Journal of Chromatography, 526 (1990) 562–568
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5122

Note

Binding of lidocaine to plasma proteins resolved by high-performance liquid chromatography

JUNKO ABE, AKIRA ASADA and MITSUGU FUJIMORI

Department of Anesthesiology, Osaka City University Medical School, 1-4-54 Asahimachi, Abenoku, Osaka 545 (Japan)

and

SUSUMU IMAOKA and YOSHIHIKO FUNAE*

Laboratory of Chemistry, Osaka City University Medical School, 1-4-54 Asahimachi, Abenoku, Osaka 545 (Japan)

(First received September 1st, 1989; revised manuscript received November 6th, 1989)

Binding of drugs to protein influences drug pharmacokinetics and causes pharmacological effects. Most acidic drugs bind to albumin in plasma [1,2]. Basic drugs mainly bind to α_1-acid glycoprotein (AAG) [3–5]. Drugs may also bind to plasma proteins other than AAG and human serum albumin (HSA).

To investigate the protein binding of drugs, equilibrium dialysis, ultrafiltration and gel permeation chromatography have been used [6–8]. These methods measure total protein binding or binding to a single kind of protein, not the binding to a particular protein in a protein mixture. High-performance liquid chromatography (HPLC) has been used for the study of protein binding of drugs [9–12] and for the resolution and the purification of proteins [13–18].

In this study, we applied HPLC to the study of drug binding to plasma proteins and tried to elucidate what proteins in plasma bound to lidocaine.

EXPERIMENTAL

Materials

HSA and human AAG were purchased from Sigma (St. Louis, MO, U.S.A.). Lidocaine hydrochloride was donated by Fujisawa Pharmaceutical (Osaka, Japan). Fresh human plasma was obtained from healthy adult men, centrifuged and dialyzed against saline.

0378-4347/90/$03 50

HSA was removed by use of Blue Sepharose CL-6B (Pharmacia, Uppsala, Sweden). Human plasma was diluted twice with 0.05 *M* Tris–HCl, pH 7.0, containing 0.1 *M* KCl, and was passed through a Blue Sepharose CL-6B column equilibrated with the same buffer. The pass-through fraction was collected, concentrated and dialyzed against saline. Protein standards for molecular mass calibration were obtained from Boehringer (Mannheim, F.R.G.).

Protein binding and HPLC

Proteins such as plasma, HSA or AAG were dissolved in 0.1 *M* phosphate buffer, pH 7.4 (HPLC buffer), and incubated with lidocaine dissolved in HPLC buffer for 30 min at 37 °C. The incubation mixture (500 μl) was injected onto an HPLC apparatus equipped with a gel-permeation column (TSK-G3000SW, 60 cm × 7.5 mm I.D., Tosoh, Tokyo, Japan). HPLC was performed at a flow-rate of 0.5 ml/min with HPLC buffer containing lidocaine (5 μg/ml) at room temperature. The effluent was collected in 0.5-ml fractions until all proteins were eluted. Protein, AAG and lidocaine concentrations in each fraction were assayed and plotted. The peak of lidocaine indicates bound lidocaine.

Assay methods

Lidocaine was measured by fluorescence polarization immunoassay (TDX systems, Abbott Diagnostics, Chicago, IL, U.S.A.) [16]. AAG was assayed on single radial immunodiffusion plates, such as NOR-Partigen-AAG (Hoechst, Frankfurt, F.R.G.). The protein concentration was measured with a protein assay kit (Bio-Rad, Richmond, CA, U.S.A.). The amount of bound lidocaine in each tube was calculated as follows: amount bound lidocaine (μg) = 0.5 ml × [concentration lidocaine (μg/ml) in each tube − concentration lidocaine (μg/ml) in each tube in the experiment without proteins]. The amount of bound lidocaine in fraction A was calculated as the total amount of bound lidocaine eluted at 22–24 min.

RESULTS

In the HPLC method for the drug-binding study, the binding ratio of each protein depends on the drug concentration in the eluent [10–12]. We used a fixed concentration of lidocaine in the mobile phase (5 μg/ml) only to compare the binding ratios of lidocaine to some plasma proteins. The HPLC column was equilibrated with buffer containing 5 μg/ml lidocaine overnight before injection of the incubation mixture of lidocaine and protein. The elution profile of bound lidocaine synchronized with that of AAG (Fig. 1). Almost all the lidocaine injected was recovered. When an incubation mixture of HSA and lidocaine was injected, a peak of bound lidocaine hardly appeared at the retention time of HSA, indicating that the binding capacity of lidocaine to HSA was low. The retention time of HSA on gel-permeation HPLC (36.8 min) was very

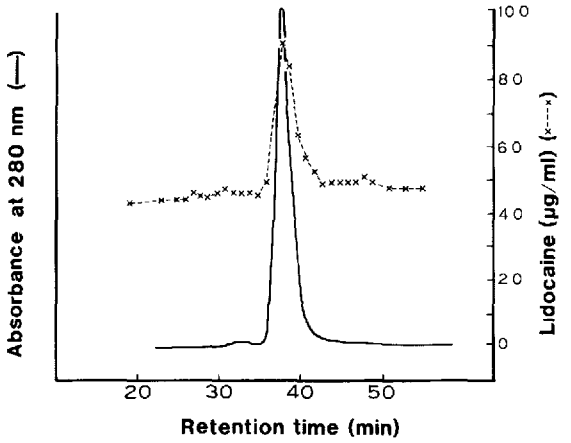

Fig. 1. HPLC profiles of α_1-acid glycoprotein and lidocaine. A mixture (500 μl) of α_1-acid glycoprotein (2.5 mg) and lidocaine (25 μg) was incubated for 30 min at 37 °C and then injected onto an HPLC apparatus with a TSK-G3000SW column. HPLC was performed at a flow-rate of 0.5 ml/min with 0.1 *M* phosphate buffer, pH 7.4, containing lidocaine (5 μg/ml) at room temperature. The lidocaine concentration in the eluted fraction was assayed by TDX systems.

close to that of AAG (37.4 min). With these HPLC conditions, the retention time of the standard proteins was as follows: cytochrome C (MW 12 500), 49.4 min; chymotrypsinogen A (MW 25 000), 46.8 min; ovalbumin (MW 45 000), 39.5 min; bovine serum albumin (MW 68 000), 36.3 min; aldolase (MW 158 000), 33.8 min; catalase (MW 240 000), 28.6 min; ferritin (MW 450 000), 24.4 min. It was difficult to resolve HSA and AAG as separated peaks by HPLC with a gel-permeation column.

When AAG (2.5 mg) was mixed with various amounts of lidocaine (6.25, 12.5, 25 or 50 μg), the amount of lidocaine bound to AAG was constant (8.2 ± 0.63 μg). From the value of lidocaine of 8.2 μg ($3.5 \cdot 10^{-8}$ mol), the molar ratio of lidocaine bound to AAG was calculated to be 0.8. In the same way, HSA was incubated with lidocaine (6.25, 12.5, 25 or 50 μg). The amount of lidocaine bound to HSA (4 mg) was 1.3 ± 0.37 μg ($0.55 \cdot 10^{-8}$ mol). The molar ratio of bound lidocaine to HSA was calculated to be 0.16. From these results, the binding capacity of AAG to lidocaine was found to be higher than that of HSA.

When 0.5, 1.25 or 2.5 mg of AAG was incubated with 10 μg of lidocaine, the amount of lidocaine bound to AAG was 1.8, 4.3 or 8.8 μg, respectively (Table I). The coefficient of correlation between AAG and bound lidocaine was 0.9989. When AAG incubated with lidocaine in the presence of 5 mg of HSA was injected, bound lidocaine was 1.9 μg with 0.5 mg of AAG, 5.0 μg with 1.25 mg of AAG and 9.7 μg with 2.5 mg of AAG, which increase was proportional to the amount of AAG. When 5 mg of HSA was incubated alone, 1.5 μg of lidocaine were bound to HSA.

Lidocaine binding to plasma proteins was also studied. On the chromato-

TABLE I

EFFECT OF HUMAN SERUM ALBUMIN (HSA) ON THE LIDOCAINE BOUND TO α_1-ACID GLYCOPROTEIN (AAG)

The different amounts of AAG were incubated with lidocaine (10 μg) in the absence or presence of HSA (5 mg).

AAG (mg)	Lidocaine bound (μg)	
	−HSA	+HSA
0.5	1.8	1.9
1.25	4.3	5.0
2.5	8.8	9.7

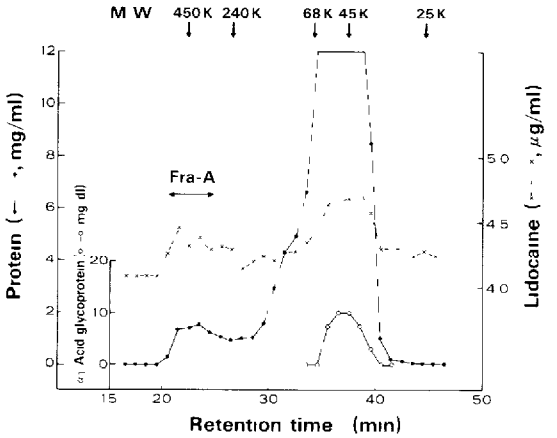

Fig 2. HPLC profile of plasma incubated with lidocaine. Plasma (500 μl) incubated with lidocaine (50 μg) was injected onto an HPLC apparatus with a TSK-G3000SW column. HPLC was performed at a flow-rate 0.5 ml/min with 0.1 *M* phosphate buffer, pH 7.4, containing lidocaine (5 μg/ml) Molecular mass was calibrated with protein standard markers: ferritin (450 000), catalase (240 000), bovine serum albumin (68 000), ovalbumin (45 000), chymotrypsinogen A (25 000). Fraction A (Fra-A) is the eluate of 22–24 min.

gram of plasma, AAG was not clearly separated from HSA. The major peak of bound lidocaine was eluted with the fraction in which AAG and HSA were eluted. Another peak of bound lidocaine appeared at a retention time of 23 min (fraction A in Fig. 2). To confirm that lidocaine did bind to fraction A protein, the binding of lidocaine to plasma from which albumin had been removed by Blue Sepharose chromatography was studied. This was done because a larger amount of fraction A can be applied on a HPLC column. The elution profile of bound lidocaine with plasma treated with Blue Sepharose is shown in Fig. 3. The amount of bound lidocaine in fraction A and the AAG fraction was 0.72 and 1.10 μg, respectively. The molar ratio of lidocaine bound to AAG was 0.96.

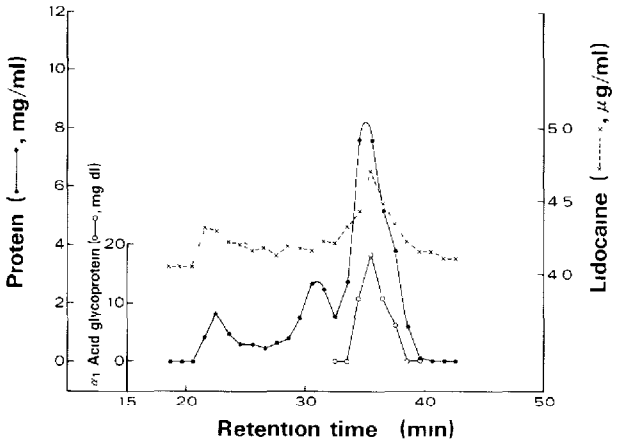

Fig. 3. HPLC profile of plasma passed through the Blue Sepharose column and incubated with lidocaine Plasma (500 μl) containing 21 mg of protein was incubated with lidocaine (50 μg) and injected onto an HPLC apparatus with a TSK-G3000SW column. HPLC was performed at a flow-rate of 0 5 ml/min with 0.1 *M* phosphate buffer, pH 7.4, containing lidocaine (5 μg/ml).

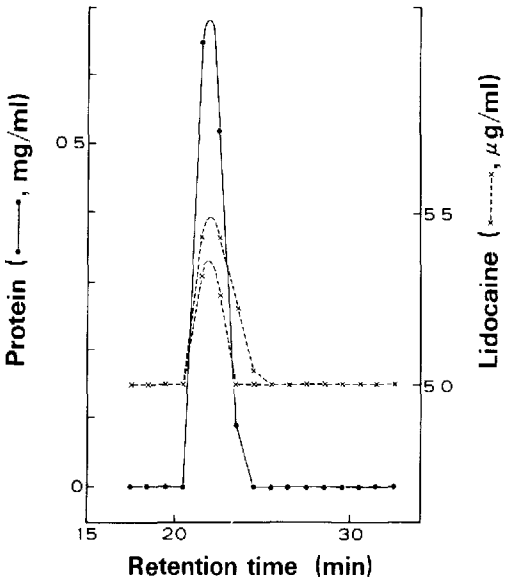

Fig. 4. HPLC profile of fraction A incubated with lidocaine. Fraction A (15 mg of protein per ml) shown in Fig 2 was obtained from plasma passed through the Blue Sepharose column. Fraction A (200 or 400 μl) was incubated with lidocaine (50 μg) and injected onto an HPLC apparatus with a TSK-G3000SW column. HPLC was performed at a flow-rate of 0.5 ml/min with 0.1 *M* phosphate buffer, pH 7.4, containing lidocaine (5 μg/ml). The upper dotted line shows lidocaine when 400 μl of fraction A were incubated with lidocaine (50 μg). The lower dotted line shows lidocaine when 200 μl of fraction A were incubated with lidocaine The solid line shows the protein concentration when 400 μl of fraction A were incubated with lidocaine (50 μg) and injected onto an HPLC apparatus.

Fraction A eluted by HPLC equipped with a gel-permeation column was collected and concentrated. Fig. 4 shows the HPLC profile of lidocaine binding to different amounts of fraction A. Lidocaine bound to 0.2 and 0.4 ml of fraction A was 0.31 and 0.56 μg, respectively. Lidocaine bound to 0.4 ml of fraction A was 1.8 times greater than that bound to 0.2 ml of fraction A, indicating that lidocaine bound to protein in fraction A varied linearly with the amount of protein present.

DISCUSSION

Lidocaine–protein binding in a mixture of AAG and HSA was dependent on the AAG concentration, not the HSA concentration. The normal mean concentration of AAG in plasma is 66 mg/dl, which is much lower than that of HSA. The AAG concentration in the plasma sometimes increases several-fold in disease. However, the plasma HSA concentration tends to decrease in many diseases, and its changes are much smaller than those of AAG [17,18]. Therefore, AAG is more important than HSA for the binding of lidocaine in the plasma.

When a mixture of AAG and HSA or plasma was injected into an HPLC apparatus equipped with a gel-permeation column, AAG and HSA could not be clearly separated, although the molecular mass of HSA is 25 000 higher than that of AAG. However, the peak of bound lidocaine was eluted at the later part of the peak of HSA and AAG. The molar ratio of bound lidocaine to AAG was higher than that to HSA. These results suggested that lidocaine binds more to AAG than HSA in the large peak fraction containing HSA and AAG during HPLC of plasma. The bound lidocaine was eluted at another position, that of fraction A, which seemed to be a lipoprotein fraction. Quinidine and imipramine have been shown to bind to lipoproteins [20,21]. In plasma passed through a Blue Sepharose column the amount of lidocaine bound to the protein of fraction A was 65% of that bound to AAG (Fig. 3). Calculated from the results of Fig. 4 the ratio of bound lidocaine to fraction A (μg/mg) was 0.96 and that to AAG was 4.6. The molecular mass of proteins in fraction A is much higher than that of AAG. Therefore, the binding capacity of lidocaine to the proteins in fraction A seems to be high.

ACKNOWLEDGEMENT

The authors thank Miss Makiko Ohki for assistance in the preparation of the manuscript.

REFERENCES

1 H.M. Solomon, J.J. Schrogie and D Williams, Biochem. Pharmacol., 17 (1968) 143

2 R. Spector, D T. Korkin and A V. Lorenzo, J. Pharm. Pharmacol., 24 (1972) 786.
3 K.M. Piafsky and O Borgå, Clin. Pharmacol. Ther., 22 (1977) 545.
4 F M Belpaire, R.A. Braeckman and M.G. Bogaert, Biochem. Pharmacol., 33 (1984) 2065
5 M.L. Kornguth, L.G. Hutchins and B.S. Eichelman, Biochem. Pharmacol., 30 (1981) 2435.
6 G.T. Tucker, R N Boyes, P.O. Bridenbaugh and D.C Moore, Anesthesiology, 33 (1970) 287.
7 J.T.F. Liao and F.W Oehme, Vet. Hum. Toxicol., 22 (1980) 151.
8 J. Bonde, N M Jensen, P. Burgaard, H.R. Augelo, N. Graudal, J.P. Kampmann and L.E. Pedersen, Pharmacol. Toxicol , 60 (1987) 151.
9 J.C K Loo, N Jordan and A.Ho Ngoc, J. Chromatogr., 305 (1984) 194.
10 S.F. Sun, S W. Kuo and R.A. Nash, J. Chromatogr., 252 (1984) 377.
11 B Sebille, N. Thuaud and J.P Tillement, J. Chromatogr., 204 (1981) 285.
12 L. Soltes, F. Bree, B. Sebille, J.P Tillement, M Durisova and T Trnovec, Biochem. Pharmacol., 34 (1985) 4331.
13 A.N Kotake and Y. Funae, Proc. Natl. Acad. Sci. U.S.A., 77 (1980) 6473.
14 Y Funae, R. Seo and S. Morimoto, Jpn. J. Clin. Chem., 11 (1982) 342.
15 Y. Funae and S. Imaoka, Biochim. Biophys. Acta, 842 (1985) 119
16 M.E. Jolley, S.D. Stroupe, K.S Schwenzer, C.J. Wana, M Lu-Steffes, H.D. Hill, S.R. Popelka, J.T. Holen and D.M. Kelso, Clin Chem., 27 (1981) 1575.
17 D.I. Freilich and E.G.V Giardina, Clin Pharmacol. Ther., 35 (1984) 670.
18 D.J Edwards, D. Lalka, F. Cerra and R.L. Slaughter, Clin. Pharmacol. Ther , 312 (1982) 62.
19 P.R. Jackson, G.T. Tucker and H.F. Woods, Clin Pharmacol Ther., 32 (1982) 295
20 A. Danon and Z. Chen, Clin. Pharmacol , 25 (1979) 316.
21 O G. Nilsen and S Jacobsen, Biochem Pharmacol., 24 (1975) 995.