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BINDING OF MEBEVERINE-HCl AND DROTAVERINE-HCl TO PLASMA PROTEINS FROM PATIENTS WITH RENAL FAILURE

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ABSTRACT

Binding of mebeverine-HCl and drotaverine-HCl to plasma proteins taken from healthy volunteers and from patients with renal failure was investigated using equilibrium dialysis technique. The concentration of total protein and albumin were determined. The study showed that the mean plasma albumin concentration was 4.3 gm% in the case of normal volunteers, and was 2.8 % in the case of patients with renal failure. On the other hand, the mean total protein concentration in plasma of normal volunteers was 6.5 %, and was 5.6 % in the case of plasma proteins of patients with renal failure. Binding study showed that the percentage bound of mebeverine-HCl was 83.51 in normal volunteers and 74.32 in patients with renal failure. The percentage bound of drotaverine-HCl was 72.45 in normal volunteers and 63.21% in patients with renal failure. This must be taken into consideration during the adjustment of the dose of both drugs.

INTRODUCTION

Mebevrine-HCl and drotaverine-HCl are widely used as antispasmodic drugs. The active form of a drug is the form which diffuse to its receptors i.e. it is the free fraction. Therefore, the pharmacological action of a compound depends on the concentration of the free form. Some pathological states quantitatively and qualitatively disturb serum proteins, thereby altering the binding of drugs[1]. Several studies have reported a significant decrease in the binding of different drugs in plasma from patients with poor renal function. [2,3] Plasma albumin binding of drugs may decrease in patients with renal failure due to hypoalbuminemia in severe nephrotic syndrome as observed in digitoxin.[4]

The binding of mebeverine-HCl and drotaverine HCl to human plasma proteins in healthy and renal diseased volunteers was investigated adopting the equilibrium dialysis technique at 37°C[5].

EXPERIMENTAL

Materials and reagents:

Blood samples from healthy and persons with renal failure were supplied from University Hospital (Zagazig). Mebeverine-HCl (E.I.P.C.O.) Company for Pharmaceutical and Chemical Industries, Egypt. Drotaverine-HCl (Alexandria Company for Pharmaceutical and Chemical Industries, Egypt). Sorensen's phosphate buffer pH 7.4[6]. Cellulose dialyser tubings, molecular weight cut off 10,000 (Union Carbide, U.S.A.)

Equipments:

Thermostatically controlled water bath with shaker (Aastell Hearson, H2130, England). Atomic absorption flame emission spectrophotometer (A.A. 640-130 Shimadzu, Japan). A pH meter (Activion Laboratory pH meter, Corning Limited, England). Centrifuge (BHG Hermle, Z230, W. Germany).

Methodology:

1. Collection of blood samples:

Blood was withdrawn from healthy persons and from patients with renal failure and kept with sodium citrate to prevent coagulation. These blood samples were centrifuged at 450 rpm. The plasma was stored at -20°C until it was used in the protein binding studies.

2. Determination of total protein concentration in collected blood samples:

This total protein procedure is a modification by Doumas[7] of biuret reaction originally described by Kingsley.[8] A purple-violet color is formed when cupric ions in an alkaline medium form a complex with the unshared pair of electrons of the nitrogen and oxygen atoms of the protein peptide bonds. The intensity of the produced color is proportional to the quantity of protein present and could be measured photometrically at 540 nm.

3. Determination of albumin concentration in collected blood samples:

Formation of albumin/bromocresol-green complex at pH 4.2 was measured photometrically at an absorbance 630 nm. [7]

4. Binding studies:

Binding studies were conducted using the equilbrium dialysis technique. Twenty cm long dialyser tubings were cut and soaked overnight in a solution of the selected buffer. These dialyser tubings were washed several times with the selected buffer then washed with deionized water. Twelve bottles of 20 cc capacity and twelve dialyser tubings were prepared for each experiment. In each bottle 5 ml of Sorensen's phosphate buffer of pH 7.4 containing the specified concentration of either mebeverine-HCl or drotaverine-HCl was placed. One of the dialyser tubing was then introduced through the bottle in such a way that both the open ends of the tube are outside the bottle. Five ml of plasma from either healthy volunteers or patients with renal failure was then placed carefully inside the dialyser tubing. The bottles were then covered with rubber caps. The bottles were allowed to be rocked in a thermostated water bath for 3 and 4 hours, to reach equilibrium for mebverine-HCl and drotaverine-HCl, respectively at 37°C. After the equilibrium time elapsed, the free drug in the bottle was determined spectrophotometrically at 262 nm[9] and 353 nm[10] for mebeverine-HCl and drotaverine-HCl, respectively. The concentration of the drugs (mebeverine-HCl and drotaverine-HCl) ranged from 2×10^{-6} M and from 2×10^{-5} M to 10×10^{-5} M, respectively.

RESULTS AND DISCUSSION

The clinical data of patients with renal failure and healthy volunteers are summarized in Table (1). In the case of the plasma of normal volunteers (N.V.), the mean plasma albumin concentration was 4.3 gm% and the mean total protein concentration was 6.5 gm%. On the other hand, for the plasma of patients with renal failure (R.F.), the mean plasma albumin concentration was 2.8 gm% and the mean

Table1: Clinical data for patients with renal failure and for normal volunteers.

Patient No.	Sex.	Age (years)		Weight (kg)		Total protein gm (%)		Serum albumin gm (%)	
		N.V	R.F	N.V	R.F	N.V	R.F	N.V	R.F
1	M	26	35	88	62	6.4	5.8	4.6	2.9
2	M	54	39	32	38	6.8	4.9	4.3	1.7
3	F	32	63	58	72	7.1	5.6	4.7	3.6
4	M	28	72	65	55	7.5	6.7	3.9	2.5
5	F	47	31	42	36	5.9	5.1	4.1	3.3
Mean		(36)	(48)	(57)	(52)	(6.7)	(5.6)	(4.3)	(2.8)

N.V = Normal volunteers.
R.F = Renal failure.

Zagazig J. Pharm. Sci., June 1995,
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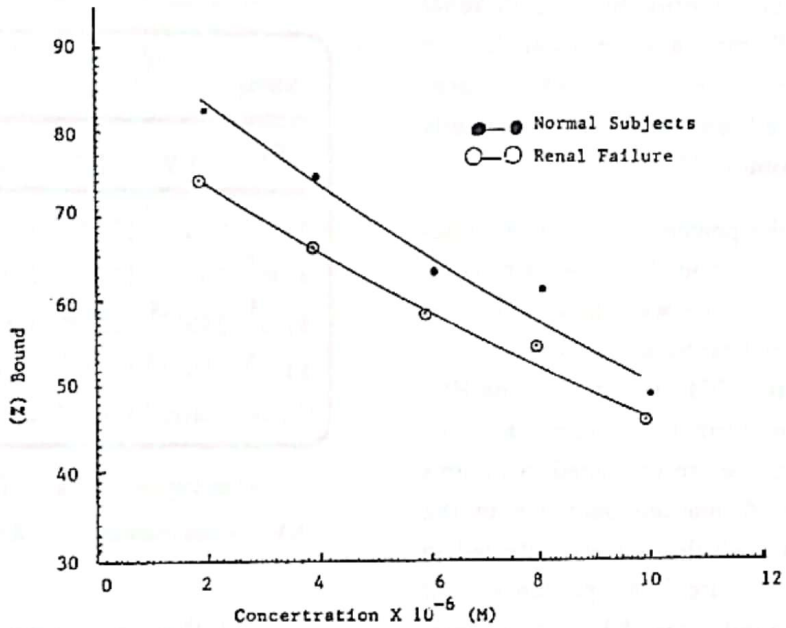

Fig. (1) : Percentage of Mebeverine hydrochloride bound to plasma portion from normal volunteers and from patients with renal failure.

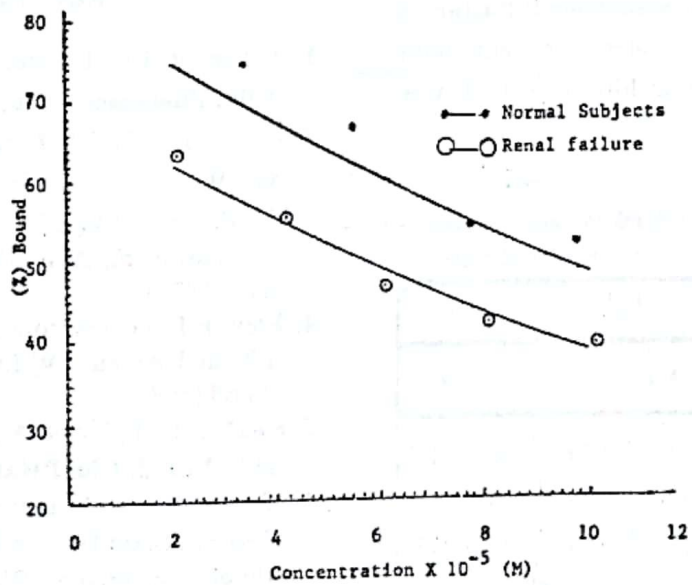

Fig. (2) Percentage of drotaverine hydrochloride bound to plasma proteins from normal volunteers and from patients with renal failure

total protein concentration was 5.6 gm %. The factors which may affect drug binding in renal failure include hypoalbuminemia, abnormal albumin configuration, hyperlipoproteinemia and displacement by accumulated endogenous compounds including drug metabolites[11].

In this work, the percentage of mebeverine-HCl and drotaverine-HCl bound to plasma proteins of patients with renal failure were less than those bound to plasma from healthy subjects. Values of percentage mebeverine-HCl and drotaverine-HCl bound in plasma from normal volunteers and from patients with renal failure are presented in Figures 1,2 and Tables 2,3. A marked decrease in the percentage bound of both drugs were detected in patients with renal failure. The percentage of mebeverine-HCl bound was 83.5 in normal volunteers and 74.3 in patients with renal failure. But the percentage bound of drotaverine was 72.45 in normal volunteers and 63.21 in patients with renal failure.

The influence of the other organic acids on the binding of drugs in plasma from patients with renal failure have been reported. These acids which have been reported to affect the plasma binding of many drugs include indoxy sulphate, 2-hydroxyhippuric acid and hippuric acid[12]. Keller et al[13]. observed that the free (unbound) fraction of furusemide was markedly greater in patients with nephrotic syndrome than in healthy subjects. It was

Table 2: Pececntage of mebeverine hydrochloride bound to plasma from normal volunteers and from patients with renal failure.

Starting concentration (M)	C_f(M)		C_b(M)		% Bound	
	N.V	R.F	N.V	R.F	N.V	R.F
2×10^{-6}	0.33×10^{-6}	0.51×10^{-6}	1.67×10^{-6}	1.49×10^{-6}	83.51	74.32
4×10^{-6}	0.97×10^{-6}	1.38×10^{-6}	3.03×10^{-6}	2.62×10^{-6}	75.84	66.64
6×10^{-6}	2.14×10^{-6}	2.48×10^{-6}	3.86×10^{-6}	3.52×10^{-6}	64.35	58.76
8×10^{-6}	3.05×10^{-6}	3.63×10^{-6}	4.95×10^{-6}	4.37×10^{-6}	67.93	54.67
10×10^{-6}	5.03×10^{-6}	5.45×10^{-6}	4.97×10^{-6}	4.55×10^{-6}	49.26	45.59

C_f = Free drug concentration C_b = Bound drug concentration
N.V = Normal volunteers. R.F = Renal failure.

Table 3: Pececntage of dortaverine hydrochloride bound to plasma from normal volunteers and from patients with renal failure.

Starting concentration (M)	C_f(M)		C_b(M)		% Bound	
	N.V	R.F	*N.V	*R.F	N.V	R.F
2×10^{-5}	0.55×10^{-5}	0.73×10^{-5}	1.45×10^{-5}	1.26×10^{-5}	72.45	63.21
4×10^{-5}	1.30×10^{-5}	1.81×10^{-5}	2.69×10^{-5}	2.19×10^{-5}	67.38	54.84
6×10^{-5}	2.42×10^{-5}	3.22×10^{-5}	3.58×10^{-5}	2.78×10^{-5}	59.67	46.36
8×10^{-5}	3.58×10^{-5}	4.66×10^{-5}	4.42×10^{-5}	3.34×10^{-5}	55.23	41.75
10×10^{-5}	5.44×10^{-5}	6.11×10^{-5}	4.56×10^{-5}	3.89×10^{-5}	45.58	38.96

C_f = Free drug concentration C_b = Bound drug concentration
N.V = Normal volunteers. R.F = Renal failure.

suggested that this effect is due to proteinuria associated with nephrotic patients[13].

In conclusion, the plasma protein binding of both mebeverine-HCl and drotaverine-HCl in the case of patients with renal failure was reduced if compared with their binding to plasma from healthy subjects. Accordingly the free fraction increased in both drugs. This must be taken into consideration during the adjustment of the dose of these drugs to patients with renal failure.

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Zagazig J. Pharm. Sci., June 1995,
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إرتباط إيدروكلوريد الميبفرين وإيدروكلوريد الدروتافيرين مع بروتينات بلازما متطوعين اصحاء ومرضى الفشل الكلوى

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تم فى هذا الجزء دراسة إرتباط كل من أيدروكلوريد الميبفرين وأيدروكلوريد الدروتافيرين مع بروتينات البلازما التى تم الحصول عليها من أشخاص أصحاء وأشخاص مرض الفشل الكلوى وقد ثبت بعد تعيين نسبة الزلال فى هذه العينات أن كمية الزلال فى بلازما مرضى الفشل الكلوى تقل عن قيمتها فى الأشخاص الأصحاء. وأوضحت تجارب الإرتباط أن كمية العقار الحرة فى حالات الفشل الكلوى تزيد عن مثيلتها فى حالات الأشخاص الأصحاء وهذا يجب أخذه فى الإعتبار عند تقدير الجرعة لكل من العقارين عند مرضى الفشل الكلوى .