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IN-VIVO SCREENING TEST FOR COMPARING ABSORPTION OF LOW AQUEOUS SOLUBLE DRUGS

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ABSTRACT

An in-vivo screening test utilizing isolated rat intestinal segments has been used successfully in comparing absorption of low aqueous soluble drugs administered in the form of aqueous suspensions. The results of attempting to manipulate the absorption of these drugs by reducing the particle size either through milling or by using the drug in the form of a melt or coprecipitate is discussed. Attention is also given to the reliability, precision and accuracy of such a method.

INTRODUCTION

Individuals familiar with drug absorption of low-aqueous soluble drugs know the importance of creating an environment in which the drug solubility rate can be increased to enhance absorption. This can be accomplished by making the particle size of the drug as small as possible either by physically milling or by using the drug in the form of melts or coprecipitates (1). In the latter, the drug precipitates in the form of small particles at the time they dissolve and the drug comes in contact with water. Also with the melts and co-precipitates the presence of the vehicle in the sus-

pension increases the solubility of the drug further increasing its solubility rate. To manipulate the solubility rates in such ways as to achieve the maximum absorption rate, it is desirable to have a reasonably quick and reliable screening test for making comparisons. In such a test it is important to simulate those conditions found in man as much as possible, therefore the use of in-vivo models should be of first choice.

Numerous model systems have been proposed and used successfully to varying degrees as recently reported by Swarbrick (2). It is the intention of this paper to report on absorption studies of low-aqueous soluble drugs administered as aqueous suspensions using a model system based on that devised by Levine and Pelikan (3). These studies were designed to show the reliability of this system for detecting different absorption rates caused by variances in the particle size of the drug and by influences of the vehicles used in the melts or coprecipitates on the solubility of the drug administered.

EXPERIMENTAL

Surgery - Adult male rats (Sprague Dawley), 250 gms or larger were fasted for 12 hours except for water prior to surgery. Under light ether anesthesia an abdominal midline incision was made and the small intestine exposed. A proximal ligature was placed loosely around the intestine approximately 5 cm. distal to the junction of the bile duct and intestine. A distal ligature was placed tightly around the intestine approximately 20 cm. distal to the proximal ligature. Care was taken not to occlude any major blood vessels supplying the segment. The drug suspension was then injected by syringe (20 gauge needle) into the intestinal segment by passing the needle into the lumen and through the loosely tied

proximal ligature. Care must be taken not to penetrate the wall of the intestine with the needle after it has passed through the ligature. The ligature was then pulled tight around the needle so that there was no backflow during the injection of the dosage. Up to 2 mls. of suspension can be injected into this length of segment. After injection as the needle is removed, the proximal ligature was further tightened and tied. The segment was replaced in the abdominal cavity and the incision was closed using 14 mm Michel Wound (Miltex Instrument Company, New York) clips. The animal was allowed to recover, which should take less than 5 minutes. After a predetermined period of time, the rat was sacrificed and the intestinal segment was removed and immediately frozen on dry ice. The segment was kept frozen until it was assayed.

The average time involved for dosing a rat was approximately 7 minutes. Two people are required at the time of injection, one for controlling the proximal ligature so that there is no backflow and the second for injection the sample, otherwise the procedure can be carried out by one person.

Preparation of Melts and Coprecipitates - The polyvinylpyrrolidinone (General Aniline and Film-molecular weight 29,000 to 32,000) coprecipitates were prepared by dissolving a weighed quantity of the drug and polyvinylpyrrolidinone in a single neck round bottom flask using methylene dichloride. This solution was flash evaporated forming the coprecipitate. The coprecipitate was then dried in a dessicator. The dried material was ground with a mortar and pestle being careful not to cause heat formation which could fuse the particles. The ground material that passed through a 60 mesh screen was used for dosing.

The polyethylene glycol (Matheson Coleman and Bell-molecular weight 15,000 to 20,000) melts were prepared by first melting the polyethylene glycol in a beaker on a hot plate. Care was taken not to heat the polyethylene glycol higher than 20°C below the melting point of the drug used. After the polyethylene glycol had melted, the drug was added and the mixture stirred slowly until the drug had dissolved. When the drug was completely in solution, the contents of the beaker were quickly transferred onto a piece of aluminum foil which had been cooled by and still in contact with dry ice. After complete solidification of the melt was obtained, it was milled in a sample mill (Chemical Rubber Company). To overcome heat generated when milling, the mill was first cooled with dry ice and secondly, pieces of dry ice were mixed with the melt as it was being milled. The milled material was rapidly screened using an 80 mesh seive. The screened material was then dried in a desiccator. This dried material was used for dosing.

Dosing - Each individual study utilized six rats. All six rats received the same dosage under the same fasting conditions. Each of the first three rats were sacrificed at the time of drug administration. The intestinal segments were immediately removed and frozen. The data from these rats were used as controls (100% unabsorbed). After the remaining three rats received their dosages they were allowed to recover from the anaesthesia. After a period of two hours, these rats were sacrificed and the intestinal segments were removed and immediately frozen.

Suspensions were made just prior to dosing. A weighed amount of the dosage form to be tested was placed in a 50 ml beaker, followed by the amount of water necessary to make the desired concentration of drug per

milliliter of suspension. Homogenous suspensions were made by using ultrasonics (Sonifier Cell Disruptor, Heat Systems - Ultrasonic Inc.). Homogeneity was maintained by a magnetic stirrer.

Assay - Reagents - All chemicals used are reagent grade (Mallinckrodt, Inc.). This also includes those used in the liquid chromatography systems.

Pentazocine (Talwin® HCl brand of pentazocine HCl - Winthrop Labs) (1,2,3,4,5,6-hexahydro-6,11,dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol) - The assay used for pentazocine hydrochloride and napsylate was adapted from that used by El-Mazati and Way (4). The frozen intestinal segments were each homogenized in 10 ml distilled water using a homogenizer (Virtis "45" Hi-Speed Homogenizer, The Virtis Company, Inc.). The homogenate was quantitatively transferred and brought to a volume of 20 ml with distilled water. Two ml of the homogenate was transferred to a 50 ml stoppered centrifuge tube containing 0.5 gm of a 1:1 mixture of sodium carbonate-sodium bicarbonate and 3 ml of distilled water. This was extracted with 25 ml of benzene by shaking on a mechanical shaker (Schaerr Shaking Machine) for 20 minutes. Ten ml of the benzene extract was further diluted to 100 ml with benzene. Ten ml of this dilution was extracted with 5.0 ml of 0.2N hydrochloric acid that has been saturated with benzene. The fluorescence at 325 nm of the acid extract was determined in a spectrophotofluorometer at an excitation wavelength of 295 nm. Concentrations of pentazocine present were calculated from a standard curve of known amounts.

4-{[6-(2-Chloro-4-methoxy)phenoxy]hexyl}-3,5-heptanedione (compound A) - The frozen intestinal segments were each homogenized as stated in the pentazocine assay. The homogenate was quantitatively transferred and

brought to a volume of 20 ml with distilled water. Five ml of the homogenate was extracted in a 50 ml stoppered centrifuge tube with 20 ml anhydrous ethyl ether by shaking for 20 minutes on a mechanical shaker. Ten ml of the ether extract was evaporated to dryness. The residue was redissolved in 1.0 ml of anhydrous ethyl ether. This solution was assayed for compound A using High Pressure Liquid Chromatography (DuPont 840, E.I. duPont de Nemours and Co., Wilmington, Del.), column-Silica (Zorbax Sil, DuPont), system - hexane: ether (7:3) with 0.1% glacial acetic acid, flow rate-0.24 ml/min. Ten mcl of the ether solution was injected onto the column. The amount of compound A was calculated by comparing peak heights of the unknowns to the peak heights of known concentrations similarly chromatographed.

1,1'-(2,5-Cyclohexadiene-1,4-diylidene-diamino) dipyrrole (compound B) - The frozen intestinal segments were each homogenized as stated above. The homogenate was quantitatively transferred and brought to a volume of 20 ml with distilled water. Four ml of the homogenate was extracted with 20 ml of anhydrous ethyl ether by shaking on a mechanical shaker for 20 minutes. Five ml of the ether extract was evaporated to dryness and the residue dissolved in 1.0 ml of chloroform. This solution was assayed for compound B by High Pressure Liquid Chromatography (Dupont 840), column-Silica (Corasil II, Waters Associates, Milford, Mass.), system - chloroform:methanol (980:20), flow rate-1.0 ml/min. Five mcl of the chloroform solution were injected on the column. The amount of compound B was calculated by peak height comparisons with known amounts of compound B.

RESULTS AND DISCUSSION

The first study was designed to show very simply that this model system can detect differences in absorption rates of a drug administered as a solution and

as a suspension. The drug chosen for this was pentazocine because its hydrochloride salt is very soluble in water whereas its napsylate salt has a much lower solubility (0.368 mg/ml in 1% gum tragacanth in water). Gum tragacanth was used because it has been used in activity screening tests and it served as a wetting agent which enabled a homogenous suspension to be made without increasing the degree of solubility of the drug to any large extent.

Pentazocine hydrochloride (10.0 mg) was administered in 2.0 ml water (5.0 mg/ml solution). This was compared to pentazocine napsylate of which 2.0 ml of a 7.676 mg/ml suspension in 1% gum tragacanth, equivalent to 10.0 mg of pentazocine hydrochloride, was administered. As an added comparison 2.0 ml of a 0.307 mg/ml solution of pentazocine napsylate in 1% gum tragacanth equivalent to 0.4 mg pentazocine hydrochloride was also given.

As the data shown in Table 1 indicates, 100% of the pentazocine hydrochloride and napsylate solutions were absorbed in the 2 hour time period whereas only 17.9% of the pentazocine napsylate suspension was absorbed. A considerable amount (73%) of the absorbed pentazocine was from dissolution of the suspended napsylate salt since only 4.8% of the pentazocine was initially dissolved in the vehicle. This shows that the technique should detect absorption differences due to dissolution effects. Therefore this data indicates that this model system and experimental procedure can show differences in absorption and that the data obtained has reasonable precision and accuracy.

To further prove the system several batches of a drug (compound B) that has very low aqueous solubility (<0.5 mcg/ml) were run. These batches have varying average particle sizes (Table 2) some of which were milled. Two ml of a 1.5 mg/ml suspension of each batch

TABLE 1

Amount of Pentazocine Hydrochloride and Pentazocine Napsylate Unabsorbed

Dosage	Absorption Time (hrs.)	Mg. Drug Unabsorbed
Pentazocine Hydrochloride solution (10 mg/2 ml)	0	8.2, 9.0, 8.2 (100%±3.1%)[a]
	2	0, 0, 0 (0%±0%)
Pentazocine Napsylate suspension (15.35 mg/2 ml) equivalent to 10 mg/2 ml Pentazocine Hydrochloride)	0	11.0, 9.6, 12.8 (100%±8.3%)
	2	9.8, 9.2, 8.6 (82.1%±3.8%)
Pentazocine Napsylate solution (0.614 mg/2 ml equivalent to 0.4 mg/2 ml Pentazocine Hydrochloride)	0	0.64, 0.54, 0.60 (100%±4.9%)
	2	0, 0, 0 (0%±0%)

[a]Mean percent unabsorbed relative to zero time ± standard error.

in 1% gum tragacanth were administered. The data shown in Table 3 suggests that there is a relationship between particle size and absorption. This data also further substantiates the reliability of this test even when small differences in absorption are to be detected.

The use of melts and coprecipitates to enhance absorption were studied using Compound B. Two ml of a suspension in water containing 12.0 mg of a Polyvinylpyrrolidinone coprecipitate (25% compound B) was administered. This was compared to a Polyethylene glycol melt (25% compound B) by similarly dosing 2.0 ml of a suspension in water containing 12.0 mg of the melt. The coprecipitate's and the melt's dosage of 2.0 ml were both equivalent to 3.0 mg of compound B.

TABLE 2

Particle Size and Aqueous Solubility of Compound B

Compound B	Average Particle Size (microns)[a]	Solubility (mcg/ml)
Lot d	21.0	<0.5
Lot e	2.85	<0.5
Lot f	2.40	<0.5
Lot h	9.80	<0.5
Polyvinylpyrrolidinone Coprecipitate (25% drug) - 0.45% Polyvinylpyrrolidinone solution	16.2	3.98
Polyethylene glycol Melt (25% drug) - 0.45% polyethylene glycol solution	12.0	1.82
Polyethylene glycol Melt (25% drug) - 0.90% Polyethylene glycol solution	-	3.08
Polyethylene glycol Melt (25% drug) - 2.25% Polyethylene glycol solution	-	7.60
Polyethylene glycol Melt (25% drug) - 4.50% Polyethylene glycol solution	-	19.70
Lot f in 0.45% Polyethylene glycol solution	-	<0.5
Lot f in 2.25% Polyethylene glycol solution	-	<0.5

[a]Average particle size was determined by the Coulter Counter, Model TA.

The data obtained (Table 3) indicates that dosing a low water soluble drug in the form of a melt or co-precipitate can increase the absorption rate but not necessarily as one might predict from particle size or

TABLE 3

Amount of Compound B Unabsorbed

Dosage	Absorption Time (hrs)	Mg Drug Unabsorbed
Lot d suspension 3.0 mg/2 ml	0	2.84, 2.92, 2.73 (100%)[a]
	2	2.84, 2.87, 2.76 (99.7%)
Lot e suspension 3.0 mg/2 ml	0	2.82, 2.90, 2.70 (100%)
	2	2.54, 2.56, 2.77 (93.2%)
Lot f suspension 3.0 mg/2 ml	0	3.14, 3.14, — (100%)
	2	3.00, 2.91, 2.89 (93.7%)
Lot h suspension 3.0 mg/2 ml	0	2.92, 3.03, 2.86 (100%)
	2	2.89, — , 2.97 (99.7%)
Polyvinylpyrrolidinone Coprecipitate (25% drug) suspension 12.0 mg/2 ml equivalent to 3.0 mg compound B/2 ml	0	2.70, 2.86, 2.84 (100%)
	2	2.48, 2.40, — (87.1%)
Polyethylene glycol Melt (25% drug) suspension 12.0 mg/2 ml equivalent to 3.0 mg Compound B/2 ml	0	3.17, 3.15, 3.20 (100%)
	2	1.78, 1.87, 1.85 (57.7%)
Polyethylene glycol Melt (40% drug) suspension 18.75 mg/2 ml equivalent to 7.5 mg Compound B/2 ml	0	7.60, 7.55, 7.50 (100%)
	2	4.57, 5.14, 5.03 (65.0%)
Mixture-3.0 mg Compound B Lot h and 4.5 mg Polyethylene glycol in 2 ml, suspension	0	3.01, 3.01, 3.00 (100%)
	2	2.86, 2.80, 2.92 (95%)

[a]Mean percent unabsorbed relative to zero time.

by the solubility influence of the vehicle. For example the particle size of the coprecipitate and melt are higher (Table 2) than those of the batches used in the previous study with the exception of Lot d. That being the case it can be reasoned that the percent absorbed would be low. The data obtained disproves that. The

solubility data relates somewhat better to the results. The solubilities of the drug from the Polyvinylpyrrolidinone coprecipitate and the Polyethylene glycol melt is greatly increased therefore it could be expected to find an increased absorption rate. This is found to be true but the solubility of the drug from the Polyvinylpyrrolidinone coprecipitate is about double that from the Polyethylene glycol melt, therefore it could be expected that the absorption rate of the Polyvinylpyrrolidinone coprecipitate should be double that of the melt which is not the case, it is about one third that of the melt. Granted the particle size of the drug from the coprecipitate is larger but in comparing the particle sizes of the previous studies the influence on the absorption rate might not be expected to be that great. There must be some other factor influencing the absorption rate other than the two parameters viewed in this paper. Whether it is the crystal form of the drug precipitated from the coprecipitate or melt or the influence of intestinal fluid or something else is an open question and grounds for further studies.

It is also interesting to note from Table 2 that as the concentration of the Polyethylene glycol in solution increases, the solubility of the drug from the melt increases. Therefore increasing the amount of Polyethylene glycol per dosage should increase the absorption rate. It should be also noted that the solubilities obtained from the melts can not be obtained by simply dissolving the drug in a solution of Polyethylene glycol. The increased solubility is a definite characteristic of the melt.

To show that the increased absorption rate of the drug from the Polyethylene glycol melt is not a result of an influence of the Polyethylene glycol alone a study was done comparing a melt to a physical mixture

of the drug and Polyethylene glycol. In these tests a mixture of compound B, Lot h and Polyethylene glycol (40% compound B) was compared to a Polyethylene glycol melt (40% compound B). There appears to be a great influence on absorption due to the Polyethylene glycol as indicated by the 5% absorption of the mixture as compared to practically no absorption with the gum tragacanth suspension of Lot h, however, it is nowhere near as pronounced as the 35% and 42% absorption with the melts. It is therefore clear that this dramatic increase in absorption is a characteristic of the melt itself.

A solution of a drug (compound A) has been compared to a melt. In this case 2.0 ml of a 2 mg/ml solution of compound A in 20% polysorbate 80 was administered. This was compared to a 2.0 ml dose of Polyethylene glycol melt (25% compound A) having 16.0 mg of Polyethylene glycol melt per 2.0 ml water, equivalent to 4.0 mg of compound A in 2 ml water. Also for comparison, 2.0 ml of a 2 mg/ml aqueous suspension of compound A was run. From the data in Table 4 it is easily seen that the absorption rate of a drug, having a low water solubility (<20 mcg/ml), administered in the form of a melt can equal that of a solution. This study also illustrates that absorption from suspensions can be measured with this in-vivo technique for drugs with very low solubilities.

CONCLUSION

The use of the isolated rat intestinal segment has been shown to be useful and dependable in detecting differing absorptions of low aqueous soluble drugs administered in suspension form. Data obtained indicates that by presenting the drug in as fine a particle size as possible, drug absorption can be enhanced. The particle size can be adjusted by milling or by precipita-

TABLE 4

Amount of Compound A Unabsorbed

Dosage	Absorption Time (hrs)	Mg Drug Unabsorbed
suspension - 4.0 mg/2 ml	0	4.0, 3.9, — (100%)[a]
	2	3.7, 3.5, 3.5 (89.2%)
solution - 4.0 mg/2 ml of 20% polysorbate 80 in water	0	3.0, 4.0, 4.0 (100%)
	2	2.8, 2.8, 2.7 (70.0%)
Polyethylene glycol Melt suspension 16.0 mg/2 ml equivalent to 4.0 mg Compound A/2 ml	0	3.4, 4.0, 3.4 (100%)
	2	2.8, 1.8, 2.3 (63.8%)

[a]Mean percent unabsorbed relative to zero time.

tion from a melt or coprecipitate dosage form. There may not be influence on the solubility of the drug caused by the vehicle used in making the melt or coprecipitate, therefore, the choice of the vehicle used can be critical. It is also evident that there are other factors influencing the absorption other than those studied.

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REFERENCES

1. W.L. Chiou, J. Pharm. Sci., 60, 1281 (1971).
2. J. Swarbrick, Drug Development Communications, 2(6), 439 (1976).
3. R.R. Levine and E.W. Pelikan, J. Pharmacol. Exp. Ther., 131, 139 (1961).
4. A.M. El-Mazati and E. Leong Way, ibid., 177, 332 (1971).