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Macromolecular prodrugs. II. Influence of variation in molecular weight and degree of substitution of O-benzoyl dextran conjugates on their physicochemical properties and stability in aqueous buffer and in plasma

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Summary

Physicochemical and hydrodynamic properties of benzoyl dextran conjugates with varying molecular weights and degree of substitution have been determined. The stability experiments revealed identical pH-dependence of rates of regeneration of benzoic acid from the various conjugates employed. Similar rate data were obtained in aqueous buffer (pH 7.40 and 37°C) and in human plasma indicating that hydrolytic release of benzoic acid in plasma proceeds in the absence of enzymatic catalysis. Although the limiting viscosity number for each dextran polymer decreases with increasing degree of substitution the analytical gel filtration data suggest that the hydrodynamic volumes for derivatives with degree of substitution up to at least 10% will be close to those of the parent dextrans and the pharmacokinetic fate of the derivatives are therefore expected to be quite similar to unsubstituted dextrans. The potential feasibility of employing macromolecular dextran prodrugs to obtain parenteral sustained release preparations is discussed.

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Introduction

In a previous study (Larsen and Johansen, 1985) partial benzoylated dextran T-70 derivatives were synthesized. The kinetics and mechanism of release of benzoic acid from the model macromolecular prodrugs together with the stability of the derivatives in human plasma were determined. The present study was undertaken in order to evaluate the influence of both the molecular weight and the degree of substitution on the physicochemical and hydrodynamic properties of benzoylated dextrans.

Materials and Methods

Dextran T-10, T-20, T-40, T-70, T-110 and the Sephadex types, G-10 and G-200, were obtained from Pharmacia, Sweden.

Determination of degree of substitution

The degree of substitution was determined by hydrolysis of the benzoyl dextran conjugates. The released benzoic acid was quantitated by the HPLC method previously described (Larsen and Johansen, 1985). An accurately weighted amount of the individual conjugate corresponding to about 5 mg was dissolved in 25.00 ml of 0.1 N NaOH and the solution was heated to 60°C for 10 min in a waterbath. After cooling 500 μl samples were withdrawn and added to 500 μl of 0.1 N HCl. The resulting solutions were assayed for benzoic acid by HPLC. The absence of non-covalently linked benzoic acid in the conjugates was confirmed by dissolving a weighted amount of the polymer (~ 5 mg) in 25.00 ml 0.05 M phosphate pH 6.0 and the solution was analyzed immediately for benzoic acid. In some experimental runs the carbohydrate benzoic acid ester concentration additionally was quantitated by a hydroxylamine-ferric chloride method (Notari and Munson, 1969) using ethyl benzoate as the standard. The values obtained by use of the two various methods agreed within $\pm 2\%$. The degree of substitution (DS) has been expressed as mg sodium benzoate released per mg of the conjugate.

Determination of the average molecular weights and the polydispersities of the conjugates

The determination of M_w of the conjugates was performed by analytical gel filtration. 2 ml samples corresponding to 10 mg dextran were applied to a Pharmacia column K-26/100 packed with Sephadex G-200 ($V_T = 177$ ml and $V_0 = 64$ ml) and were eluted with a 0.3% sodium chloride solution. The flow rate was kept at 4.0 $ml \cdot h^{-1}$ by means of a constant-speed peristaltic pump and the eluent was collected in 3.0 ml fractions. The dextran concentrations were determined by the anthrone method (Nordic Pharmacopoeia procedure). Well characterized dextran fractions (Nordic Pharmacopoeia standards) were used in the calibration and the linear correlation between K_{av} and log M_W within the range of M_W 110,000–7000 is presented in Fig. 1. In order to evaluate the polydispersity of the substituted dextrans M_n values were determined by end-group analysis in accordance with the Somogyi phosphate method described by Isbell et al. (1953).

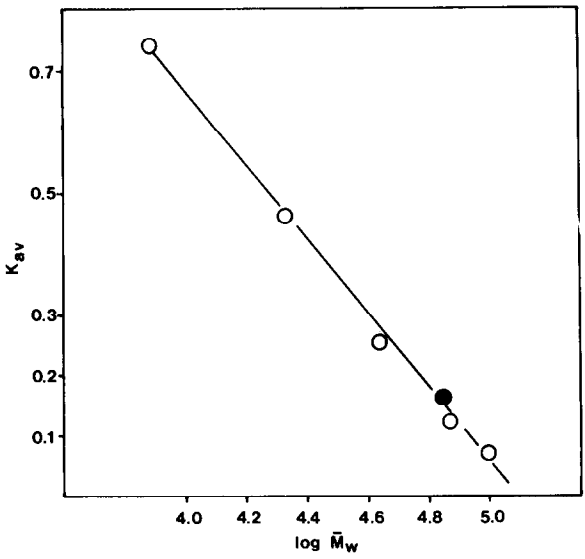


Fig. 1. Calibration curve K_{av} vs log M_w for dextrans on Sephadex G-200. Dextran standards (○) and benzoyl dextran T-70 (DS 9.1%) (●).

Viscosity measurements

The viscosities of aqueous solutions of the parent dextrans and some benzoyl dextran conjugates were measured in an Ubbelohde viscometer at 20 ± 0.01°C.

The outflow time for each solution was determined as the mean of 5 measurements. The individual determinations agreed within 0.2%. The outflow time of pure solvent was greater than 120 s indicating that the kinetic energy corrections are negligible and are therefore omitted.

The limiting viscosity number, $[\eta]$, is defined as the reduced viscosity (η_{spec}/c) at the limit of zero concentration, where the specific viscosity

$$\eta_{spec} = \frac{\eta - \eta_0}{\eta_0} = \frac{\eta}{\eta_0} - 1 \tag{1}$$

where η_0 and η are the dynamic viscosities of the pure solvent and the solution, respectively. According to Poiseuille's equation η_{spec} can be determined from:

$$\eta_{spec} = \frac{t\rho}{t_0\rho_0} - 1 \tag{2}$$

where ρ/ρ_0 is the ratio of the densities of solution and pure solvent and t/t_0 is the corresponding ratio of the respective outflow times from an Ubbelohde type viscometer. For dextran solutions the following relationship between ρ/ρ_0 and the concentration, c (in g/ml), has been found (Nordic Pharmacopoeia procedure)

$$\frac{\rho}{\rho_0} = 1 + 0.4c \tag{3}$$

The limiting viscosity number for dextran solutions has therefore been calculated as:

$$[\eta] = \lim_{c \to 0} \frac{\frac{t}{t_0}(1 + 0.4c) - 1}{c} \quad (4)$$

Determination of solubility

The solubility of conjugates with varying degree of substitution and molecular weight was assessed in aqueous buffer pH 7.40 at 25°C. Exact determination of the solubility of the conjugates failed due to the tendencies of dextrans to form gels in high concentrated solutions. However, regardless of M_w the solubility of conjugates with a degree of substitution up to 15.8% was greater than 30% w/v.

Results and Discussion

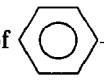
Characterization of the conjugates

One parameter in the characterization of macromolecular prodrugs is the determination of the molecular weight distribution and the average molecular weights. This being of special importance when synthesis of the conjugates involves reaction conditions which may lead to crosslink reactions. It is well known that factors influencing the therapeutic usefulness of drug carrier conjugates show molecular weight dependence as, for example, renal clearance, retention in circulation and hypersensitivity reactions. Immunogenicity reactions are one major impediment to the progress in the clinical use of macromolecular delivery systems.

Although only few hypersensitivity reactions accompany dextran therapy (Richter and Hedin, 1982), rare and sometimes life-threatening dextran-induced anaphylactoid reactions do occur (Richter et al., 1981). Allergenic properties of dextran preparations are dependent on both the degree of branching and the fraction of high molecular weight contaminants (Wold and Heen, 1978). Thus characterization of dextran conjugates must include determination of M_w as well as the polydispersity (M_w/M_n) of the preparations.

The calibration curve using dextran standard fractions (Fig. 1) can be described by the following equation:

$$K_{av} = 3.11 - 0.612 \log M_w \quad (n = 5,\ r = 0.998) \quad (5)$$

From a K_{av} value of 0.160 for a benzoyl dextran conjugate (DS = 9.1%) a weight average molecular weight of 66,500 has been calculated. Assuming that dextrans solely consist of anhydroglucose units the molar ratio of  –CO–/anhydroglucose for a conjugate with DS 9.1%, corresponds to 10.9%, from which a M_w of 70,200 is obtained. By comparison the experimental determined and the calculated

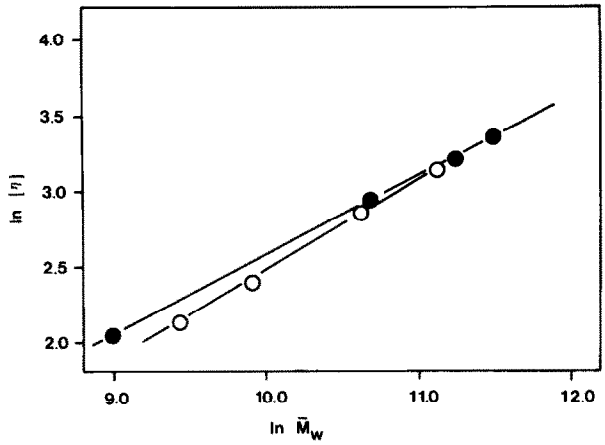


Fig. 2. A ln-plot of the limiting viscosity number $[\eta]$ against the average molecular weight, M_w for dextran standards (●) and for benzoyl dextran conjugates with DS of 4.0% (○).

M_w values agree within 5%. In case that both the shape of the molecular weight distribution and the original dextran coil structure are restored after derivatization the polydispersity of the conjugate should be equal to that of the parent dextran. According to specifications the polydispersity of dextran T-70 is 1.90. Determination of the latter parameter for the 9.1% substituted conjugate revealed a value corresponding to 1.97.

In Fig. 2. is shown the relationship between the logarithms for the limiting viscosity number and the weight average molecular weight, M_w, for dextran standards with M_w ranging from 7000 to 110,000. The relationship can be derived from the Mark-Houwink equation:

$$[\eta] = KM^a \tag{6}$$

where K and a are constants characteristic for each polymer series. The following equation is obtained:

$$[\eta] = 7.24 \times 10^{-2} \times M_w^{0.52} (ml \cdot g^{-1}) \tag{7}$$

In Fig. 2. is also shown a $\ln[\eta]$ versus $\ln M_w$-plot for benzoyl dextran conjugates with a degree of substitution of approximately 4%, where the linear curve is adequately described by:

$$[\eta] = 2.60 \times 10^{-2} \times M_w^{0.61} (ml \cdot g^{-1}) \tag{8}$$

Reported values of the constants a and K for various dextran preparations together with the results of the present study are presented in Table 1.

From Fig. 2 and by comparing Eqn. 7 and Eqn. 8 it is seen that substitution of dextrans with benzoyl groups influences the Mark-Houwink relationship. Although

TABLE 1

VALUES OF THE MARK-HOUWINK CONSTANTS a AND K FOR VARIOUS DEXTRAN PREPARATIONS

Dextran	Temp. (°C)	$[\eta] = K M_W^a$		Reference
		K (ml/g)	a	
NRRL B-512 ($M_w \leqq 100{,}000$)	25	9.78×10^{-2}	0.50	Senti et al. (1955)
B-512 Ph ($22{,}000 \leqq M_W \leqq 725{,}000$)	20	24.3×10^{-2}	0.42	Granath (1958)
B-512 F ($2{,}000 \leqq M_w \leqq 500{,}000$)	20	14.8×10^{-2}	0.48	Basedow and Ebert (1979)
($7{,}000 \leqq M_w \leqq 110{,}000$)	20	7.24×10^{-2}	0.52	Present study
Benzoyl dextran (DS = 4%) ($7{,}000 \leqq M_w \leqq 110{,}000$)	20	2.60×10^{-2}	0.61	Present study

the calculated values of the constant, a, for the two series are of the same order of magnitude, the form factor, a, for substituted dextrans is slightly increased compared to the value determined for the parent dextrans. This means that the limiting viscosity number for substituted dextrans with molecular weight above 81,000 should be greater than for pure dextrans. However, substitution with relatively lipophilic benzoyl groups is not expected to enhance the limiting viscosity number or the hydrodynamic volume of the derivatives as indicated in Fig 3. where it is seen that the hydrodynamic volume decreases with increasing degree of substitution. It is therefore assumed that, within the experimental error, the form factor is not altered significantly after derivatization having a value of approximately 0.5. The magnitude of a gives information of molecular shape in solution. Thus, a value close to 0.5 is characteristic for polymers behaving like statistic coils in solution.

While the constant a is relatively unaffected by substitution up to a DS corresponding to 10%, the constant K seems more sensitive to substitution of the polymers. The K value obtained for the parent dextrans is in good agreement with the value reported by Senti et al. (1955). The observed decrease of K for benzoylated dextrans implicates a decrease in $[\eta]$ and—as $[\eta]$ is an expression of the hydrodynamic volume—a corresponding reduction of the hydrodynamic volume of the conjugates. Introduction of benzoyl groups into the highly hydrophilic dextrans makes formation of intramolecular hydrophobic bonds possible. Furthermore, increasing lipophilicity of the conjugates leaves water an inferior solvent. These conditions are most likely responsible for the observed diminution of K. Support for this assumption is apparent from Fig. 3 where the correlation between the hydrodynamic volume for substituted dextrans (T-70) and the degree of substitution is presented. In accordance with the suggestions given above DS markedly influences

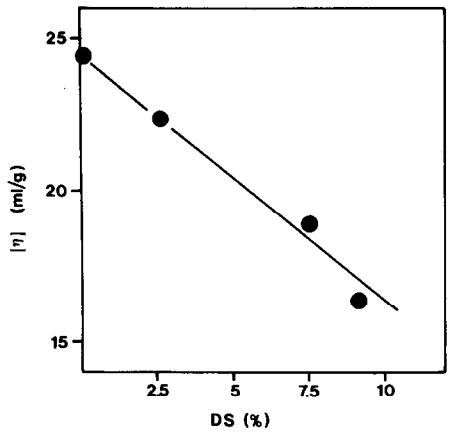


Fig. 3. Correlation between the hydrodynamic volume of substituted dextrans (T-70) and the degree of substitution.

the hydrodynamic volume, leaving $[\eta]$ almost inversely proportional to the degree of substitution in the molecular weight range studied. The constant K obtained from viscosity determinations can therefore be interpreted as a measure of the ability of the individual molecules to pack in a given solvent.

In Table 2 representative data are shown for both parent dextrans and some benzoylated dextrans. Together with the values of the limiting viscosity number, the Huggins constant, k′, as calculated from Huggins equation is also given:

$$\frac{\eta_{spec}}{c} = [\eta] + k'[\eta]^2 c \qquad (9)$$

TABLE 2

HYDRODYNAMIC VOLUME $[\eta]$ AND HUGGINS CONSTANT, k′, FOR UNSUBSTITUTED DEXTRANS AND PARTIALLY BENZOYLATED DEXTRANS IN AQUEOUS SOLUTION

Dextran	M_n (parent dextran)	M_W (parent dextran)	DS (%)	$[\eta]$ ($ml \cdot g^{-1}$)	k′
T-7	4530	7700	0	7.61	1.10
T-40	26200	43900	0	19.2	0.85
T-70	39000	75200	0	24.7	0.78
T-110	76110	99680	0	29.0	0.60
T-70	34600	65600	2.6	22.4	0.87
T-70	34600	65600	3.7	22.6	0.57
T-70	34600	65600	7.5	18.9	1.02
T-70	34600	65600	9.1	16.4	1.30
T-20	16450	20400	4.4	11.1	2.34
T-40	28000	41000	4.5	18.2	0.72

k′ is partly a measure of hydrodynamic interactions between the molecules in solution (Miller, 1968). Although no adequate theoretical treatment of k′ is available some empirical information is deducible. From Table 2 it can be seen that an increment in DS for substituted dextrans causes a corresponding increase in Huggins constant. According to Miller (1968) such an enhancement of k′ often is due to a change in the solvent from a good to a poor one. As water is the solvent in all cases, water can be regarded as a less good solvent for substituted dextrans, this being in good agreement with our findings concerning the reduction in hydrodynamic volume with increasing DS.

It is important here to emphasize that the dextrans used in this study possess polydispersities from 1.3 to 1.9, thus revealing relatively broad molecular weight distributions. In such cases the molecular weight average in the Mark-Houwink equation is neither a number (M_n) nor a weight average (M_w), but a so-called viscosity average (M_v). M_v can be calculated assuming that the molecular weight distribution follows a Lansing-Kraemer distribution (Senti et al., 1955; Granath, 1958). The mentioned reservation is generally important to recall when the Mark-Houwink equation is used to determine average molecular weights for similar polymers. However, in a study on solution properties of dextrans Granath (1958) has determined the constants K and a for dextrans with 5% branching using the Mark-Houwink relationship using both M_w and M_v. The results show that K and a are relatively insensitive to application of either M_w or M_v in the equation, when the polydispersity of the dextran fractions varied from 1.1 to 2.7. These observations are in close agreement with our results.

The distribution of substituents in the partially benzoylated dextrans has been assessed by GPC on Sephadex G-200, as described for the determination of M_w. The column effluent was assayed by both the anthrone reaction and by measuring the absorbance at 235 nm, where the latter solely is a measure of benzoyl groups present in the polymers. An almost constant ratio of A_{620} to A_{235} was found for the various fractions suggesting that the substituents are distributed uniformly between the polymer chains.

A comparison of the solution properties of benzoyl dextran conjugates and the parent polymeric compounds indicates that partial benzoylation of dextrans up to 15.8% does not result in a significant change of the conformation of the conjugates in aqueous solution. As expected, the hydrodynamic volume is more sensitive to introduction of hydrophobic substituents. The observed reduction in hydrodynamic volume, as measured by the limiting viscosity number, may therefore alter the renal excretion of the conjugates compared to pure dextrans. On the other hand, the data obtained from the analytical gel filtration suggest that partial benzoylation of dextrans only slightly influences the hydrodynamic volume. In gel permeation chromatography the gel–solution distribution coefficient of a macromolecule depends on both the molecular weight and the conformation of the polymer in solution. In the case of dextrans, having calibrated the gel for a specific polymer, the determination of the molecular weight distribution of an unknown sample is only reliable if the hydrodynamic properties of the sample are quite similar to those of the standards used in the calibration (Granath and Kvist, 1967). For a dextran con-

jugate with degree of substitution corresponding to 9.1%, the experimentally determined M_w from gel filtration was only 5% lower than the theoretically calculated value, reflecting that partial substitution of dextran in this case does not result in a significant change of the hydrodynamic volume. The apparent discrepancy between the data from viscosity measurements and gel filtration may most likely be attributed to the different conditions under which the influence of the degree of substitution on the hydrodynamic volume has been assessed. Determination of the limiting viscosity number is performed in dilute aqueous solution, whereas in gel filtration the formation of intramolecular hydrophobic bonds is suppressed by the interaction of the conjugates with the hydrophilic gel chains. With the hydrophilic environment in vivo in mind, it is to be expected that the hydrodynamic properties of the conjugates will be close to those of the parent dextrans in vivo.

Besides glomerulus filtration one other parameter dictating the pharmacokinetic fate of macromolecular conjugates after parenteral administration is the extent of uptake by cells of the reticuloendothelial system in the liver and the spleen. Clinical dextran (e.g. dextrans with M_w of 40,000 and 60,000, respectively) is mainly excreted exponentially by the kidney, and only a small proportion is taken up by tissues (Arturson and Wallenius, 1964; Schwarz et al., 1981; Köhler et al., 1974). In patients with normal renal function the latter authors have determined a dextran 40 plasma half-life of about 11 h, while the 12 h urinary recovery was 48%. The negligible uptake of clinical dextrans by phagocytic cells is most likely due to their hydrophilic non-ionic character. Similarly, in case of colloidal carrier particles, it has been suggested that more hydrophilic non-charged particles should be expected to remain in circulation for longer periods of time compared to rather hydrophobic particles (Van Oss et al., 1975). The data of the present study suggest that after partial derivatization of dextrans with relatively lipophilic substituents the hydrodynamic properties of the conjugates are comparable to those of the parent dextrans indicating that similar pharmacokinetic profiles may be expected in vivo following parenteral administration.

The degradation experiments have been carried out as described in a previous paper (Larsen and Johansen, 1985). The kinetics of release of benzoic acid from benzoyl dextran conjugates with varying average molecular weights and degree of substitutions have been determined in aqueous solution in the pH-range 7.0–9.5 at 60°C. In Fig. 4 the rate data are summarized showing the pH dependence of the logarithm of the buffer independent first-order rate constants for cleavage of the ester bond. The slope of the pH-rate profile is 0.99 indicating that the hydrolysis is specific base-catalyzed. As seen from Fig. 4, the rates of release of benzoic acid from the conjugates are independent of both the average molecular weight and the degree of substitution. Using the previously determined activation energy for the second-order rate constant, k_{OH}, the half-lives at pH 7.40 and 37°C for representative conjugates have been calculated and are presented in Table 3 together with the corresponding experimental rate data for degradation of the conjugates in 80% human plasma at 37°C. Practically identical stabilities of the various conjugates in aqueous solution and in plasma are observed revealing that hydrolysis in human plasma proceeds without enzyme catalysis.

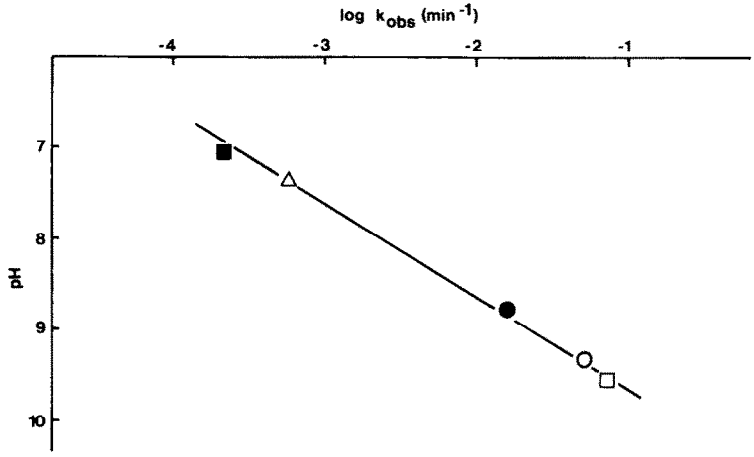


Fig. 4. pH–rate profile for hydrolysis of benzoyl dextrans at 60°C and $\mu = 0.5$. ○, T-70 (DS = 9.1); ●, T-10 (DS = 4.1); □, T-20 (DS = 3.8); ■, T-40 (DS = 4.6); △, T-110 (DS = 4.2).

Parenteral sustained release preparations using dextrans as intermediate carriers

A substantial number of prolonged action products is available on the market today. Formulations have been brought about by means of the conventional galenic approach or by more sophisticated drug delivery systems, the latter also encompassing the chemical approach (prodrugs) (Sinkula, 1978). Potential as well as realized advantages for the application of parenteral sustained release products have been discussed by Ballard (1978) and Lee and Robinson (1978). In the design of controlled release formulations macromolecular prodrugs may add one further dimension to the prodrug approach. Similar to low molecular weight prodrugs the regeneration of the drug compound from the macromolecular conjugate is affected by enzymatic or non-enzymatic hydrolytic mechanisms, dictated by the nature of the established bond between the carrier and the drug. However, in the case of

TABLE 3

STABILITY OF BENZOYL DEXTRANS WITH VARYING MOLECULAR WEIGHT AND DEGREE OF SUBSTITUTION (DS) IN 0.05 M PHOSPHATE BUFFER pH 7.40 AND IN 80% HUMAN PLASMA (37°C)

Benzoyl dextrans		$t_{1/2}$ (h) pH 7.40	$t_{1/2}$ (h) plasma
Dextran	DS		
T-70	3.4	186	199
T-70	9.1	180	183
T-70	15.8	182	190
T-10	4.1	178	192
T-20	3.8	180	188
T-40	4.6	182	195
T-110	4.2	180	182

macromolecular prodrugs the disposition and persistence within the body may be determined solely by the pharmacokinetic properties of the polymeric transport group from which the drug is released in a predictable manner.

Using dextrans as macromolecular carriers, depot effect has been demonstrated (Sezaki and Hashida, 1984), but it has to be emphasized that the localization of conjugates following the distribution phase varies with both the site of administration and the physicochemical and hydrodynamic properties of the derivatives. The extravascular mobility of dextrans decreases with increasing molecular weight of the polymer. Thus, injection of a conjugate within the vicinity of the target tissue may provide an appropriate depot from which the therapeutic agent is activated slowly. Although the hydrolysis rate of drugs linked directly to dextran through an ester linkage to some extent is influenced by the chemical structure of the individual compounds, the results of the present study clearly indicate that feasible sustained release preparations for drugs containing a carboxylic acid functional group can be obtained by employing dextrans as intermediate carriers, where half-lives for release of the therapeutic agents can be expected to correspond to approximately 8 days. Kojima et al. (1980) have attached mitomycin C to dextran via an amide linkage. In mice the same group (Hashida et al., 1981) has studied the therapeutic efficacy of the conjugate after intratumoral injection. The antineoplastic agent is released by non-enzymatic hydrolysis with a half-life of 24 h and the conjugate showed a superior effect on subcutaneously implanted B-16 melanoma compared to free mitomycin C. The significant increase of the life-span was ascribed to the sustained pharmacological activity of the conjugate. Hashida et al. (1984) have investigated the disposition and pharmacokinetics of mitomycin C derivatives using dextrans with molecular weights of 10,000, 70,000 and 500,000, respectively, after intravenous administration. By use of radiolabelled conjugates, it was found that radioactivity rapidly accumulated in the reticuloendothelial systems of the liver, spleen and lymph. In comparison to clinical dextrans the pharmacokinetic profiles of the mitomycin C dextran conjugates are altered dramatically. The phagocytic engulfment of the derivatives may most likely be attributed to their physicochemical properties. Mitomycin C is attached to the dextrans via an ϵ-aminocaproic acid spacer arm. The spacer amino group is linked to dextran by CNBr activation. As discussed in a previous paper (Larsen and Johansen, 1985), this gives rise to a polycationic character of the conjugates due to formation of N-substituted isoureas and N-substituted imidocarbamates. Furthermore, hydrolytic release of mitomycin C from the conjugates results in liberation of free spacer arm carboxylic acid groups, which will contribute to the total charge of partly hydrolyzed conjugates at physiological pH. As has been shown for colloidal particles (Van Oss et al., 1975; Illum and Davis, 1984) the charge of the colloids is an important determinant in phagocytic uptake. The polycharged nature of the mitomycin derivatives may therefore be responsible for the endocytic activity of the reticuloendothelial system. However, after intravenous injection almost similar sustained plasma levels of free mitomycin C were detected regardless of the carrier size although the concentration time profiles of the drug varied with the molecular weight of the dextrans. Although the overall electric charge of the cell surface is rather electronegative, thus

absorbing/adsorbing polycations more strongly (Drobnik and Rypácek, 1984), the assessment of the therapeutic feasibility of using charged dextran conjugates has to await future investigations due to the little information available about the influence of both the nature of charge and the total amount of charge on the uptake of macromolecular conjugates by the reticuloendothelial systems.

Regarding the pharmacokinetic profiles of clinical dextrans together with the hydrodynamic properties of partly benzoylated dextrans it should be possible to design dextran derivatives circulating in the vascular system for a given period of time continuously releasing the active agent. The circulating half-lives for dextrans vary with the molecular weight of the polymer. Plasma half-life for dextran 40 is 10–11 h (Köhler et al., 1974; Schwarz et al., 1981) and for dextran 60, 42 h (Schwarz et al., 1981), whereas the intravascular half-lives for dextrans with molecular weights below 30,000 are less than 1 h (Arturson and Wallenius, 1964). In order to obtain reproducible pharmacological responses, also the polydispersity of the conjugates have to be considered. Injection of dextran derivatives with relatively broad molecular weight distribution may contain a high molecular weight fraction which will be taken up by the reticuloendothelial system (Persson, 1952) and slowly metabolized by dextranases (Ammon, 1963). Besides phagocytic elimination it has been shown that the molecular weight distribution of dextran in plasma after intravenous injection is subject to a rapid change in the high-molecular weight direction because of the rapid transcapillary disappearance of the lowest molecular part into the extravascular space and the urine (Arturson and Wallenius, 1964).

The potential utility of using macromolecular compounds as carriers for various drug substances has attracted great interest in recent years. Most efforts have been directed towards biological evaluation of the conjugates, while basic characterization of derivatives has received only little attention. Recognizing that the therapeutic efficacy of macromolecular conjugates to a great extent is governed by the physicochemical and hydrodynamic properties of the derivatives, such as rate of regeneration of the drug, hydrophilic/lipophilic balance, charge and hydrodynamic volume, further progress in the macromolecular prodrug approach may arise from optimizing the physicochemical properties of the conjugates.

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