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Pulmonary absorption of carboxyfluorescein in the rat

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The pulmonary absorption of the fluorescent marker 6-carboxyfluorescein (CF) has been characterized. CF was administered intratracheally (i.t.) as a fluid instillate to pentobarbitone-anaesthetized rats at doses of 0·5 and 2 mg kg^{-1}. The absorption was characterized by both model-independent and model-dependent pharmacokinetic analyses of blood concentration data with reference to previous intravenous (i.v.) studies. The mean fraction available (F) of CF was 90 and 112% with a mean absorption time of 107 and 109 min for the lower and higher doses, respectively. The terminal half-life for the i.t. administered CF (73 and 83 min for the 0·5 and 2 mg kg^{-1} doses, respectively) was significantly longer ($P < 0·001$) than after i.v. dosing (18 min). This indicates a slow pulmonary absorption of CF. Blood concentration-time profiles could not be adequately described by models involving a simple first-order absorption process; a model incorporating two simultaneous first-order inputs gave a much better description, its absorption rate constants differing by almost two orders of magnitude.

The lung lacks many of the characteristics associated with the gastrointestinal tract which decrease the bioavailability of some orally administered compounds, yet the potential of the lung as a route of administration for systemically acting drugs has not been fully investigated. Most studies have used an analysis of amounts of drug remaining in the respiratory tract to characterize absorption from the lung (Burton & Schanker 1970, 1974 a,b,c; Lanman et al 1973; Hemberger & Schanker 1983). However, the disappearance from lung by apparent first- or zero-order kinetic processes has not been correlated with circulating drug levels. Another approach to studying lung absorption has been to use pharmacokinetic analysis of blood concentrations in the determination of systemic availability and absorption rates (Brown et al 1981; Fuller & Collier 1983; Trnovec et al 1984).

The pharmacologically inert material 6-carboxyfluorescein (CF) has been extensively used to study the effects of liposome encapsulation on drug disposition. We have characterized the absorption of CF from the lung by the pharmacokinetic analysis of blood level data. For such an analysis the intravenous (i.v.) disposition of the marker needs to be defined and ideally have a dose-independent profile. CF is known to have a linear disposition following i.v. administration (Woolfrey et al 1985) and can be routinely assayed in plasma to adequately low concentrations (Senior & Gregoriadis 1982).

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Materials and methods

CF (Eastman-Kodak, Rochester N.Y.) was purified using the charcoal/LH-20 procedure of Ralston et al (1981). Pentobarbitone was prepared in 0·9% saline and administered intraperitoneally to male Wistar rats (200–250 g) at a dose of 67 mg kg^{-1}. After 1 to 1·5 h, anaesthesia was maintained by subsequent doses of 8 mg kg^{-1} every 15 to 20 min or as needed. A carotid artery was cannulated with PP50 polythene tubing (Portex, Hythe) and a 2·5 cm section of PP250 then inserted 0·6 cm below an incision made between the 4th and 5th tracheal caudal rings. To make these studies comparable with previous i.v. investigations (Woolfrey et al 1985), a jugular vein was tied-off with thread. 100 μl doses of CF dissolved in isotonic phosphate buffer, pH 7·4 were instilled into the lungs through a length of PP50 inserted through the tracheal cannula to a depth of 2·1 cm from the tracheal incision. The fluid was administered over 10 to 20 s while the rat was maintained at an angle of 80°. One minute after administration, the animal was returned to an angle of 10° and the PP50 tubing withdrawn. 75 μl samples of blood were taken from the carotid artery at various times after dosing, diluted with 3 ml of isotonic phosphate buffered saline, pH 7·4 containing 0·01% potassium chloride, then centrifuged to remove red blood cells. The concentration of CF in the supernatant was determined by fluorimetry using excitation and emission wavelengths of 490 and 517 nm, respectively. During the experiment the body temperature of each rat was monitored by a rectal thermometer and maintained at 37 °C by heat from an incandescent lamp suspended above the animal.

The absorption function of individual blood concentration-time profiles was determined using both model-dependent and model-independent techniques with reference to previous i.v. studies (Woolfrey et al 1985). For the model-dependent analyses the non-linear least squares regression program, NONLIN-74 (Metzler et al 1974) was used to fit either Models I or II (Fig. 1) to the individual profiles. 95% population limits of the disposition parameters derived from i.v. studies (Woolfrey et al 1985) were used as parameter bounds in the non-linear regression analyses. The quality of the fits was

assessed by weighted residuals analysis, Akaike's Information Criterion (AIC) (Yamaoka et al 1978a) and the F-ratio test (Mandel 1964). Model-independent analysis of the absorption function involved calculating the mean residence time of CF at the absorption site (t_a) (Yamaoka et al 1978b). Areas under the curve (AUC) were calculated using the linear trapezoidal rule. Since pharmacokinetic parameters tend to be geometrically distributed (Jusko 1980; Sheiner & Beal 1981, 1982), a log-normal transformation was applied to the mean pharmacokinetic parameters and was used in all statistical tests (5% significance level).

Results

Fig. 2 shows the mean blood concentration-time profile for CF when administered intratracheally (i.t.) to rats at doses of 0·5 and 2 mg kg^{-1}. For comparison the mean i.v. profile resulting from 0·5 mg doses of CF has been included.

Models I and II (Fig. 1) were fitted to the individual blood concentration-time profiles with Model II giving the best fits assessed by F-ratio tests ($P < 0.05$ to $P < 0.001$) and AIC values. AIC values for Model II were typically twice those obtained by fitting Model I. The superior fit of Model II to the data was further supported by weighted residuals analysis and by inspection of the predicted blood concentration-time profiles. As a result Model II was used for the model-dependent analysis of blood concentrations.

Table 1 compares the mean pharmacokinetic parameters for CF when administered i.t. at doses of 0·5 and 2 mg kg^{-1}. The mean fraction available for each of the two i.t. doses of CF was 90 and 112%. The values of k_{31} and k_{41} differed by two orders of magnitude with the major fraction of the CF dose absorbed from compartment 3 of Model II, at rates determined by k_{31}. Since this was the predominant absorption process, the reciprocal of the mean absorption time ($1/t_a$) was similar to k_{31}. Comparison of the terminal half-life (73 and 83 min at 0·5 and 2 mg kg^{-1}, respectively) with that following i.v. administration (18 min, Woolfrey et al 1985) indicates a prolonged absorption. There were no significant differences between doses for any of the pharmacokinetic parameters suggesting dose-independent absorption and disposition.

Table 1. Mean pharmacokinetic parameters for CF when administered i.t. to rats at doses of 0·5 and 2 mg kg^{-1}.

Parameters	Dose of CF (mg kg^{-1}) 0·5 (n = 5)	2 (n = 5)
F (%)	89·94 (141·8/57·06)	112·09 (138·7/90·58)
k_{31} (min^{-1})	0·009 (0·013/0·006)	0·013 (0·028/0·006)
k_{41} (min^{-1})	0·71 (1·27/0·39)	0·73 (1·99/0·27)
t_a (min)	106·91 (144·35/79·20)	108·86 (151·04/75·49)
Terminal half life (min)	73·12 (83·77/63·83)	83·13 (108·4/63·77)
Dose normalized AUC (μg ml^{-1} min^{-1})	42·73 (67·4/27·08)	53·24 (81·50/34·77)
% of AUC extrapolated	25·8	25·4

Upper and lower 95% confidence limits are shown in parentheses. k_{31} and k_{41} are for Model II depicted in Fig. 1. No significant differences in parameter estimates were found between the different doses. (Student's *t*-test).

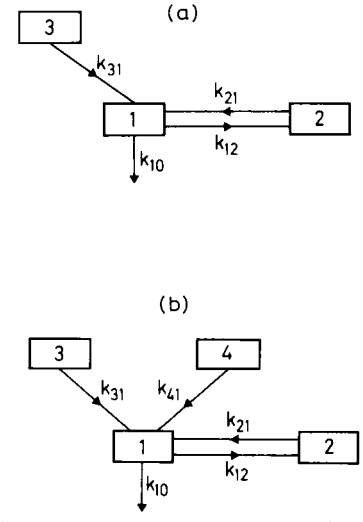

FIG. 1. (a) Model I: single first-order input into compartment 1 with two compartment disposition. (b) Model II: two first-order inputs into compartment 1 with two compartment disposition.

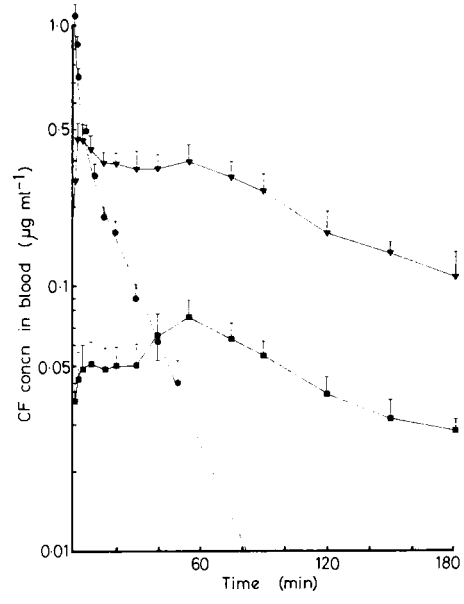

FIG. 2. Blood concentration time profiles of CF in rats after administration of doses of 0·5 and 2 mg kg^{-1}. Values shown are mean ± s.e.m. ■ 0·5 mg kg^{-1} i.t. (n = 5). ▼ 2 mg kg^{-1} i.t. (n = 5). ● 0·5 mg kg^{-1} i.v. (n = 6).

Discussion

The long mean absorption time after i.t. administration of CF indicates a slow absorption. Also since the blood concentrations declined with a clearly defined half-life significantly longer than that following i.v. dosing, this indicates a prolonged first-order absorption process. This type of 'flip-flop' pharmacokinetics following pulmonary administration has been reported for sodium cromoglycate (Brown et al 1981; Fuller & Collier 1983). As CF has been reported to have a dose-independent disposition (Woolfrey et al 1985), the dose-independence of mean absorption time and fraction available suggests a first-order absorption mechanism. The very short time in which peak concentrations were reached after i.t. dosing is incongruous with a slow singular first-order absorption process. This is demonstrated by the inability of Model I to describe the peak and pre-peak concentration data adequately. Model II gave a much better description to the data, however, the estimates for absorption rate constants were associated with large standard errors. This is mainly a result of the relatively large number of parameters in the model. Such a situation questions the reliability of the estimates for k_{31} and k_{41} and for this reason a model-independent analysis of the absorption function was carried out.

Studies have determined that materials may pass through the lung epithelium by a variety of mechanisms including active transport (Moss & Ritchie 1970; Enna & Schanker 1973), diffusion (Enna & Schanker 1972a) and by passage through the lipoid regions of the membrane (Burton & Schanker 1974c). Sodium cromoglycate and phenol red have been reported to be absorbed from the rat lung by both active transport and passage through the pores of the epithelium membrane (Gardiner & Schanker 1974). As the above compounds and CF are anionic materials with similar molecular weights, they might be expected to be absorbed by similar mechanisms. This is consistent with the proposed absorption model for CF involving two simultaneous inputs into the central compartment. The lung epithelium has been shown to contain at least three different populations of pore sizes (Enna & Schanker 1972b) and would therefore allow the passage of molecules with different molecular masses. It is unlikely that CF passes through the lipid region of the membrane as many studies have confirmed this route is limited to lipophilic compounds (Enna & Schanker 1972b; Schanker 1978).

In conclusion, this work has determined that the pulmonary absorption of aqueous solutions of CF in the rat is efficient with a fraction available close to unity. The absorption process is complex and the simplest representation involves two simultaneous inputs into the central compartment.

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