5-FLUOROURACIL DERIVATIVES WITH SERUM PROTEIN BINDING POTENCIES

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To develop an optimal delivery system for 5-fluorouracil (5FU) using serum protein as a drug carrier, a series of its benzyl derivatives was synthesized. Then their binding to the serum protein was investigated by equilibrium dialysis. The benzyl derivatives of 5FU were strongly bound to rat plasma protein or human serum albumin. The bound percentage increased with increasing hydrophobicity. It was suggested that the benzyl derivative of 5FU existed in the blood as a complex with serum albumin and circulated for a long time as a polymeric drug does.

KEYWORDS 5-fluorouracil; protein binding; albumin; drug delivery system

5-Fluorouracil (5FU) has a high anti-tumor activity and is widely used in cancer chemotherapy. However, since it is quickly eliminated from the body, mainly by metabolism in the liver,[1] we need to develop a drug delivery system (DDS) for its clinical use. Much research concerning a DDS for 5FU has been reported, especially that related to synthetic or natural polymers in which 5FU is grafted onto the polymer chain.[2-4] This type of drug is referred to as a polymeric drug. However, there are many problems in their clinical use: the polymeric drug may induce antigen toxicity,[5] it may be trapped by the reticuloendothelial system and accumulated in the liver or spleen,[6,7] or the way of administration may be restricted.[8] All the problems are caused by introducing the high molecular weight compound into the body.

In this study, we have developed a new drug delivery system for 5FU using serum protein such as albumin. Our strategy is as follows. A prodrug of 5FU with a high binding ability to serum albumin are synthesized. When administered, it would form a complex with the albumin in the blood, and circulate in the body as a polymeric drug does. Finally, the 5FU would be released from the complex at an appropriate rate by cleavage of the prodrug. This system should be thought to have some advantages in comparison with the polymeric systems,[9,10] since the synthesized prodrug is still a low-molecular-weight compound.

For the basic method using serum albumin as a drug carrier, poorly hydrolyzable derivatives were synthesized in the first stage. According to the procedure shown in Fig. 1, a benzene moiety was attached to 5FU by the reaction of the sodium salt of 5FU with the corresponding benzyl halide in N,N-dimethylformamide at 60°C for 24 h. After repeated recrystalization from ethanol and water, five kinds of benzyl derivatives of 5FU[11] were obtained. These were used for the following experiments. To determine the poorly hydrolyzable property of the benzyl derivatives, the hydrolysis reaction of Tegaful[12], 5FUB and 5FUMCB were performed in isotonic phosphate buffer (pH 7.4) at 37°C as shown in Fig. 2. In contrast to Tegaful, the benzyl derivatives suffered substantially no hydrolysis. Therefore, the cleavage to 5FU from the benzyl derivatives can be ignored at least in the isotonic phosphate buffer within 48 h. From the partition coefficients (Table I), the hydrophobicity of the compounds used here are in the following order; 5FUDCB > 5FUMCB > 5FUMB > 5FUMOB > 5FUB > Tegaful > 5FU. Their protein binding properties were determined by equilibrium dialysis using rat plasma or buffered 4.5 % human serum albumin (HSA) solution (Table I). It was found that the bound percentage of the derivatives or the 5FU increased in the order of their hydrophobicity as reported by Laznicek et al..[13] This protein binding phenomenon of the benzyl derivatives of 5FU were also found spectroscopically (data not shown). From these facts, it appears that the high protein binding ability can be induced in 5FU by attaching the benzene moiety, especially the chlorosubstituted one. This suggestion seems to be very important in thinking about more improved prodrug in further developing our system. It was also apparent that, among the five derivatives reported here, 5FUMCB, which has a monochlorobenzene moiety,

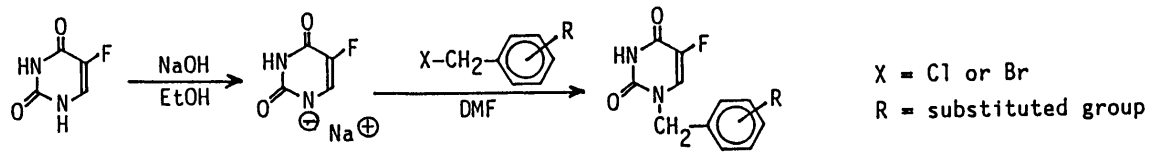

Fig. 1. Synthetic Procedure for the Benzyl Derivatives of 5FU[11]

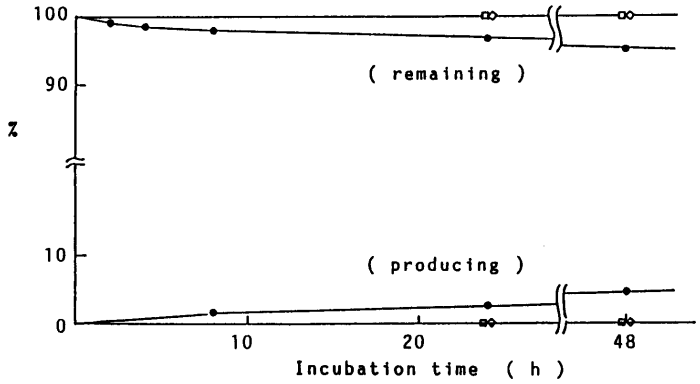

Fig. 2. Conversion of the Benzyl Derivatives of 5FU and Tegaful into 5FU
The hydrolysis reaction was effected with isotonic phosphate buffer (pH 7.4) at 37°C. The remaining intact 5FU derivatives and the 5FU produced were detected by HPLC (see legend of Table I); (●) Tegaful, (□) 5FUB, (◇) 5FUMCB.

was optimal as the model compound for our DDS system because of its solubility as well as its protein binding potency.

5FU, Tegaful and 5FUMCB were administered intraveneouly to rats. Their half-life ($T_{1/2}$) and the apparent distribution volume (Vd) were calculated from the time-course of their plasma concentration. The concentrations of these compounds were determined by HPLC (see legend of Table I) after treating the rat plasma with methanol. The detailed experimental methods as well as the results will be reported elsewhere. The $T_{1/2}$ of 5FU, Tegaful and 5FUMCB in the plasma were 0.17 h, 5.38 h and 27.8 h, respectively. Thus 5FUMCB remains in the blood stream much longer than 5FU or Tegaful. From this data and the high protein-bound percentage in vitro, it appears that 5FUMCB is also bound to serum protein (almost albumin) in vivo and is circulated in the body as a complex with albumin like a polymeric drug. Further, the Vd of 5FUMCB was less than half that of 5FU or Tegaful. This may also be explained by assuming that 5FUMCB exists predominantly as a complex with albumin in the plasma.

From the results obtained in this paper, we obtained the basic information for using the serum protein as a drug carrier. That is, we can improve the protein binding ability by attaching a benzene or a chlorosubstituted benzene moiety to 5FU. These 5FU derivatives may bind to serum protein in vivo as well as in vitro, and may be circulated in the body like a polymeric drug. Investigations to determine both their distribution in the body and their metabolism in the liver or blood as well as clinical testing are currently under way.

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Table I. Aqueous Solubility, Partition Coefficients and Serum Protein Binding of 5FU and 5FU Derivatives

Compound	Aqueous solubibity (mg/ml)	Partition coefficient	Protein binding (bound %)	
			Rat plasma	4.5% HSA
5FU	13.0	0.106	0	-
Tegaful	19.5	0.331	22.9 ± 0.38	-
5FUB	0.494	7.841	52.7 ± 1.46	59.6 ± 0.14
5FUMOB	0.444	11.02	74.3 ± 0.82	85.2 ± 1.15
5FUMB	0.282	31.38	95.9 ± 0.79	91.7 ± 0.42
5FUMCB	0.0817	43.37	97.3 ± 0.12	94.8 ± 0.03
5FUDCB	0.0329	91.53	97.2 ± 0.13	95.4 ± 0.46

The aqueous solubility of the compound was determined at 22°C by adding excess amounts of phosphate-NaCl isotonic buffer (isotonic buffer) at pH 7.4. The apparent partition coefficient was determined in an octanol-isotonic buffer system. Detailed methods for measuring both parameters were described by Buur and Bundgaard.[14] The protein binding study of the 5FU and 5FU derivatives in vitro was performed by equilibrium dialysis at 37°C using the procedure described by Sugiyama.[13] Rat plasma or buffered 4.5 % HSA solution containing 0.1 mM of the compound was dialyzed against an equal volume of isotonic buffer for 4 h. The concentration of 5FU and its derivatives on both sides were determined using reversed phase HPLC (LC-6A, Shimadzu Co., Japan) equipped with a UV detector (SPD-6A, Shimadzu Co., Japan). The analytical conditions were as follows: Column, Cosmosil $5C_8$ (15 cm x 4.6 mm I.D., Nakarai Tesque Co., Japan); Mobile phase, mixture of methanol-0.05M acetate buffer (pH 5.0); Wave length of the detector, 262nm.

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11) The abbreviations of the benzyl derivatives of 5FU, their analytical data and mp are as follows: 5FUB, 1-benzyl-5-fluorouracil, Calcd. for $C_{11}H_9O_2N_2F$, C 60.00%, H 4.12%, N 12.72%, Found C 60.27%, H 4.23%, N 12.71%, mp=175.3°C; 5FUMOB, 1-(4-methoxybenzyl)-5-fluorouracil, Calcd. for $C_{12}H_{11}O_3N_2F$, C 57.60%, H 4.43%, N 11.20%, Found C 57.35%, H 4.43%, N 10.96%, mp=166.1°C; 5FUMB, 1-(4-methylbenzyl)-5-fluorouracil, Calcd. for $C_{12}H_{11}O_2N_2F$, C 61.54%, H 4.74%, N 11.96%, Found C 61.59%, H 4.81%, N 11.79%, mp=183.7°C; 5FUMCB, 1-(4-chlorobenzyl)-5-fluorouracil, Calcd. for $C_{11}H_8O_2N_2ClF$, C 51.88%, H 3.17%, N 11.00%, Found C 51.97%, H 3.43%, N 10.97%, mp=189.4°C; 5FUDCB, 1-(2,4-dichlorobenzyl)-5-fluorouracil, Calcd. for $C_{11}H_7O_2N_2Cl_2F$, C 45.70%, H 2.44%, N 9.69%, Found C 45.75%, H 2.66%, N 9.64%, mp=190.4°C.
12) 1-(2-Tetrahydrofuranyl)-5-fluorouracil, gift from Mitsui Co. Ltd. (Tokyo, Japan).
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