약 제 학 회 지
J. Korean Pharm. Sci
9 (1) 7~14 (1979)

Sdtuy on Hydrophobic Drug-Soluble Carrier Coprecipitates (III)[1)]

Diuretic Effects of Furosemide-PVP Coprecipitate

Sang Chul Shin*

Furosemide-PVP共沈物의 利尿效果에 關한 硏究

申 祥 澈

서울大學校 藥學大學

(Received Feb. 25, 1979)

The relative efficacy on the renal function of rabbits by oral administration of furosemide and 1 : 2 furosemide-PVP coprecipitate was compared by measuring the urine volume in response to maximal response and the amounts of electrolytes excreted in urine.

The furosemide produced a rapid onset, short duration of diuresis, in contrast, the 1 : 2 furosemide-PVP coprecipitate, a rapid onset, significantly larger magnitude, and longer duration of diuresis and therefore the bioavailability of furosemide from the coprecipitate were increased significantly. The average urine volume and the amount of sodium and potassium excreted in urine were increased about 2.9-, 14.8-, and 1.8-fold from furosemide, and about 6.2-, 24.2-, and 3.6-fold from 1 : 2 furosemide-PVP 40,000 coprecipitate, rerpectively, comparing by their control values.

One of most efficient diuretics in current therapeutic use, furosemide[1-12)] 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid, is a potent nonmercurical diuretic of the sulfonamide group.

The physicologic-pharmacologic properties and clinical use of furosemide have been studied extensively[13-17)].

* College of Pharmacy, Seoul National University

1) Partial fulfillment of Doctor of Pharmaceutical Science degree requirement to the Graduate School, Seoul National University

In a series of works in this laboratory[18,19] it is shown that the coprecipitates with PVP increase the dissolution rate and equilibrium solubility of furosemide. The diuretic effects of furosemide might be different from its coprecipitates.

Concerning with enhanced dissolution rate and equilibrium solubility of furosemide from the 1 : 2 furosemide-PVP coprecipitate, a study of furosemide was undertaken to explore its effects on renal function.

The relative efficacy of the renal function of rabbits by oral administration of furosemide and 1 : 2 furosemide-PVP coprecipitate were compared by measuring the volume of urine in response to maximal response and the amonunts of electrolytes excreted in urine. The ratio of these response an index of physiological availability.

EXPERIMENTAL

Materials-The furosemide (USP XIX) (Teva middle east pharm. & chemical works), polyvinyl pyrrolidone (average mol. wt., about 40,000) were pharmaceutical grade. All other chemicals were reagent grade.

Apparatus-Harvard infusion pump (Harvard model 975-D), Flame photometer (Colemann).

Prepartion of Furosemide Test Systems-The 1 : 2 (w/w) ratio furosemide-PVP coprecipitate was prepared by the solvent method described in the previous paper[18-20].

Studies on the Diuretic Action of Rabbits-White, healthy rabbits weighing about 1.6 to 2.0kg were used in this study without distinguishing sexes. They were fasted overnight before experiments and not anesthetized to prevent inhibition of absorption of drugs in GI tracts. All rabbits were administered about 50 ml of water by a stomach tube at 60 minutes before experiments. The rabbits were fixed on the animal board in supine position and the tracheal T tube was inserted into trachea in order to respire easily. One of the femoral artery was inserted with polyethylene catheter to facilitate withdrawl of blood specimens. 0.9% heparin-saline (400 IU/ml) was used as an anticoagulant. The blood specimens were taken at the middle of each collection periods. The plasma after centrifuge were used for analysis, with urine, simultaneously.

Through a small, single midline incision, both urethers were exposed and cannuldate with polyethylene tube number 23 for the purpose of urine collection. The urine from both kidneys was collected in a graduated cylinder at 20 minute intervals. The urine collections were measured for volume and electrolyte concentration.

To replace the urinary loses of water, isotonic infusion solution composed of 3% glucose, 0.3% sodium chloride, and 0.25% creatinine was given into a branch of jugular vein with a speed of about 32ml/hr via a Harvard pump established for two subjects maintaining for the continuous infusion through the whole experiments. The creatinine was added in infusion solution to monitor the rate of glomelular filtration (GFR).

Time was allowed for the urine to become stable after the intravenous infusion of above solution and the urine flow became almost stable after 3—4 hours. When the urine flow rate was almost stable, the collection of control urine samples was carried out twice at an interval of 20 minutes and the effective dose of furosemide at 60mg/kg and of the 1 : 2 furosemide-PVP 40,000 coprecipitate corresponding to 60mg/kg of furosemide were administered orally with about 50ml of water using stomach tube and then the samples of urine were collected continuously at 20 minute intervals for 100 minutes.

Determination of Electrolytes-Each plasma and urine specimens was analyzed for sodium and potassium concentrations using Colemann flame photometer by internal standard flame photometry. The glomelular filtration rate was determined by means of creatinine clearance. The creatinine in urine and plasma specimens was determined by the Phillips mehod[21].

RESULTS AND DISCUSSION

Effect of Furosemide on the Renal Function of Rabbits-The effects of furosemide by oral administration at 60 mg/kg on the renal function of rabbits are shown in Table I. The physiological availability of furosemide was evaluated by the increase in urine flow, and the electrolytes excreted in urine. The data excreted in urine during 20 minute periods before and after administration of furosemide were averaged. The result shows that the furosemide produced a prompt, profound diuresis, and the excretion of potassium and sodium and the urine volume were all increased significantly by furosemide.

During 20 minutes of the first collection period, the urine volume was increased about 2.2-fold, and C_{Cr}, meaning the glomelular filtration rate in this period, was decreased about 30% comparing with control period, respectively. However, the amounts of Na^+ ($U_{Na}V$) excreted in urine were increased about 5.9-fold and, those of K^+ (U_KV), about 2.9-fold.

And during the next 20 minutes of 2nd period, it was increased about 5.9-in urine volume, about 21.73- in $U_{Na}V$, and about 2.8-fold in U_KV respectively. The maximal response of oral administration of furosemide at 60 mg/kg to rabbits receiving a saline infusion appeared in the 2nd period. During the 2nd period showing the maximal diuresis, the reaborption rates of $Na^+$and K^+ were decreased to $86.93\pm2.20\%$ and $51.00\pm3.35\%$, comparing with their control periods, $99.27\pm0.53\%$ and $90.0\pm3.44\%$, respectively.

Afterthe 2nd period, the increasing rates of $U_{Na}V$, and U_KV were somewhat decreased and the inhibition of reabsorption rate of Na^+ and K^+ in renal tubules weakened, and the GFR was decreased continuously not with respect to the urine volume. The observation in this study demonstrates that the furosemide is a diuretic whose action is rapid in onset, short in duration, and extremely potent.

Effect of 1 : 2 Furosemide-PVP 40,000 Coprecipitate on the Renal Function of

Table Ⅰ—Effect of Furosemide on the Renal Function of Rabbits

	Control Periods	Experimental Periods (minutes)				
		0～20	20～40	40～60	60～80	80～100
Vol(ml/20min)	2.43±0.55	5.30±2.63	14.27± 6.11	12.83± 6.39	8.03± 2.94	6.93± 3.26
C_{Cr}(ml/min)	8.13±1.19	5.80±1.34	6.04± 1.36	5.71± 1.41	4.99± 1.31	4.38± 1.06
$U_{Na}V$(μEq/min)	1.61±0.22	9.43±4.60	34.99±13.12	41.58±23.24	23.71±12.50	17.52± 8.29
Rab_{Na}(%)	99.27±0.53	90.2 ±3.03	86.93± 2.20	88.67± 1.62	90.57± 1.98	90.77± 3.13
U_KV(μEq/min)	2.24±0.96	6.33±3.66	8.18± 5.28	6.43± 4.34	5.32± 3.78	4.81± 3.14
Rab_K(%)	90.00±3.44	53.50±4.77	51.00± 3.35	55.40± 3.10	53.90± 2.78	50.90± 3.78

The furosemide was orally administered at 60 mg/kg and the values represent the means± standard errors from 4 experiments.
Abbreviations: Vol, urine flow rate;
C_{Cr}, clearance of creatinine meaning glomerular filtration rate;
$U_{Na}V$ and U_KV, amounts of sodium and potassium excreted in urine;
Rab_{Na} and Rab_K, fraction of reabsorbed sodium and potassium which were filtered by glomeruli.

Table Ⅱ-Effect of 1 : 2 Furosemide-PVP 40,000 Coprecipitate on the Renal Function of Rabbits

	Control Periods	Experimental Periods (minutes)				
		0～20	20～40	40～60	60～80	80～100
Vol(ml/20min)	1.78±0.44	4.10±1.49	11.60± 4.16	16.70± 6.04	16.66± 5.20	15.14± 4.91
C_{Cr}(ml/min)	8.76±2.11	9.24±2.96	8.92± 3.04	9.95± 3.48	7.99± 1.96	7.79± 2.33
$U_{Na}V$(μEq/min)	1.94±1.11	15.05±7.24	43.45±23.04	64.89±29.65	63.87±24.97	57.28±22.53
Rab_{Na}(%)	99.90±0.04	98.90±0.27	99.50± 1.44	94.60± 1.84	93.70± 1.23	94.20± 0.45
U_KV(uEq/min)	2.09±0.95	8.81±4.90	11.15± 4.43	11.43± 4.35	8.55± 2.68	8.51± 2.94
Rab_K(%)	92.80±1.96	77.60±8.25	65.60± 0.93	65.50± 4.42	68.40± 3.62	67.30± 4.04

The 1 : 2 furosemide-PVP 40,000 coprecipitate corresponding to 60 mg/kg of furosemide was orally administered, and the values represent the means ±standard errors from 6 experiments. The other abbreviations are as shown in Table Ⅰ.

Rabbits-The quantity of 1 : 2 furosemide-PVP coprecipitate, equivalent to 60 mg/kg of furosemide was administered in order to investigate the effects on the renal function of rabbits. The results from six experiments were illustrated in Table II.

The 1 : 2 furosemide-PVP 40,000 coprecipitate produced a large magnitude, long duration diuresis. However, during the first 20 minute period, the urine volume was increased about 2.3-, and the $U_{Na}V$, about 7.8-fold, but, the increasing rate was lower than that of furosemide only. The urine volume was increased with progress of time and showed the maximal response during the 3rd, and 4th periods. In these periods, the reabsorption rate of Na^+ was inhibited to 93.9% ± 1.23%, comparing its control period 99.9 ± 0.04%.

During the 3rd period showing maximal diuresis, the increasing rate in urine volume by 1 : 2 forosemide-PVP 40,000 coprecipitate was about 9.4- comparing its control per-

iod, in contrast, that by furosemide during the 2nd period of maximal response was about 4.5-fold.

Comarison on the Renal Function between Furosemide and 1 : 2 Furosemide-PVP 40,000 Coprecipitate-The percent changes by furosemide and 1 : 2 furosemide-PVP 40,000 coprecipite are shown in Fig. 1 as various parameters.

The relative efficacy of test preparations was compared by measuring the urine volume in response to maximal diuresis and the amounts of electrolytes excreted in urine by the two drugs. The ratio of these response provides and index of physiological availability.

The excretion data obtained for 5 periods were averaged and the standard error was calculated respectively (Table III and Fig. 2). The average urine volume and the amount of sodium and potassium excreted in urine were increased about 2.9-, 14.8-, and 1.8-fold from furosemide, and about 6.2-, 24.2-, and 3.6-fold from 1 : 2 furosem-

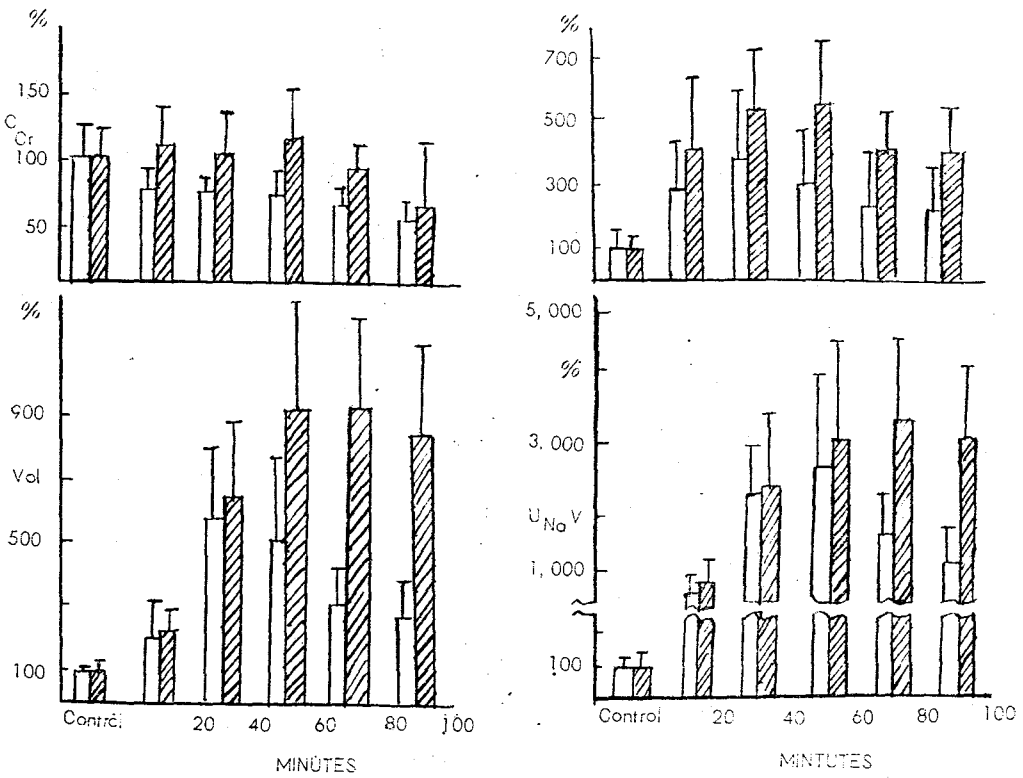

Figure 1—Effect of furosemide and 1 : 2 furosemide-PVP 40,000 copredipitate on the renal function of rabbits as various parameters.
The control values are calculated to 100 and the percent changes by furosemide and 1 : 2 furosemide-PVP 40,000 coprecipitate are depicted.
Mean values and standard errors were obtained from Table III and IV, respectively.
The open columns represent the changes by furosemide, and shaded columns, by 1 : 2 furosemide-PVP 40,000 coprecipitate. The other abbreviations are as shown in Table I.

Table Ⅲ Effect of Furosemide and 1 : 2 Furosemide-PVP 40,000 Coprecipitate on the Renal Function of Rabbits

	Drugs	Control Period	Experimental Period	% Increase
Vol (ml/20min)	Furosemide	2.43±0.55	9.47±4.30	287.71%
	Coprecipitate	1.78±0.44	12.86±3.30*	622.47%
$U_{Na}V$ (μEq/min)	Furosemide	1.61±0.22	25.44±8.06	1480.12%
	Coprecipitate	1.94±0.95	48.91±14.70	2421.13%
U_KV (μEq/min)	Furosemide	2.24±0.96	6.21±2.40	177.23%
	Coprecipitate	2.09±0.95	0.69±2.21	363.64%

The abberviations are as shown in Table III.
*, significant at $P<0.05$

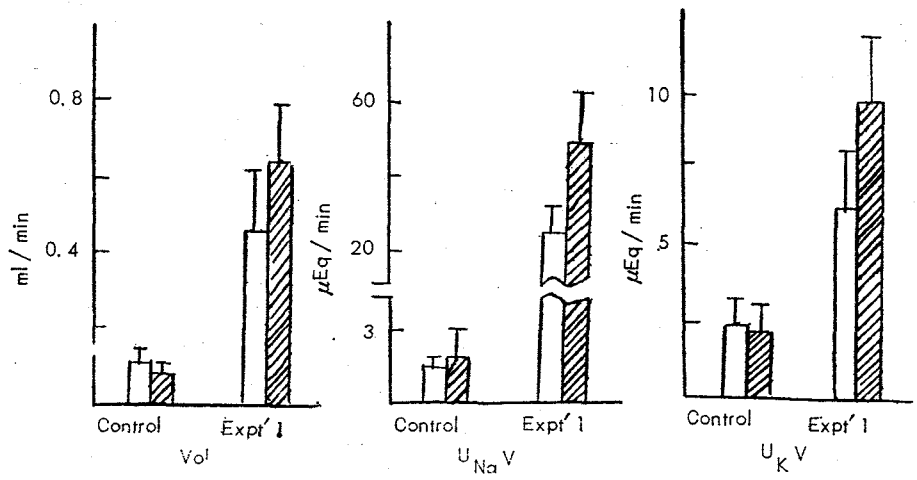

Figure 2-Comparison on the effects of furosemide and 1 : 2 furosemide-PVP 40,000 coprecipitate. The open columns represent the changes by furosemide, and the shaded columns, by 1 : 2 furosemide-PVP 40,000 coprecipitate.

ide-PVP 40,000 coprecipitate, respectively, comparing by their control values. In other words the total urine volume, sodium, and potassium excreted in urine within 100 minutes from the 1 : 2 furosemide-PVP coprecipitate were increased about 2.2-, 1.6-, and 2.0-fold comparing with those from furosemide.

The results show that furosemide produced a rapid onset, short duration of diuresis, in contrast, the 1 : 2 furosemide-PVP 40,000 coprecipitate, a rapid onset, larger magnitude, and longer duration of diuresis than furosemide only, and therefore the bioavailability of furosemide from the coprecipitate were increased significantly.

Suki et al[22]. demonstrated that the greater diuretic action of furosemide is not the simple result of marked in hibition of sodium transport at the same site in the renal tubules where other sulfonamide diuretics act, but also ascending limb of Henle's loop of renal tubules is a major site of furosemide action. Ludens[23] reported that furos-

emide shows a transient increase in RBF in dogs, however, there is little information of increase in GFR, rather decrease in GFR[22,24].

From these observations of furosemide and the coprecipitate, similar pattern of diuretic response in urine volume, $U_{Na}V$, and U_KV was also shown, on the other hand, somewhat different diuretic response in C_{Cr} was observed control and experimental periods of 1 : 2 furosemide-PVP 40,000 coprecipitate. In relation to the marked increase in urine volume by 1 : 2 furosemide-PVP 40,000 coprecipitate comparing that by furosemide only (Fig. 2), it is suggested that the diuretic action of 1 : 2 furosemide-PVP coprecipitate might be explained that it inhibits sodium reabsorption in renal tubules and not produce the decrease of GFR.

CONCLUSIONS

The present study on the diuretic effects for the furosemide test preparations could be summarized as follows:

1. The average volume and the amount of sodium and potassium excreted in urine are increased about 2.9-, 14.8-, and 1.8-fold from furosemide, and about 6.2-, 24.2-, and 3.6-fold from 1 : 2 furosemide-PVP 40,000 coprecipitate, respectively, comparing by their control values.

2. The increasing rate in urine volume showing maximal diuresis is about 5.9-fold during the 2nd period by furosemide only comparing the control period, while that is about 9.4-fold during 3rd period by 1 : 2 furosemide-PVP coprecipitate.

3. The furosemide produces a rapid onset, short duration of diuresis, in contrast, the 1 : 2 furosemide-PVP coprecipitate, rapid onset, significantly larger magnitude, and longer duration of diuresis, and therefore the bioavailability of furosemide from coprecipitate were increased significantly.

ACKNOWLEDGMENTS

Received January 19, 1979, from the college of Pharmacy, Seoul National University. The author adequately express his appreciation to Dr. Chong-Hak Woo, Dr. Shin-Keun Kim, Dr. Min-Hwa Lee, Dr. Nak-Doo Kim in Seoul National University and Dr. Sug-Tea Ko in Choseon University for their helpful discussions.

REFERENCES

1) W.G. Walker, *Med. Clin. North America*, **51**, 1277 (1967).
2) R. Muschaweck and P. Hadju, *Arz. Forsch.*, **14**, 44 (1964).
3) G. Malnic, F.L. Vieira, and W. Fill., *Rev. Ass. Med. Bras.*, **12**, 287 (1966).

4) E.M. Garcia, D.J.C. Prata, L.V. Carvalho, *O. Hospital*, **70**, 265 (1966).
5) A.D. Ferreira, *ibid.*, **70**, 1057 (1966).
6) A. Heiland, K. Klutsch, and F. Suzuki, *Drug Research*, **14**, 713 (1964).
7) E. Sabbaga, S. Koperstych, and C.F. Villela, *O. Hospital*, **67**, 175 (1965).
8) A. Rosenkranz, Weiner Med. *Wochenschrift* **14**, 236 (1964).
9) K. Laderbogen, M. Hudopohl, *Die Medizinische Welt*, **17**, 182 (1965).
10) H.F. Schaefer, *ibid.*, **16**, 922 (1964).
11) W. Stokes and L.C.A. Nunn, *Brit, Med. J.*, **2**, 910 (1964).
12) W. Statson, F. Cannon, H.O. Heneman, *Circulation*, **34**, 910 (1966).
13) J.B. Hook, A.H. Michael, J. Brody, and H.E. Williamson, *J. Pharmacol. Exp. Ther.*, **154**, 667 (1966).
14) J.B. Hook and H.E. Williamson, *J. Pharmacol. Exp. Ther.*, **148**, 88 (1965).
15) B. Calesnick, J.A. Christensen, and M. Richter, *Proc. Soc. Exp. Biol. Med.*, **123**, 17 (1966).
16) A.W. Forrey, B. Kimpel, A.D. Blair, and R.E. Cutler, *Clin. Chem.*, **20**, 152 (1974).
17) P. Hajdu and A. Haussler, *Arz. Forch.*, **14**, 709 (1964).
18) S.C. Shin, M.H. Lee, and C.H. Woo, *J. Kor. Pharm. Sci.*, **6**, 48 (1976).
19) S.C. Shin, *Arch. Pharm. Res.*, **2**, 35 (1979).
20) *Ibid.*, **2**, 49 (1979).
21) R.A. Phillips, Inpeterson and Van Slyke, *Quantitative Clinical Chemistry*, Vol. 2, Williams & Wilkins, Baltmore, 1943.
22) W. Suki, F.C, Rector, Jr., and D.W. Seldin, *J. Clin. Invest.*, **44**, 1458 (1965).
23) J.H. Ludens, J.B. Hook, M.J. Brody, and H.E. Williamson, *J. Pharmacol. Exp. Ther.*, **163**, 456 (1968).
24) S.T. Ko, *J. Health Fellowship Foundation*, **4**, 1 (1973).