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Di-iodo-L-tyrosine-labelled Dextrans as Molecular Size Markers of Nasal Absorption in the Rat

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Abstract—A series of fractionated di-iodo-L-tyrosine-labelled dextrans (DIT dextrans), with a narrow range of number average molecular weights from 1260 to 45 500 Da, was administered intranasally and intravenously to anaesthetized rats. The nasal absorption of these compounds ranged from 0·6 to 52·7%. There was an inverse relationship between molecular size and the proportion of an intranasal dose absorbed. The study demonstrated the usefulness of DIT dextrans as molecular weight markers and confirmed the relationship between molecular size and nasal absorption for highly water soluble compounds. These results also supported the proposition that there is a continuous range of aqueous pores in the nasal mucosa.

In recent years the nose has been recognized as an important alternative route for the delivery of therapeutic peptide and protein drugs (Chien & Chang 1987). However, much has still to be investigated about the mechanisms by which such hydrophilic compounds cross the nasal membrane. One factor of importance is the molecular size. To perform an investigation on the influence of molecular size for drug transport of peptides, model compounds can be used but these should satisfy the following criteria; be members of a homologous series of hydrophilic compounds with similar physicochemical properties; be physiologically, pharmacologically and chemically inert and thus not perturb the biological system; be of a well-defined molecular size; and be easy to analyse.

Dextrans have several of these ideal properties but suffer from two major drawbacks. Generally, commercially available dextrans are heterogeneous mixtures containing a wide range of molecular sizes, and the analysis of low concentrations of dextran is problematic. Fractionated di-ido-L-tyrosine (DIT)-labelled dextrans, produced by activating dextran with 4-nitrophenol chloroformate followed by reaction with di-iodo tyrosine and purified by gel filtration (Vansteenkiste et al 1991), have many of the qualities of ideal molecular size markers, without the problems associated with standard dextrans. They are members of a homologous series, they are hydrophilic, they are chemically and biologically inert, the individual molecular sizes are closely defined, and, by radioiodinating the di-iodo-L-tyrosine, they are easy to analyse. Moreover, these DIT dextrans are highly water soluble and available in a wide size range which brackets the molecular size of many biologically active compounds.

To date, the nasal absorption of similar molecules of different sizes has been studied by Maitani et al (1989) and by Donovan et al (1990). Maitani et al (1989) followed the absorption of fluorescein isothiocyanate (FITC) and diethylaminoethyl (DEAE) dextrans with molecular weights (mol. wt) of 4100, 9000, 17 500 and 6000, 9000, 17 200, respectively, using enhancers in order to obtain measurable absorption. Donovan et al (1990), used heterogeneous mixtures of polyethylene glycols (PEGs) 600, 1000 or 2000.

The purpose of the present study was to investigate the influence of molecular size, over a wide range, of a well defined model compound, on transport across the nasal mucosa. Several DIT dextrans with mol. wts from 1260 to 45 500 Da were radioiodinated, administered intranasally and intravenously to anaesthetized rats and the plasma concentrations of radioactivity followed in serial plasma samples. By comparison of the intranasal and intravenous results the proportion of the intranasal dose absorbed was calculated.

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Materials and Methods

Materials

Thirteen DIT dextrans carefully fractionated by gel permeation chromatography (Table 1), with number average mol. wts from 1260 to 45 500 Da, were synthesized, purified and characterized as described previously (Vansteenkiste et al 1991). The dextrans had ratios of weight average mol. wt to number average mol. wt of less than 1·6, indicating a narrow range of sizes within each fraction. These DIT dextrans were radioiodinated with ^{125}I (Amersham International, Amersham, Bucks, UK) using a simple exchange reaction similar to that described by Hupf et al (1978). Concentrations of DIT dextran after iodination were measured spectrophotometrically at the predetermined absorption maximum of the compound (in the region of 220 nm), using a Perkin Elmer spectrophotometer.

All other reagents were of, at least, standard laboratory grade.

Preparation of animals

Adult male Wistar rats (260 ± 60 g, Nottingham University) were prepared for dosing as previously described (Fisher et al 1985, 1987) using a simplified version of the Hirai et al (1981) technique. Briefly, an anaesthetized animal was placed on its back, a cannula was inserted into the trachea to maintain respiration, and the oesophagus was occluded by tying it onto this cannula. With the oesophagus blocked there was no loss of the dose to the gastrointestinal tract (Fisher et al

Table 1. DIT dextrans administered intravenously and intranasally, mean doses administered, mean AUC_{0-360} values and mean proportions intransally absorbed. (All mean values are ± s.d.)

Compound	Number average mol. wt (Da)	Intravenous		Intranasal		Intranasal dose absorbed (%)
		Dose (mg kg^{-1})	AUC_{0-360} (μg mL^{-1} min)	Dose (mg kg^{-1})	AUC_{0-360} (μg mL^{-1} min)	
1	45 500	2·0±0·1	1395±186	2·2±0·1	9·3±3·9	0·6±0·3
2	33 410	1·5±0·1	1144±136	1·4±0·1	9·4±2·9	0·9±0·3
3	22 650	1·5±0·1	978±311	1·8±0·1	11·6±2·5	1·0±0·2
4	17 760	2·8±0·1	851±62	3·6±0·3	27·0±5·4	2·5±0·5
5	20 750	4·1±0·2	820±99	4·5±0·2	24·5±5·0	2·8±0·6
6	14 900	3·1±0·2	572±71	2·8±0·1	19·9±7·4	3·9±1·3
7	9800	1·2±0·1	167±19	1·3±0·1	11·4±6·6	6·4±2·2
8	9060	2·3±0·2	253±81	2·3±0·1	6·8±1·3	2·7±0·5
9	3400	3·3±0·2	609±67	3·2±0·2	238·5±28·3	40·1±4·6
10	2300	3·0±0·1	519±66	3·2±0·1	144·2±21·1	26·7±3·8
11	1625	1·2±0·1	276±64	1·4±0·1	66·5±9·0	21·9±2·0
12	1380	2·5±0·1	425±35	2·4±0·1	153·0±4·2	36·7±2·1
13	1260	2·0±0·1	455±40	2·0±0·1	235·0±33·7	52·7±7·6

1985). The carotid artery was cannulated for the collection of serial blood samples. Intranasal doses (50 μL) were administered directly into the nasal cavity, via a nostril, using a microsyringe fitted with approximately 40 mm of cannula tubing (0·28 × 0·61 mm, i.d. × o.d., Portex, Hyde, Kent, UK) attached to a needle. Intravenous doses (500 μL) were administered via an indwelling needle in the caudal vein. Groups of at least three animals were used. Blood samples were collected up to 360 min after dosing.

Determination of radioactivity

Plasma was separated by centrifugation, and the concentration of ^{125}I present in 200 μL samples determined by gamma counting (Compugamma, LKB-Wallac, Turku, Finland).

Stability of the DIT dextrans

The stability of the DIT dextrans was determined by incubating samples in phosphate buffered saline pH 7·4 at 37°C for 72 h. No di-iodo-tyrosine was released. The stability of selected radioiodinated DIT dextrans was determined by incubating samples in fresh heparinized rat plasma at 37°C for 6 h. Little radioiodine (mean 1·7%) was lost from the radioiodinated DIT dextrans.

Calculations

Areas under plasma concentration vs time curves from 0 to 360 min (AUC_{0-360}) were calculated using the trapezoidal method. The proportion of the intranasal dose absorbed was calculated by comparison of intravenous and intranasal AUC_{0-360}, and adjusting for any differences in dose.

Results and Discussion

Dextran is hydrolysed only slowly in many animal species (Gronwall 1957; Gruber 1969) including the rat (Vars et al 1952). Eventually glucose is formed which can be incorporated into many body constituents (Gray 1953). In the present work DIT dextrans and radioiodinated DIT dextrans have

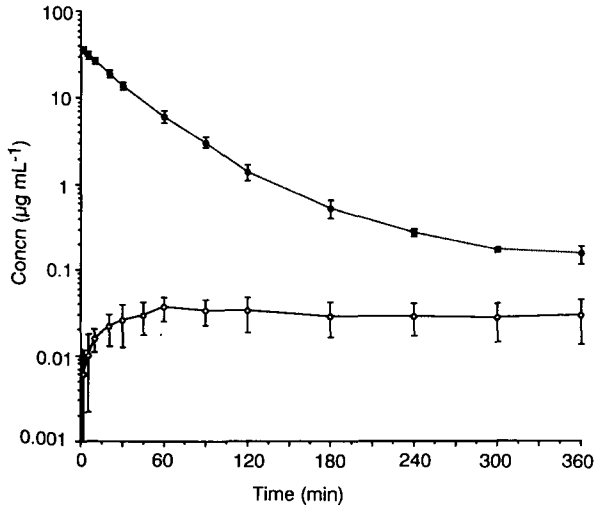

FIG. 1. Mean plasma concentrations (μg mL^{-1} ± s.d.) of DIT dextran compound 1 (45 500 mol. wt) after intravenous (●) or intranasal (○) administration to rats (2·2 mg kg^{-1}).

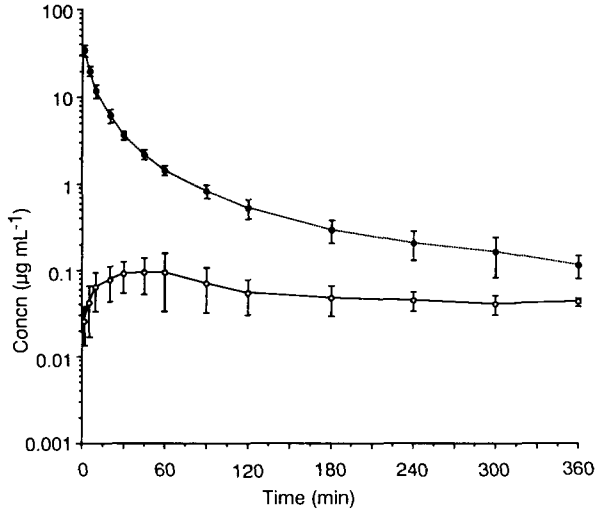

FIG. 2. Mean plasma concentrations (μg mL^{-1} ± s.d.) of DIT dextran compound 6 (14 900 mol. wt) after intravenous (●) or intranasal (○) administration to rats (2·8 mg kg^{-1}).

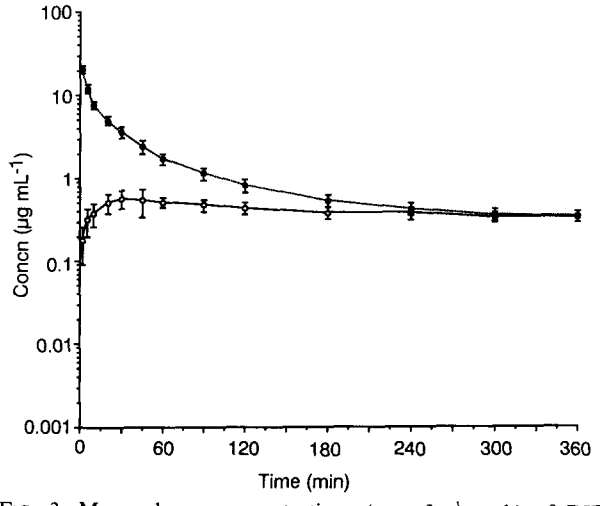

FIG. 3. Mean plasma concentrations ($\mu g\ mL^{-1} \pm$s.d.) of DIT dextran compound 10 (2300 mol. wt) after intravenous (●) or intranasal (○) administration to rats (3·2 mg kg^{-1}).

been shown to be stable. Therefore, plasma concentrations of radioactivity represented plasma concentrations of intact DIT dextran. The mean plasma concentrations of DIT dextrans, following intravenous or intranasal administration of representative compounds (compounds 1, 6, 10 and 13), are shown in Figs 1–4.

Following intravenous administration, the plasma concentrations of DIT dextran, for all mol. wts, showed a biphasic decline. This is a similar pattern to that reported previously in rats by Hanahoe & Wright (1983) for unfractionated dextran of 70 000 mol. wt, and Fisher (1987) also for unfractionated dextran of 70 000 mol. wt. All other animal species studied, principally dogs, have shown a similar biphasic decline for unfractionated dextrans with mol. wts ranging between 10 000 and 125 000 Da (Wasserman & Mayerson 1952; Terry et al 1953; Grotte 1956; Arturson & Wallenius 1964; Lami 1965). Two of these reports (Grotte 1956; Arturson & Wallenius 1964) suggest that the rapid early decline was due to the clearance of low mol. wt dextran, present in the heterogeneous test material, from the plasma to extravascular space and urine. This suggestion could not be valid in the present study as the DIT dextrans had a narrow molecular size distribution and, moreover, the rapid early decline was seen with all molecular sizes investigated.

After intranasal administration, the plasma concentrations of DIT dextran rose to a broad peak or plateau at about 60 min after dosing and remained at the plateau concentration, or declined only slowly, for the remainder of the experiment. This general type of plasma profile was seen for all thirteen DIT dextrans studied. A similar plasma profile was also seen previously when a radiolabelled dextran of 70 000 Da was administered to rats (Fisher 1987). As the molecular size of the DIT dextran decreased an increase in the actual plasma concentrations was achieved.

The number average mol. wts, the mean $AUC_{0\text{-}360}$ values following intravenous and intranasal administration and the mean proportion of the dose absorbed, are shown in Table 1. Where mean values are shown, the standard deviation is also shown. This illustrates that, despite some variation between groups, there was an increase in the amount of material absorbed intranasally as the molecular size decreased (from $0{\cdot}6 \pm 0{\cdot}3\%$ for the 45 500 mol. wt to $52{\cdot}7 \pm 7{\cdot}6\%$ for the 1260 mol. wt). The proportion of the dose absorbed intranasally is likely to be an underestimate, since at 360 min plasma concentrations of DIT dextran had not significantly declined from their plateau levels. Fisher (1987) showed that 1·8% of the dose was absorbed from an intranasally administered dextran with a mol. wt of 70 000. However, this material represented a wide range of molecular sizes and when plasma samples were analysed only about 33% of the material detected was of high mol. wt. Therefore, an absorption of approximately 0·6% agrees well with the present study.

Fig. 5 illustrates the number average mol. wt vs the proportion of the dose intranasally absorbed plotted on log: log axes. An inverse relationshp between size and absorption is clearly demonstrated, the line of best fit having a correlation coefficient of −0·96. There is evidence that unsubstituted dextrans in solution with a mol. wt of greater than 2000 Da assume a random coil shape; below this size

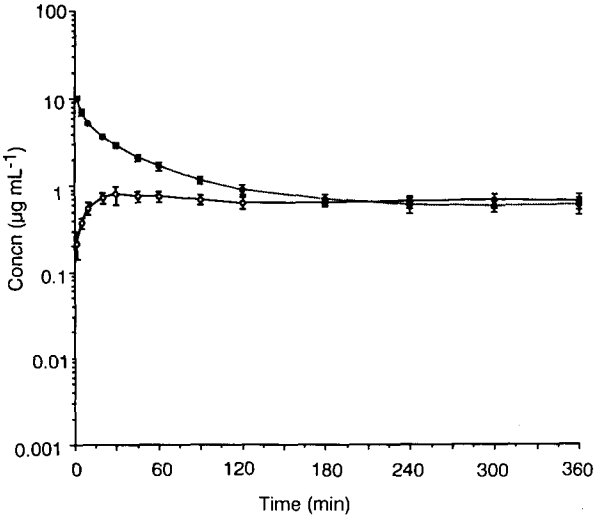

FIG. 4. Mean plasma concentrations ($\mu g\ mL^{-1} \pm$s.d.) of DIT dextran compound 13 (1260 mol. wt) after intravenous (●) or intranasal (○) administration to rats (2·0 mg kg^{-1}).

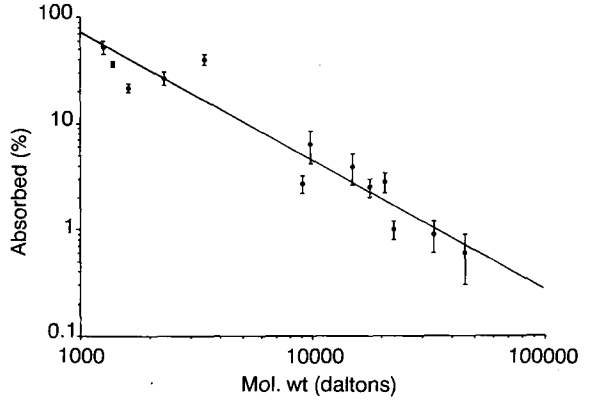

FIG. 5. Correlation between log of the proportion of an intranasal dose absorbed and log of the number average mol. wt.

dextrans have a rodlike shape (Gekko 1971; Basedow & Ebert 1979). However, this transition does not appear to affect the mol. wt/absorption relationship obtained in this study. The mol. wt/absorption relationship has been suggested previously (Fisher et al 1987), but, in that work, the mol. wt markers were not ideal. Maitani et al (1989) studied the effect of molecular size on nasal absorption of powders by rabbits. This author used fluorescein isothiocyanate (FITC) dextrans with mol. wts of 4100, 9000, 17 500, and diethylaminoethyl (DEAE) dextrans with mol. wts of 6000, 9000, 17 200, as neutral and charged macromolecules, respectively. As the molecular size of the dextran increased the plasma concentrations detected decreased, supporting the current results. However, in these studies (Maitani et al 1989) no absorption of dextran was seen without the addition of sodium glycocholate as an enhancer. Kotani et al (1983) showed no nasal absorption of FITC dextran by the rat. These results do not agree with the results obtained in the present study where all sizes of DIT dextran were seen to be absorbed to a greater or lesser extent. Also, the effect of the enhancer used by Maitani et al (1989) on the membrane is unknown. Donovan et al (1990) studied the effect of molecular size on nasal absorption with unfractionated polyethylene glycols (PEGs). After dosing with PEG 600, 1000 or 2000, urine was collected for 6 h and the concentration of PEG oligomers present was analysed. Donovan et al (1990) did show a molecular size/nasal absorption relationship for the smaller molecular sizes and narrower mol. wt range (approx. 450–2250 Da) that they used. As in the present study, Donovan et al (1990) saw no obvious molecular size cut-off. In absolute terms when similar sized PEGs and DIT dextrans are compared, the proportion of PEG absorbed (Donovan et al 1990), is lower than the proportion of DIT dextran absorbed (this study).

The non-ideal compounds used earlier by Fisher et al (1987) were small in number and furthermore covered a large mol. wt range. They were differently charged and some had pharmacological activity. In addition some were metabolized and the larger compounds were very heterogeneous. All compounds were found to be absorbed, but the resulting mol. wt vs proportion absorbed relationship could, because of the above factors, be described as fortuitous. In the present study DIT dextrans have been used as molecular size markers, and as a consequence, most of the possible limitations of the previous study have been resolved. A larger number of compounds was used and the differences in size between compounds were relatively small. Moreover, the range of molecular sizes represented by each compound was small. Being members of an homologous series the DIT dextrans had similar physicochemical properties. They were inert and carried a small but uniform charge. Indeed, for in-vivo and in-vitro work, these fractionated DIT dextrans are probably the most useful mol. wt markers yet to be described.

The confirmation of the inverse relationship between molecular size and nasal absorption supports the idea that water soluble compounds cross the nasal mucosa mainly by diffusion (Fisher et al 1987). Further support for this idea was presented by McMartin et al (1987), who looked for correlations between published values for the nasal absorption of compounds, and the physicochemical properties of those compounds. They found the best correlation was between molecular size and nasal absorption, consistent with nonspecific diffusion through aqueous channels. The presence of such aqueous channels or pores in the nasal mucosa has been suggested by Hirai et al (1981), Kaneo (1983) and Hayashi et al (1985). On the other hand Gibson & Olanoff (1987) suggested that pores do not exist. In the present study, the continuity of this absorption vs size relationship between 45 000 and 1260 Da indicates that any such aqueous pores must have a wide distribution of sizes, or must be dynamic in nature. This idea is supported by the lack of molecular size cut-off, also observed by Donovan et al (1990). Such uncertainty can only be resolved when further work has been done, especially the visualization of the movement of compounds across the nasal mucosa at a cellular level.

In conclusion, the study described here illustrates the usefulness of fractionated DIT dextrans as mol. wt markers, and confirms the relationship between molecular size and nasal absorption for highly water-soluble compounds.

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