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Note

Possible role of serum protein binding to improve drug disposition

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Abstract

In order to investigate the possibility of using serum protein-binding as a tool for developing drug delivery systems, lipophilic derivatives of 5-fluorouracil (5FU), in which a benzene moiety was attached to the N1 position of 5FU, were synthesized and their protein binding potencies and in vivo pharmacokinetic properties were studied. A good relationship was observed between the lipophilicity of derivatives and their binding potency to rat or human serum albumin. The apparent distribution volume of the 5FU derivative after intraveneous injection into rats was diminished while the unbound fraction of derivatives in the plasma decreased. The biological half-life was expressed as a function of both unbound clearance and protein binding potencies of the derivatives. Among the derivatives synthesized, the mono- or dichloro substituted forms showed greater protein binding potency and a dramatically prolonged blood concentration in vivo. The chemical modification of 5FU using these substituted groups should be useful to improve the in vivo disposition of 5FU.

Key words: Drug delivery system; 5-Fluorouracil; Protein binding; Drug disposition

Serum protein binding is one of the most important factors to define the disposition of many drugs in the body. Drug molecules which exist as a complex with serum protein, such as with albumin in the blood stream, are considered not to be able to penetrate the wall of blood vessels of most organs. Drugs which possess high binding affinity to serum protein often show a long biological half-life with a relatively small volume of distribution. In the case of warfarin, more than 99% of the drug binds to albumin in the blood and is eliminated slowly from the body, while less than 1% of the total drug can express the pharmacologic effects (Yakobi and Levy, 1975).

On the other hand, there are several drugs which involve problems in their use due to their rapid elimination from the body as a result of fast metabolism or excretion. 5-Fluorouracil (5FU), a widely used anti-tumor drug, has been reported to have a very short half-life of less than 10 min, mainly due to metabolism in the liver (Fraile et al., 1980). Since frequent administration in order to maintain the desired blood concentration of 5FU strenghthens its adverse reaction, many in-

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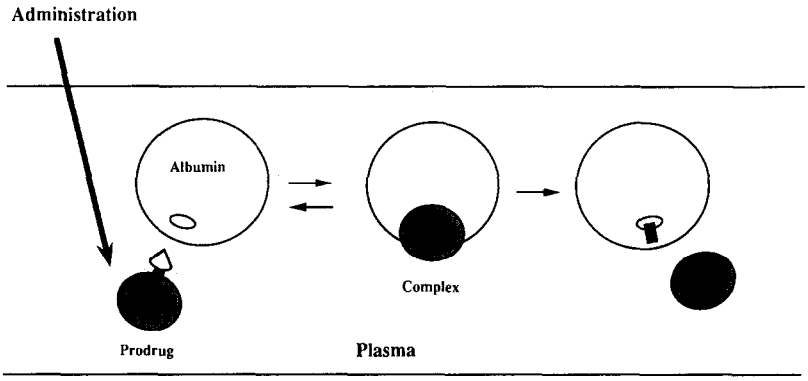

Fig. 1. Strategy for drug delivery system utilizing drug-protein binding.

vestigations have been undertaken to improve its pharmacokinetic properties. Utilizing some polymers as a drug carrier is one of the possible ways of prolonging the blood concentration of such drugs (Akashi et al, 1989; Ouchi, 1990; Ouchi et al., 1990). Hashida et al. (1984) reported that a polymeric prodrug of mitomycin C grafted onto dextran or polyethylene glycol was retained in the blood stream for a considerable length of time and gradually released the active moiety via a hydrolysis reaction. These prodrugs also have the ability to accumulate at the tumor site (Takakura et al., 1987a). The latter authors demonstrated the higher therapeutic efficacy of the prodrug compared to the parent drug itself on tumor-bearing mice (Takakura et al., 1987b). In those reports, it is obvious that the most distinct advantage of the polymeric prodrug is its ability to keep the drug in the blood for a prolonged time.

Therefore, if the drug which binds to the serum

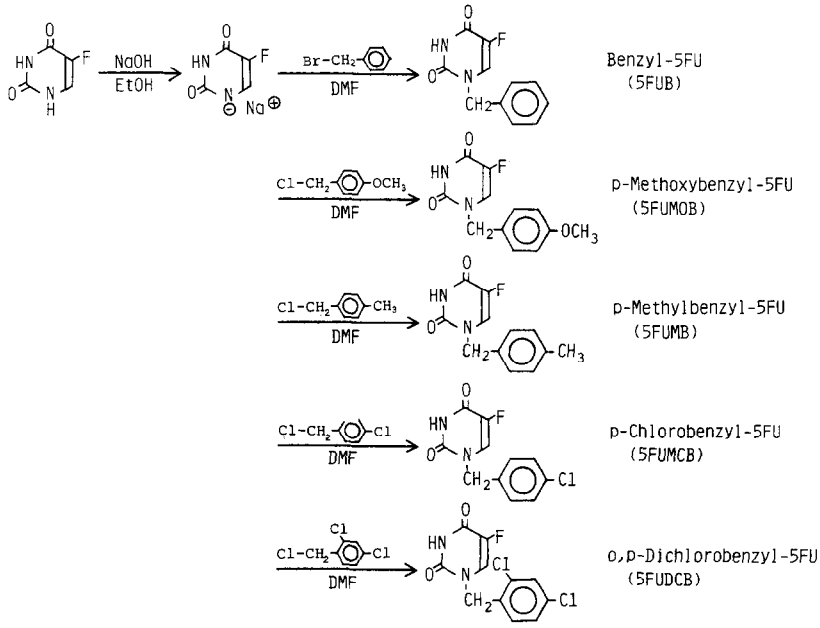

Fig. 2. Synthetic pathways of 5FU derivatives and their designations.

protein acts as the polymeric drug in the body, it might be possible to utilize drug-protein binding to improve drug disposition. Our new strategy is illustrated in Fig. 1 where newly synthesized prodrug which has a high binding affinity to the serum protein is expected to be retained in the blood for a longer time than its parent drug. The parent drug is then released gradually by the hydrolysis reaction. In this system, low molecular weight prodrugs are administered into the body and, thereafter, bind to the endogenous protein in the blood. Therefore, this system can avoid many problems occurring during the administration of exogenous macromolecules into the body, such as: (1) the induction of antigen toxicity; (2) extensive trapping by the reticuloendothelial system; and (3) the restricted route for administration.

To develop the new drug delivery system (DDS) based on the above idea, 5FU was chosen as a model drug and, as a first step, several of its non-hydrolyzable derivatives were synthesized. We have previously reported a method for synthesis and their physicochemical properties (Yamashita et al., 1989). Further investigations were performed on their in vivo disposition after intravenous injection into the rat. In this paper, the possibility of employing protein binding as a tool for DDS is discussed from the standpoint of pharmacokinetic aspects.

5FU and its prodrug tegafur were kindly donated by Mitsui Pharmaceutical Co., Ltd (Tokyo, Japan). All other reagents were analytical grade. A benzene moiety having several substituted groups was attached to the N1 position in 5FU by reaction of the sodium salt of 5FU with the corresponding benzyl halide in *N,N*-dimethylformamide (DMF) at 60°C for 24 h. After repeated recrystalization from ethanol and water, five kinds of benzyl derivatives of 5FU were obtained (Yamashita et al., 1989). Their molecular structures and designations are depicted in Fig. 2. The purity of products determined by HPLC was more than 99%.

The aqueous solubility and the partition coefficient of each derivative including 5FU and tegafur were determined according to the method of Buur and Bundgard (1984). The solubility was measured at 20°C by adding an excess amount of the compound in pH 7.4 phosphate-NaCl isotonic buffer (isotonic buffer). The partition coefficient of each derivative was determined in an *n*-octanol/isotonic buffer system at 20°C.

The in vitro protein binding study of 5FU derivatives was performed at 37°C using the equilibrium dialysis method described in the previous paper (Yamashita et al., 1989). Each derivative dissolved in isotonic buffer (0.1 mM) was dialysed against rat plasma or 4.5% human serum albumin (HSA) solution for 4 h employing a cellulose dialysis tube (Spectrapor, Mol. Wt cut off 1000, Spectrum Medical Industries, Inc., LA, U.S.A.) with a dialysis chamber (Abe Science, Chiba, Japan).

Male Wistar rats (200–250 g body weight) were used to determine the pharmacokinetic parameters of derivatives under anesthetized conditions. The femoral artery of rats was cannulated with a polyethylene tube for the collection of blood samples. The test compound dissolved in isotonic buffer was then administered into the femoral vein and the blood was taken at appropriate intervals from the femoral artery. The dose administered of each compound was 125 μg/rat except 5FUMCB (75 μg/rat) and 5FUDCB (30 μg/rat), because of the low water solubility of these two compounds. Blood samples were centrifuged for 5 min and the supernatant was mixed with twice its volume of methanol. The mixed solution was vortexed and centrifuged for 5 min to remove proteins and the concentration of derivatives in the supernatant was determined. From the time course of the plasma concentration after intraveneous (i.v.) injection, the pharmacokinetic parameters were calculated according to a nonlinear least-squares fitting method using the MULTI program developed by Yamaoka et al. (1981).

The concentrations of all derivatives including 5FU and tegafur were determined using an HPLC system (LC-6A, Shimadzu Co., Japan) equipped with a variable-wavelength UV detector (SPD-6A, Shimadzu Co., Japan). The HPLC conditions used here were as follows: column, Cosmosil $5C_{18}$ (15 cm × 4.6 mm i.d., Nakarai Tesque Co., Japan); mobile phase, mixture of methanol and pH 5.0

Table 1
Physicochemical property and protein binding of 5FU derivatives

Abbreviation	Mol. Wt	Solubility (mg/ml)	PC [a]	Protein binding (bound %) [b]	
				Rat plasma	4.5 % HSA
5FU	130	13.0	0.106	~0	~0
Tegaful	200	19.5	0.331	22.9±0.4	–
5FUB	220	0.494	7.841	52.7±1.5	59.6±0.1
5FUMOB	250	0.444	11.02	74.3±0.8	85.2±1.2
5FUMB	234	0.282	31.38	95.9±0.8	91.7±0.4
5FUMCB	255	0.0817	43.37	97.3±0.1	94.8±0.1
5FUDCB	289	0.0329	91.53	97.2±0.1	95.4±0.5

[a] Partition coefficient measured in *n*-octanol/buffer system.
[b] Bound % of each derivative was expressed as the mean ± S.E. of three experiments.

phosphate buffer (5:95 for 5FU, 10:90 for tegafur, 40:60 for 5FUB and 5FUMOB, 45:55 for 5FUMB, 55:45 for 5FUMCB and 5FUDCB); wavelength, 262 nm.

All derivatives synthesized remained stable in buffer solution or in rat plasma over the 24 h incubation period. Also, no free 5FU appeared in the blood after i.v. injection of any derivatives (data not shown), suggesting that they were not hydrolyzed to 5FU even under in vivo condition.

The attachment of a benzene moiety to 5FU reduced the water solubility with enhancing the partition coefficient into *n*-octanol as depicted in Table 1. Substituent groups at the *para* position of the benzene ring enhanced lipophilicity in the order of -Cl > $-CH_3$ > $-OCH_3$ > -H. Addition of another Cl at the *ortho* position (5FUDCB) doubled the partition coefficient compared to the monochloro derivative (5FUMCB). The clinically used prodrug of 5FU, tegafur, which utilizes the sugar molecule as the substituent, showed similar solubility with a slightly higher partition coefficient compared to 5FU.

Protein binding of 5FU derivatives in rat plasma or buffered 4.5% HSA solution was determined in vitro. As expected from the high water solubility, 5FU has no affinity to the serum protein. The percentage of the derivatives bound increased in accordance with the order of their lipophilicity in both solutions (Table 1). In Fig. 3, the binding potency (as unbound drug fraction) of the 5FU derivatives is plotted vs their lipophilicity (as the logarithm of the partition coefficient). In both solutions, almost the same correlation was observed between these two parameters. In this series of derivatives, three compounds, 5FUMB, 5FUMCB and 5FUDCB, showed high affinity (more than 90% bound) to serum protein.

Many reports have demonstrated the existence of specific binding sites on the albumin molecule. According to the classification of Hansen (1981), in which six different sites were presented, most drugs bind mainly to region 6 on the albumin. However, it is also recognized that the specificity of each binding site is not strongly restricted, and

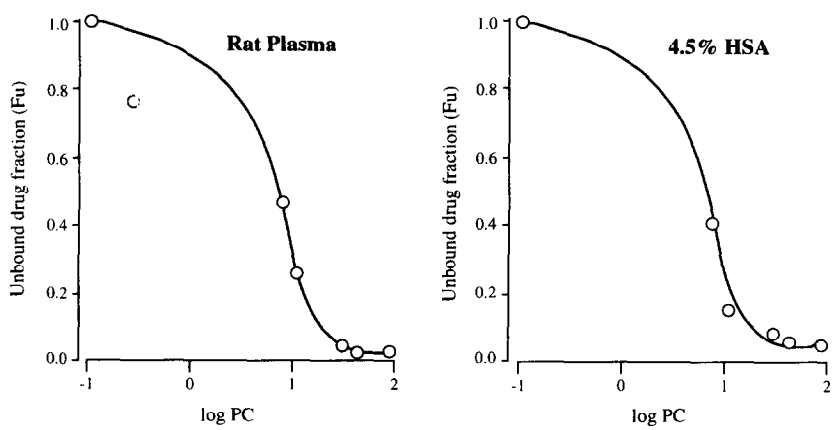

Fig. 3. Relationship between unbound fraction and lipophilicity of 5FU derivatives. Unbound fraction of each derivative was calculated from in vitro equilibrium dialysis experiment using rat plasma or 4.5% human serum albumin (HSA).

usually serum albumin readily forms complexes with lipophilic substances through hydrophobic binding. Therefore, we started this project from the synthesis of lipophilic derivatives of 5FU using a benzene moiety. Although the predominant binding site of 5FU derivatives was not clear, it is obvious from Fig. 3 that, among compounds having similar structures, lipophilicity is the most important factor in defining the protein binding potency. Laznicek et al. (1987) reported similar results concerning the plasma protein binding-lipophilicity relationship among 11 organic acids (benzoic and phenylacetic acid derivatives). This indicates a simple and easy way for the design and synthesis of a prodrug having a high affinity to albumin. Binding to other serum proteins such as α-acid glycoprotein should be less important than that to albumin, since (1) the amount of albumin in the serum is much greater than that of other proteins and (2) no significant differences were observed between the percent binding in rat plasma and that in HSA solution. Assuming that the binding affinity of derivatives to rat serum albumin and HSA is the same, only 2–3% of the total binding of 5FUMB, 5FUMCB and 5FUDCB might correspond to binding to other proteins in rat plasma.

The pharmacokinetic parameters of 5FU derivatives, apparent distribution volume (V_d), total body clearance (CL_{tot}) and biological half-life ($t_{1/2}$), determined after i.v. injection are summarized in Table 2. As is well known, 5FU was rapidly eliminated from the body with a half-life of about 10 min. 5FUMCB and 5FUDCB showed much longer half-lives compared to the other compounds, due to their low values of CL_{tot}. 5FUMB and 5FUMOB had fairly short half-lives despite their high protein binding.

V_d of derivatives was diminished with decrease in their unbound drug fractions. As a basic rule in biopharmacy, only drugs that exist free from serum protein binding are considered to distribute to organs. The linear correlation between the unbound fraction and V_d of each derivative shown in Fig. 4 clearly reflects this rule, i.e., the serum protein acts as the retainer of drug within the blood vessel. This is a very important advantage of our system, since drugs distributed to the normal organ usually induce an adverse reaction, such as a myelosuppresive action of 5FU. Assuming that the binding of derivatives to the albumin in the plasma and in the interstitual fluid is the same, strongly bound derivatives (5FUB, 5FUMCB and 5FUMDB) were considered to distribute only in the extracellular fluid (plasma and interstitual fluid), and not into the cells of organs, as their V_d (about 200 ml/kg) coincided well with the total volume of the extracellular fluid which can be roughly estimated as 20% of the body weight. In addition, the V_d values of the above three derivatives were compatible with that of

Table 2
Pharmacokinetic parameters of 5FU derivatives after intraveneous injection to rat

Abbreviation	F_u [a]	V_d (ml/kg)	CL_{tot} (ml/h)	$t_{1/2}$ (h)
5FU	~1	586.5±82.3	807.7 ±43.4	0.17±0.01
Tegaful	0.771	421.8±15.6	12.29 ± 1.29	5.38±0.23
5FUB	0.473	350.8±18.4	3.44 ± 0.07	14.9 ±0.50
5FUMOB	0.257	218.4±13.0	8.56 ± 0.51	3.88±0.04
5FUMB	0.041	194.7±10.2	6.28 ± 0.31	4.69±0.46
5FUMCB	0.027	197.1± 2.3	1.02 ± 0.08	27.8 ±2.1
5FUDCB	0.028	195.0± 1.9	0.668± 0.066	47.0 ±5.1

Pharmacokinetic parameters of each derivative were expressed as the mean±S.E. of at least three experiments.
[a] Free fraction of each derivative in rat plasma calculated from in vitro experiment.

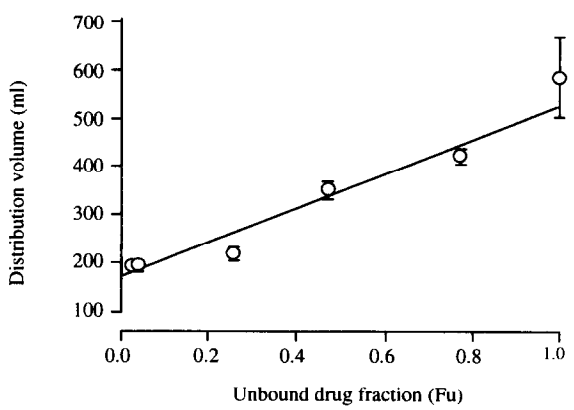

Fig. 4. Relationship between distribution volume and unbound fraction of 5FU derivatives. Unbound fraction of each derivative was calculated from in vitro equilibrium dialysis experiment using rat plasma. Distribution volume was expressed as the mean with S.E. of at least three experiments.

Table 3
Unbound clearance of 5FU derivatives

Abbreviation	Unbound clearance (ml/h)
5FU	807.7
Tegaful	15.94
5FUB	7.27
5FUMOB	33.41
5FUMB	153.2
5FUMCB	37.78
5FUDCB	23.86

warfarin (162.4 ml/kg) measured by the same method in rats.

On the other hand, the half-life of derivatives cannot be correlated simply to their protein binding affinity. 5FU is rapidly metabolized in the liver mainly at the N1 position of its pyrimidine ring. In all derivatives synthesized, this metabolic step was completely blocked by attaching the benzene moiety at the N1 position through an N-C bond. This should be the first reason for the increased half-life in all derivatives. The next factor affecting the half-life of derivatives would be the in vivo stability of a substituted group itself. Unbound clearance, calculated simply as CL_{tot}/F_u (Table 3), is convenient to explain this idea. Among the derivatives, 5FUMB has the highest value of unbound clearance, providing the reason for its short half-life despite its high protein binding affinity. A methylene group on the benzene ring has often been referred to as 'active methylene' and is known to be readily oxidized in the liver to a carbonyl group. Chlorpropamide, a kind of oral hypoglycemic drug, is an analog of tolbutamide in which the methylene group on the benzene ring of tolbutamide has been substituted by chloride. This molecular modification resulted in a longer half-life of chlorpropamide (about 33 h in human) compared to tolbutamide (about 6 h) (Jackson and Bressler, 1981). The stabilizing effect of halogen substitution (obstructive halogenation) was utilized here in 5FUMCB and 5FUDCB and successfully prolonged the half-life of both derivatives.

It is also obvious from Tables 2 and 3 that high protein binding, and thus the low unbound fraction level in the plasma, contributed to the longer half-lives of 5FUMCB and 5FUMDB as well as the low unbound clearance. Rowland and coworkers (Hall and Rowland, 1984; Rowland, 1984; Rowland et al., 1984) investigated the effect of protein binding on the hepatic and renal clearance of several drugs and demonstrated that the clearance (organ clearance) of drugs with low unbound clearance depends on plasma binding. However, if the unbound clearance is high, the elimination becomes blood flow-limited and relatively insensitive to a change in binding.

In conclusion, it has been revealed that the derivatives which possess both high protein binding potency and low unbound clearance should be useful for our system to achieve a prolonged half-life with reduced distribution to organs. Based on the results described above, we are now conducting a subsequent experiment where prodrugs of 5FU, which are hydrolyzed at the proper rate and gradually release 5FU into the blood, are being synthesized.

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