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Mean residence time for drugs subject to reversible metabolism

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Conventional methods based on moment analysis lead to biased estimates of the mean residence time for drugs subject to reversible metabolism. A stochastic approach is described which enables unbiased estimation of the mean residence time.

Although metabolism is normally considered to be irreversible there are several instances in which it has been demonstrated to be reversible. Examples include prednisolone (Meikle et al 1975), methylprednisolone (Ebling et al 1985; Ebling & Jusko 1986), clofibrate (Faed & McQueen 1979) and sulindac (Duggan et al 1980). A particularly intriguing example is the interconversion of the enantiomers of aryl propionic acids (Hutt & Caldwell 1983) whereby the inactive *R*-enantiomer is converted to the active *S*-enantiomer, although the reverse process seems to be less important. Reversible metabolism complicates pharmacokinetic analysis and caution must be exercised in estimating parameters such as clearance (Wagner et al 1981) and bioavailability (Hwang et al 1981). In the light of the recent controversy surrounding mean residence time (Benet 1985), the purpose of the present work was to define mean residence time for drugs undergoing reversible metabolism.

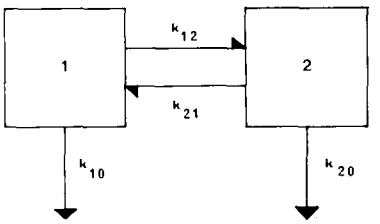

FIG. 1. Model for reversible metabolism. Compartment 1 represents the drug and 2 the metabolite.

Theory.

The simplest model of a drug subject to reversible metabolism is shown in Fig. 1 where 1 represents the drug and 2 the metabolite. The rate equations describing the kinetics of the drug and metabolite are given below

$$V_1 \cdot \frac{dC_1}{dt} = -k_{11}.V_1.C_1 + k_{21}.V_2.C_2 \quad (1)$$

$$V_2 \cdot \frac{dC_2}{dt} = k_{12}.V_1.C_1 - k_{22}.V_2.C_2 \quad (2)$$

where $k_{11} = k_{10} + k_{12}$, $k_{22} = k_{20} + k_{21}$, V and C are the volume of distribution and the concentration, respectively, of either the drug or metabolite. Units of concentration and dose are molar. Note that the notation k_{12} for the transfer of material from compartment 1 to 2 is opposite to the convention adopted by researchers in engineering (see Godfrey 1983).

Integrating equations (1) and (2) from 0 to ∞ with simultaneous bolus injection of both drug and metabolite results in equations (3) and (4)

$$AUC_1 = \frac{D_1.k_{22} + D_2.k_{21}}{V_1.|K|} \quad (3)$$

$$AUC_2 = \frac{D_1.k_{12} + D_2.k_{11}}{V_2.|K|} \quad (4)$$

where $|K| = k_{11}.k_{22} - k_{12}.k_{21}$

AUC denotes area under the curve and D the dose. In a similar fashion if equations (1) and (2) are first multiplied by time, t, and integrated, expressions for the area under the first moment curve (AUMC) can be derived (equations (5) and (6)).

$$AUMC_1 = \frac{(D_1.k_{22} + D_2.k_{21}).k_{22} + (D_1.k_{12} + D_2.k_{11}).k_{21}}{V_1.[|K|]^2} \quad (5)$$

$$AUMC_2 = \frac{(D_1.k_{22} + D_2.k_{21}).k_{12} + (D_1.k_{12} + D_2.k_{11}).k_{11}}{V_2.[|K|]^2} \quad (6)$$

Conventionally, mean residence time (MRT) is calculated as the quotient of AUMC and AUC (Yamaoka et al 1978)

$$MRT = \frac{AUMC}{AUC} \quad (7)$$

However if elimination does not occur exclusively from the systemic circulation this prescription is not valid (Veng Pedersen & Gillespie 1984). This is effectively the situation with reversible metabolism in which the metabolite compartment in Fig. 1 can be viewed as the 'tissue compartment' in the conventional 2-compartment model.

Consequently a different approach is required, which is conveniently provided by a stochastic description of the system (Hearon 1981; Eisenfeld 1981). If $p_{ij}(t)$ is defined as the probability that a particle initially starting in compartment j will be located in compartment i at time t, it can be shown that the time history of p_{ij} is determined by rate equations analogous to equations

(1) and (2) (Covell et al 1984). Therefore the mean residence time in compartment i for particles originating in compartment j, v_{ij}, is

$$v_{ij} = \int_0^\infty p_{ij}(t).dt \quad (8)$$

In the case of the model shown in Fig. 1, v_{11} is the MRT for the drug after administration of the drug and v_{12} is the MRT for the drug after administration of the metabolite. If the drug and metabolite are administered simultaneously the MRTs are given by (Eisenfeld 1981)

$$MRT_1 = \frac{D_1.v_{11} + D_2.v_{12}}{(D_1 + D_2)} \quad (9)$$

$$MRT_2 = \frac{D_1.v_{21} + D_2.v_{22}}{(D_1 + D_2)} \quad (10)$$

These formulae are only appropriate when, after administration into compartment j, a particle has a finite probability of reaching compartment i.

The expressions for v_{ij} for the model in hand are

$$v_{11} = \frac{k_{22}}{|K|} \quad (11)$$

$$v_{12} = \frac{k_{21}}{|K|} \quad (12)$$

$$v_{21} = \frac{k_{12}}{|K|} \quad (13)$$

$$v_{22} = \frac{k_{11}}{|K|} \quad (14)$$

Some special cases are of interest:

(i) $D_1 = D_2$

Equations (9) and (10) reduce to

$$MRT_1 = \frac{k_{22} + k_{21}}{2.|K|} \quad (15)$$

$$MRT_2 = \frac{k_{12} + k_{11}}{2.|K|} \quad (16)$$

whereas the quotient of AUMC and AUC gives

$$\frac{AUMC_1}{AUC_1} = \frac{k_{22}^2 + k_{21}.k_{22} + k_{12}.k_{21} + k_{11}.k_{21}}{|K|.(k_{21} + k_{22})} \quad (17)$$

$$\frac{AUMC_2}{AUC_2} = \frac{k_{12}.k_{22} + k_{12}.k_{21} + k_{11}.k_{12} + k_{11}^2}{|K|.(k_{11} + k_{12})} \quad (18)$$

(ii) $D_2 = 0$ (Drug only administered)

$$MRT_1 = \frac{k_{22}}{|K|} \quad (19)$$

$$MRT_2 = \frac{k_{12}}{|K|} \quad (20)$$

$$\frac{AUMC_1}{AUC_1} = \frac{k_{22}^2 + k_{12}.k_{21}}{|K|.k_{22}} \quad (21)$$

$$\frac{AUMC_2}{AUC_2} = \frac{k_{11} + k_{22}}{|K|} \quad (22)$$

(iii) $D_1 = D_2$, $k_{21} = 0$ (the case of the propionic acid enantiomers when administered as a racemate)

$$MRT_1 = \frac{AUMC_1}{AUC_1} = \frac{1}{k_{11}} \quad (23)$$

$$MRT_2 = \frac{k_{11} + k_{12}}{2.(k_{11}.k_{22})} \quad (24)$$

$$\frac{AUMC_2}{AUC_2} = \frac{k_{12}.k_{22} + k_{11}.k_{12} + k_{11}^2}{(k_{11} + k_{12}).k_{11}.k_{22}} \quad (25)$$

Note that equation (23) cannot be derived from equation (9) as administration of the metabolite (e.g. the *S*-enantiomer of the propionic acid) does not lead to the production of the drug (i.e. the *R*-enantiomer).

Discussion

An estimate of the discrepancy that can arise in the calculation of MRT can be made using the reversible metabolism of sulindac to its active sulphide metabolite as an example. From the rat data of Duggan et al (1980) the four rate constants, k_{11}, k_{12}, k_{21} and k_{22} can be estimated to be 0·037, 0·0088, 0·015 and 0·033 min^{-1}, respectively. The MRTs calculated using equations (15) and (16) are 22·2 and 21·1 min for the drug and its metabolite, respectively. The corresponding values calculated using equation (17) and (18) are 43·6 and 42·6 min, clearly an overestimation.

The MRT in the presence of peripheral elimination can be calculated in a model-independent fashion using the following relationship (Veng Pedersen & Gillespie 1984)

$$MRT = \frac{\int_0^\infty Ab.dt}{Dose} \quad (26)$$

where Ab is the total amount of drug in the body. It can be verified that the MRT calculated using equation (26) gives the same result as equation (9). However, in general, it is difficult to measure the total amount of drug in the body. When metabolism is reversible, a biased estimate of MRT may result from the conventional calculation using equation (7). A correct estimate of MRT can be obtained but only when a detailed knowledge of the disposition of both the drug and metabolite exists.

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Anti-inflammatory activity of a polysaccharidic fraction of *Echinacea angustifolia*

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The anti-inflammatory activity of a polysaccharidic fraction (EPF) obtained from *Echinacea angustifolia* roots has been examined using the carrageenan paw oedema and the croton oil ear test. EPF (0·5 mg kg^{-1} i.v.) almost inhibited the carrageenan-induced oedema over 8 h and furthermore, EPF, topically applied, inhibited mouse ear oedema induced by croton oil. EPF also reduced the leukocytic infiltration of the croton oil dermatitis, evaluated both as peroxidase activity and histologically. After topical application EPF appears to be slightly inferior in potency to indomethacin. The results suggest that the anti-inflammatory activity of *E. angustifolia* resides in its polysaccharidic content.

Root preparations of *Echinacea purpurea* and *Echinacea angustifolia* have been used for the treatment of wounds, burns and other cutaneous affections, and to treat disorders such as viral infections, cutaneous illnesses and acute and chronic disease due to a deficiency of immunological responses. Also the cosmetic use of *Echinacea* preparations is widespread.

Polysaccharides present in the preparations were thought responsible for some responses. Bonadeo et al (1971) proposed that the roots' skin-repairing action may be due to the formation of a complex between hyaluronic acid and a polysaccharidic principle of the plant content.

Wagner et al (1984) demonstrated that a polysaccharidic fraction of *Echinacea purpurea* possessed immunostimulating properties. Purified polysaccharides obtained from *E. purpurea* were shown also to activate macrophages strongly (Stimpel et al 1984).

We found (Tragni et al 1985) that a partially purified aqueous extract of *E. angustifolia* (EAE), topically applied, inhibited the croton oil-induced oedema in mouse ear and that the intravenous administration of the extract inhibited carrageenan-induced oedema in the hind paw of the rat.

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The aim of the present work has been to verify if the activity can be ascribed to the polysaccharidic content of the plant.

Materials and methods

Chemicals. λ-Carrageenan, croton oil, indomethacin and guaiacol (*o*-methoxyphenol) were purchased from Sigma Chemical Co., St. Louis, MO, USA, hexadecyltrimethylammonium bromide (HTAB) was from Eastman Kodak Co., NY, USA, and ketamine hydrochloride from Parke Davis, Milan, Italy.

Preparation of the polysaccharidic fraction (EPF). The polysaccharidic fraction was obtained as described by Wagner et al (1984): 400 g of powdered roots of *E. angustifolia* yielded 1·2 g of EPF.

Animals. Male Sprague-Dawley rats (200 g) and male CD1 albino Swiss mice (28–32 g) (Charles River, Calco, Italy) were maintained on a standard laboratory diet with free access to tap-water. The animals were kept for at least two weeks at constant temperature (22 ± 1 °C) and humidity (50–60%) in an artificially illuminated room in the dark from 1900–0700h.

Carrageenan paw oedema. Paw oedema was induced by injection of 50 μL of 1% λ-carrageenan in 0·9% sodium chloride in the plantar region of the right hind paw of rats. Oedema was determined immediately after injection and at 1 h intervals thereafter, using a mercury plethismograph, as described by Winter (1965). EPF was injected intravenously 1 h before carrageenan injection at two doses: 0·5 or 0·1 mg kg^{-1}. Control animals received saline i.v.

Croton oil dermatitis. The inflammation was induced in anaesthetized mice (ketamine HCl 150 mg kg^{-1} i.p.) by application of 35 μg of croton oil dissolved in 15 μL of acetone to the inner surface of the right ear. EPF, and indomethacin as reference drug, were dissolved in the