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Short Communication

Microscopic determination of drug solubility in plasma and calculation of injection rates with a plasma circulatory model to prevent precipitation on intravenous injection

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1. Introduction

Slow IV infusion of poorly water soluble drugs is common practice for the estimation of various intrinsic pharmacokinetic/pharmacokinetic/pharmacodynamic parameters. For those compounds which have low solubility in plasma relative to their dose, the potential exists for precipitation in the vein during an IV injection or infusion. No method has been published, to our knowledge, where the solubility of a compound in plasma at 37 °C was rapidly measured and safe injection rates were calculated. This information is highly useful in the early evaluation of the activity, toxicity, metabolism and absolute bioavailability of a compound. The measurement of various intrinsic pharmacological parameters after IV administration is possible for low water soluble compounds depending upon the dose, infusion rate, solubility in plasma, plasma flow rate and solvent.

Although the problem of compound precipitation in veins has been recognized for a long time, an adequate solution has not been presented. Crystallization of diphenylhydantoin from a co-solvent, when added to a 5% solution of plasma protein fraction, was demonstrated in 1974 by Schroeder and DeLuca [1] using an in vitro injection system. Further, they calculated that at a 1 ml min^{-1} infusion rate of a 50 mg ml^{-1} diphenylhydantoin solution the blood flow rate in a large vein would be too slow to prevent precipitation. Precipitation of diazepam and alprazolam was reported after injections into saline or 5% dextrose [2], or after addition to plasma [3]. It has been suggested that thrombophlebitis from IV injections of diazepam might be due to diazepam crystals when the injection rate is too rapid or the venous blood flow is slow [4]. This has been cited as a possible reason for the positive correlation between delayed diazepam peak plasma concentration and the incidence of pain and irritation determined at the injection site [5].

A description is given here of a simple and quick method for approximating the solubility and dose delivery rate of poorly water soluble compounds. Maximum safe injection rates (mass per unit time) were calculated for several compounds. Based upon the dose and solution concentration, the infusion rate (volume per unit time) that will not result in precipitation was determined. Any co-solvent toxicity had to be considered in the volume calculations. Recirculating compounds in the blood are of minor importance except for long term infusions and for those compounds which have very low distributive volumes.

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2. Materials and methods

Proprietary compounds (WINs 49596, 61605 and 63843) were obtained from Sanofi Winthrop, Collegeville, PA and solubilized in polyethylene glycol 400 (PEG-400) at 10 mg ml^{-1} concentrations for the following experiments. Diazepam, a product of Elkins-Sinn, Inc., Cherry Hill, NJ, was supplied in 10 mg ml^{-1} sealed ampoules.

2.1. Solubility in plasma

Dog plasma (Rockland Inc., Gilbertsville, PA) containing sodium heparin, was heated to 37 ± 0.05 °C in 2 ml aliquots in 8 ml test tubes using jacketed beakers connected to a constant temperature water circulator (VWR model 1115, Scientific Corp., Niles, IL). The drug solutions were added to the 37 ± 0.05 °C plasma in 10 µl aliquots. The samples were then vortex mixed and a drop of the plasma–drug mixture was transferred via heated capillary transfer pipette to a 37 ± 0.05 °C glass microscope slide in a hot stage cell (Mettler FP 82 and FP 80, Mettler Instrument Corp., Hightstown, NJ) for microscopic examination (320 ×, Leitz Laborlux 12 POL microscope, Upstate Technical Equipment Co., Dwight Park Drive, Syracuse, NY) for precipitates. The plasma sample was also visually examined for opalescence and/or precipitation as was the blank plasma before use. This method is similar to that described for human gastrointestinal fluids [6].

The experiment was repeated with blank solvent in dog plasma so that any PEG-400 effects on dog plasma could be distinguished from visible drug interactions with plasma components. The experiment was also conducted in water to determine the magnitude of the difference between drug solubility in water and plasma. WINs 49596 and 61605 were previously tested in human gastric and intestinal fluids using this technique [6]. Diazepam was used, as other investigators have reported on this compound [2,3]. Solubility in plasma was calculated from the amount of compound added to the plasma until precipitates were observed microscopically, and the precipitate type was noted.

2.2. Drug IV infusion

Since stationary plasma does not mimic the in vivo plasma infusion situation, the drug solutions were injected into 37 ± 0.05 °C dog plasma, flowing at 60 and 40 ml min^{-1} by a peristaltic pump (Cole Parmer Instrument Co., Barringtom, IL) through a # 16 silastic tubing as a vein (Masterflex, Cole Parmer). The silastic tubing inlet end was placed in the plasma reservoir in a jacketed beaker connected to a VWR-1145 water circulator (Polyscience Corp., Niles, IL) set at 37 °C. The drug solutions were infused with a 341A syringe pump (Sage Instruments, Cambridge, MA) and either a 1 or 2.5 cm^3 syringe fitted with at 22 gauge needle that was inserted into the tubing "vein" at the outlet side of the peristaltic pump. The infusion times were calculated for a 20 mg dose of each drug with Eq. (1), using the microscopically determined solubility concentration in plasma at which oily droplets were first observed and plasma flow rates of either 60 or 40 ml min^{-1}. These injection rates were bracketed to determine the accuracy of the solubility determination, and the probable accuracy of the injection rate. The capillary tip of a heated (37 ± 0.05 °C) disposable transfer pipette was inserted into the tubing at a point 10 cm below the needle injection site, to remove plasma samples from inside the silastic "vein". The collected plasma portions were immediately placed on the heated hot stage slide and examined by microscopy for precipitates. Instantaneous equilibrium of the injected drug in plasma was assumed in the calculation:

$$\text{maximum infusion time (min)} = \frac{\text{total dose (mg)}}{\text{plasma solubility (mg ml}^{-1}\text{)} \times \text{venous plasma flow rate (ml min}^{-1}\text{)}} \quad (1)$$

3. Results and discussion

The structures, and some physical properties of WINs 49596, 61605, 63843 and diazepam are given (Fig. 1).

3.1. Solubility in dog plasma

At the highest level of PEG-400 in a blank plasma sample (2.7% v/v), a slight opalescence

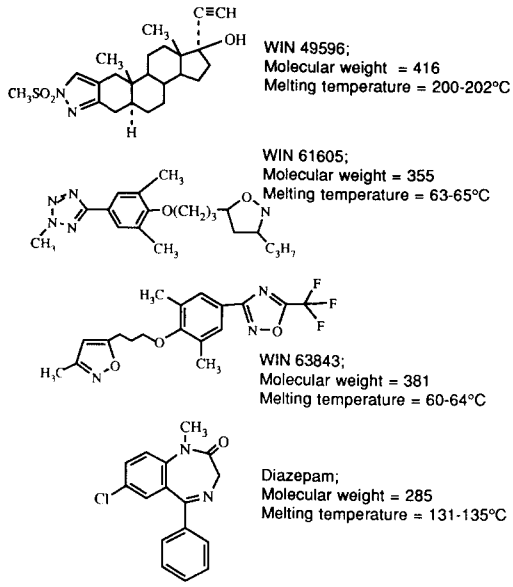

Fig. 1. Structure, and physical properties of WINs 49596, 61605, 63843 and diazepam.

was observed in plasma but no precipitates were seen by microscopy. At this concentration PEG-400 effects on the plasma were not expected to interfere with solubility determinations. The compound concentrations at which some oily droplets (3–5 μm diameter) began to form in plasma, or precipitation occurred, are listed below with the water solubilities to show the great magnitude of differences in solubility due to plasma protein binding (Table 1). The approximate 37 °C (±0.05 °C) solubilities are considered to be slightly less than the lowest range values.

WINs 49596 and 61605 formed oily droplets in human gastric and intestinal (GI) fluids, but at much lower concentrations than in plasma [6]. The oily droplet phenomena for other compounds have been reported previously [6–8]. In water, WIN 49596 formed amorphous particles and the other compounds formed crystals [6]. The oily droplets in blood plasma were identical to those in the GI fluids, and did not coalesce or aggregate. Solubility varied widely between different human GI fluids [6], but because of the limited availability, blood plasma was pooled and variations with individual plasma samples were not tested here. At higher drug concentrations, the compounds precipitated as crystals in the plasma. The blood plasma solubilities of all four compounds were markedly higher than in water.

Table 1
WINs 49596, 61605, 63843 and diazepam; 37 °C plasma and aqueous solubilities at 37 °C determined by hot stage microscopy, and precipitate types

WIN compound	Drug in plasma concentration (mg ml^{-1})	Precipitate formation in plasma	Aqueous solubility (mg ml^{-1})
49596	605–625 725–750	Oily droplets Amorphous, then crystals	<10
61605	640–660 990–1020	Oily droplets Crystals	<30
63843	80–100 200–240	Oily droplets Crystals	<2
Diazepam	50–80 180–210	Oily droplets Crystals	<1

3.2. Drug IV infusion

The critical parameters which determine whether a compound will precipitate during IV injection are solubility in plasma, drug dose and plasma flow rate in the vein. In this model, the silastic tube flow rate is used in place of the venous flow rate. The approximate plasma solubility results make it possible to calculate the maximum infusion rate for any vein or artery when the appropriate flow rates for those vessels are known (Eq. (2)). Conversely, knowing the experimentally determined value for the infusion rate at which precipitation starts to occur and the solubility in plasma, the necessary venous flow rate can be calculated:

$$\text{maximum infusion rate (mg min}^{-1}) = \text{Plasma solubility (mg ml}^{-1}) \times \text{venous plasma flow rate (ml min}^{-1}) \quad (2)$$

As an example, for WIN 49596, at a 20 mg dose (2 mg kg^{-1} for a 10 kg dog), 33.1 ml of plasma is necessary to dissolve the drug (20 mg per 0.605 mg ml^{-1}). At a flow rate of 60 ml min^{-1}, 0.55 min of plasma flow (33.1 ml per 60 ml min^{-1}) is necessary to prevent precipitation. Therefore, 20 mg of compound over a 0.55 min infusion period should not precipitate; this is 36.4 mg min^{-1} (20 mg per 0.55 min). At the calculated infusion times in Table 2, no solid precipitates formed. At reduced infusion rates no precipitates were detected. As instantaneous mixing may not be occurring and ensure a high degree of safety it

Table 2
IV injection rates (calculated maximum and experimental) of a 20 mg dose of each of three WIN compounds (49596, 61605 and 63943 at 10 mg ml^{-1} in PEG-400) and diazepam (5 mg ml^{-1}) in dog blood plasma, flowing at 60 and 40 ml min^{-1}

Compound	Plasma flow rate (ml min^{-1})	Calculated max. inf. rate (mg min^{-1})	Experimental inf. rate (mg min^{-1})	Plasma observations
WIN 49596	60	36.4	30.8	No precipitates
			43.5	Very few amorphic particles
			57.1	Amorphic particles & crystals
	40	24.2	28.8	No precipitates
			36.4	Amorphic particles & crystals
WIN 61605	60	38.4	57.1	No precipitates
			80.0	Amorphic particles & oily droplets
	40	35.6	30.8	No precipitates
			40.0	Very few amorphic particles
WIN 63843	60	4.80	4.80	No precipitates
			5.50	Very few amorphic particles & oily droplets
	40	3.20	3.20	No precipitates
			3.50	Very few amorphic particles & oily droplets
Diazepam	60	3.00	3.00	No precipitates
			3.50	Very few amorphic particles
	40	2.00	2.00	No precipitates
			2.50	Very few amorphic particles

would be prudent to further reduce this infusion rate to insure that the drug will not precipitate during injection. These calculations were applied to the remaining compounds and a 20 mg dose of each. Plasma flow rates of 40 and 60 ml min^{-1} were used in the model, because they are in line with flow values in blood vessels that might be used in toxicology, metabolism or pharmacological studies [9,10].

Because no precipitates were observed when drugs were injected at the calculated rates (Table 2), the solubility in plasma was verified with the flowing plasma model. The precipitate type in flowing plasma differed from the type in stationary plasma, oily droplets versus amorphic particles (Tables 1 and 2). This shows the need to mimic the in vivo situation as much as possible when preparing to test compounds biologically. The solvent volume and toxicity must be considered in each experiment. In our experience, PEG-400 has been, in most cases, an excellent solvent of low toxicity.

4. Conclusions

A rapid microscopic procedure was developed for approximating drug solubilities in plasma at 37 °C. Using plasma flow rates which are comparable to those in veins, an in vitro model was devised which can detect precipitation. Using the drug plasma solubility values and the plasma flow rate, the maximum infusion rate (mg min^{-1}) can be calculated at which no precipitation should occur. At this rate, no precipitation was observed for four compounds of diverse chemical structures and widely different plasma solubilities. At higher infusion rates, amorphous particles or crystals were formed in the plasma. Therefore, it appears that this procedure can be useful for approximating a safe, non-precipitating infusion rate for compounds of low solubility in plasma. However, it is recommended that a safety factor of four be used to insure that no precipitation will occur.

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