr., respectively. This does not imply that 3 mcg./ml. is a therapeutic plasma level for infections in dogs; it is merely a cutoff point for this comparative study.

Determination of Pharmacokinetic Constants—Plasma data can be described by the $A \xrightarrow{k_a} B \xrightarrow{k_e} C$ consecutive first-order model, where A represents the amount of drug at the injection site, B the amount of drug in the central body compartment (blood and rapidly equilibrating tissues), and C the amount of drug eliminated from the central compartment at any time. The absorption rate constant, k_a, and the elimination rate constant, k_e, govern the drug-transfer rate from the depot and central compartment, respectively.

Semilogarithmic plots of plasma concentration *versus* time were made. An estimate of the elimination rate constant, k_e, was made from the terminal log linear segment of the data given for the administration of dihydrostreptomycin sulfate in water. The assumption was then made that the different formulations should in no way alter the elimination rate constant. The only changes that would normally be expected would be variations in the time and magnitude of the peak blood levels, and these factors are the result of changes

[2] Wesson Oil, Hunt-Wesson Foods, Fullerton, Calif.

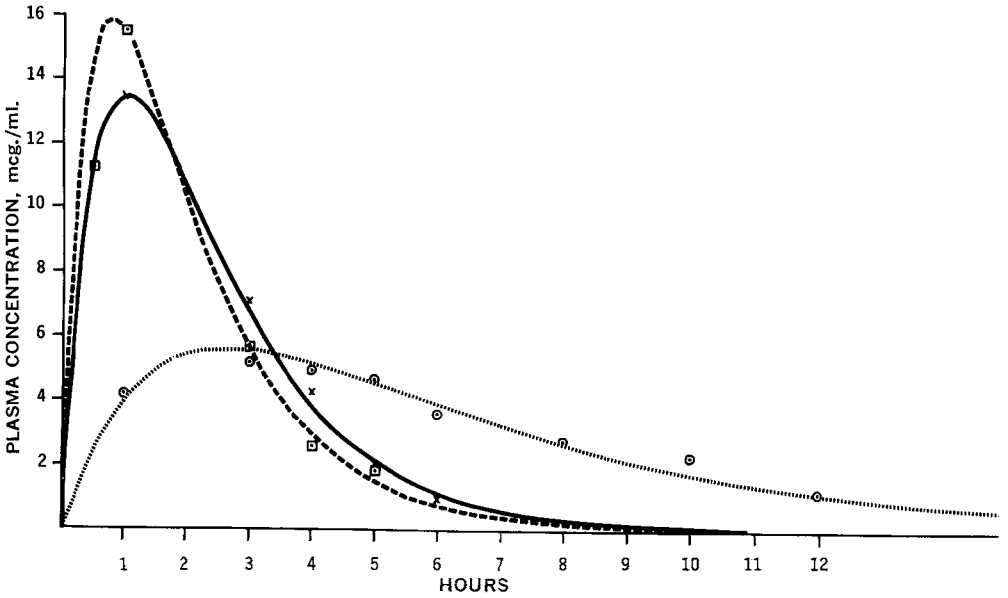

Figure 1—*Concentration of dihydrostreptomycin in the plasma of dogs as a function of time after intramuscular administration of 4 mg./kg. of dihydrostreptomycin (as sulfate) in water (⊡) and in cottonseed oil (×) and of 4 mg./kg. of dihydrostreptomycin (as pamoate) in cottonseed oil (⊙). Dotted lines give one-compartment open-model fits.*

in the magnitude of the absorption rate constant, k_a, for the different formulations.

Using this approach of maintaining k_e constant, k_a values for the sulfate data could be obtained graphically by the "feathering" technique, but the pamoate data could not be treated this way because dihydrostreptomycin was more slowly absorbed from this form. Therefore, the k_a and k_e values obtained by this graphical technique were used in an analog computer simulation of the $A \rightarrow B \rightarrow C$ model. This permitted estimation of k_a for the pamoate form. The values of k_a and k_e are given in Table I. These values are only estimates of the true values because there was a lack of data points to characterize completely the blood level curve prior to and after attainment of peak levels. Nevertheless, the values obtained seem to be reasonably good estimates and give both a magnitude and direction to the characterization of the effect of formulation on the blood levels.

Table I also gives the area under the blood level *versus* time curves following administration of dihydrostreptomycin. The area under these curves can be taken as a measure of the availability of drug from its dosage form, especially in the present case where elimination of the drug occurs by a first-order process, and the dosage forms are given by the same route of administration. All areas are expressed as a percentage of the actual area under the curve for dihydrostreptomycin sulfate in water, which was assumed to be the most "available" formulation.

The areas under the blood level *versus* time curves were obtained by plotting the plasma concentration *versus* time on cartesian coordinate graph paper, connecting each successive two points by a straight line using dark ink and using a line follower to trace the line and generate a voltage which was fed into an analog computer and integrated as a function of time.[3]

If elimination is assumed to be a first-order process, the areas under the blood level *versus* time curves should be proportional to dose if all formulations are equally available (26). The data in Table I indicate that this is the situation with the dihydrostreptomycin formulations, because the same dose of dihydrostreptomycin given as pamoate or sulfate salt gives the same area under the blood level *versus* time plot; this shows that the pamoate salt is just as "available" as the sulfate salt. However, the pamoate formulation differs from the others because it is more slowly absorbed.

SUMMARY

A dihydrostreptomycin pamoate suspension in cottonseed oil (dosed intramuscularly) maintained dog plasma levels above 3 mcg./ml. longer than equivalent doses of a dihydrostreptomycin sulfate suspension in cottonseed oil or a solution in water.

Plasma data can be described by the $A \xrightarrow{k_a} B \xrightarrow{k_e} C$ model.

The pamoate salt was as available as the sulfate salt, but it was absorbed more slowly.

[3] The line follower was a Moseley type F3B used in conjunction with a Moseley 2D-2 X-Y recorder and a Pace TR-10 analog computer, Electronic Associates, Inc., West Long Branch, N. J.

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