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Comparative Bioavailability of Two Furosemide Formulations in Humans

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Abstract □ Twelve healthy male volunteers participated in a balanced crossover comparison of a brand-name and generic furosemide formulations. Each treatment was given as a single 40-mg tablet following an overnight fast. Furosemide concentrations in plasma and urine were determined up to 24 h after treatment; urine output and urinary sodium excretion were also measured. In comparison with the brand-name tablets, generic furosemide was significantly less bioavailable. Using a 95% confidence interval approach, generic furosemide gave up to 66% lower maximum furosemide plasma levels, up to 52% less area under the plasma level curve to infinite time, and up to 37% less urinary recovery of furosemide. Comparison of the effect of the two treatments was a less sensitive measurement of bioequivalence. Confidence intervals for differences in urinary output and sodium excretion over the period of maximum effect (0–4 h) were, however, asymmetrical, and pharmacodynamic differences between treatments were significant at the 10% level.

Keyphrases □ Furosemide—comparative bioavailability, brand-name and generic formulations, humans, plasma and urine □ Bioavailability—comparative, furosemide formulations in humans, plasma and urine □ Formulations—comparative bioavailability, brand-name and generic furosemide in humans, plasma and urine

During 1979, generic formulations of furosemide tablets which were not legally marketed became available to U.S. physicians. Shortly after these tablets were introduced, reports began to appear of diuretic ineffectiveness of some of the products. The Food and Drug Administration became aware of the problem and took steps to prevent further clinical use of unauthorized furosemide tablets (1).

With the above background in mind, it was felt that it would be useful to compare the relative bioavailability of the brand-name tablet formulation of furosemide available in the United States and one of the abovementioned generic furosemide tablets. This paper presents the results of a crossover study in healthy volunteers with comparative pharmacokinetic and pharmacodynamic measurements.

EXPERIMENTAL

Subjects—Twelve healthy male volunteers aged 18–42 years, within 10% of ideal body weight, received treatment on two occasions with 1-week between doses. A comprehensive checkup, including clinical examination, clinical chemistry/hematology evaluation, and urinalysis revealed no evidence of cardiac, respiratory, hepatic, or renal disease. The project was subject to ethics review, and each subject gave his signed informed consent.

Treatment—Furosemide (40 mg) was administered as a single tablet on two occasions 1 week apart to each subject, according to a balanced crossover design. Treatments were designated product I[1] and product II[2] respectively. Product I (the brand-name tablet) contained an average of 39.6 mg of furosemide/tablet; product II (the generic tablet) contained an average of 40.3 mg of furosemide/tablet. Therapy was given by mouth with 100 mL of water following an overnight fast, 1 h before a standard tea/toast breakfast. Oral fluid supplements (150 mL of water) were given at 1, 2, 3, 4, and 6 h after treatment.

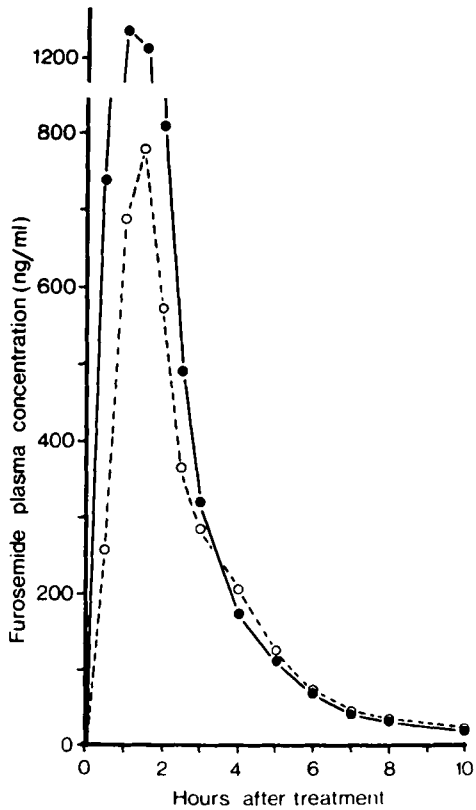

Figure 1—*Mean furosemide plasma concentration following oral treatment with product I (●) and product II (○).*

[1] Lasix, lot 600229; Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.
[2] Furosemide lot 8137-07; Pharmadyne Laboratories Inc., Elmwood Park, N.J.

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Table I—Plasma Furosemide Concentration, ng/mL

Time, h	Subject 1	2	3	4	5	6	7	8	9	10	11	12	Mean ± *SD*
						Product I							
0.5	1000	1180	1610	90	1190	2950	25	100	70	105	145	400	739 ± 893
1	1650	1560	970	1810	1630	2010	75	1870	165	130	740	2240	1236 ± 787
1.5	1110	1650	430	1460	1080	1490	1060	1710	550	1380	1500	1150	1214 ± 404
2	670	1150	340	800	570	830	720	820	430	1360	1040	940	806 ± 292
2.5	420	730	220	470	330	530	460	570	380	930	480	370	491 ± 188
3	240	460	140	290	240	380	270	370	250	630	360	220	321 ± 130
4	115	230	60	160	145	250	130	210	160	280	220	140	175 ± 63
5	50	125	50	115	70	140	50	100	230	190	145	80	112 ± 58
6	30	75	45	65	35	95	25	60	120	135	95	60	70 ± 35
7	20	45	20	40	15	50	15	40	75	110	70	30	44 ± 29
8	15	35	15	40	15	35	20	35	55	65	45	30	34 ± 16
10	<15	20	20	25	0	20	<15	25	40	45	25	20	21 ± 13
24	0	<15	0	20	0	<15	0	0	25	<15	<15	<15	— —
						Product II							
0.5	135	200	880	45	670	30	30	65	30	570	15	420	258 ± 301
1	1800	1350	740	90	1010	135	45	280	60	1580	20	1140	688 ± 665
1.5	910	1350	490	210	730	1340	380	770	105	1400	600	1080	780 ± 446
2	660	850	420	180	490	1100	420	630	100	800	620	590	572 ± 278
2.5	390	440	260	180	340	700	240	480	100	450	400	420	367 ± 158
3	300	260	185	155	280	560	160	450	95	330	330	350	288 ± 132
4	200	130	105	175	135	330	100	250	190	160	540	165	207 ± 123
5	85	65	80	120	95	150	75	160	240	105	290	80	129 ± 71
6	65	50	50	45	60	100	40	105	90	80	140	55	73 ± 30
7	45	25	35	35	45	60	30	70	65	55	75	45	49 ± 16
8	35	<15	25	20	30	40	30	55	50	40	50	30	34 ± 15
10	25	20	20	20	25	15	20	35	40	25	25	20	24 ± 7
24	<15	0	<15	0	<15	0	<15	<15	25	0	<15	<15	— —

Table II—Urinary Furosemide Excretion, mg/Sample

Time, h	Subject 1	2	3	4	5	6	7	8	9	10	11	12	Mean ± *SD*
						Product I							
0–1	6.51	3.34	5.66	1.53	5.11	5.11	0.25	1.73	—	0.23	0.84	3.41	3.07 ± 2.28
1–2	8.12	5.79	4.46	5.32	4.67	2.89	6.37	5.43	1.23	3.91	7.85	5.49	5.13 ± 1.93
2–3	2.34	2.77	1.14	1.97	4.54	1.67	2.90	1.71	2.03	2.87	2.99	1.94	2.40 ± 0.87
3–4	0.86	1.00	0.68	0.93	0.67	0.80	0.56	0.85	—	1.37	1.80	0.93	0.95 ± 0.35
4–6	0.75	—	0.44	0.52	0.85	0.63	0.75	—	3.03	0.87	1.51	0.75	1.01 ± 0.77
6–8	0.43	1.63	0.77	0.56	0.42	0.39	0.52	1.26	0.91	0.74	0.75	0.40	0.65 ± 0.37
8–10	0.20	0.45	0.27	0.17	0.29	0.20	0.29	0.25	0.47	0.38	0.29	0.20	0.29 ± 0.10
10–24	0.38	0.57	0.76	0.20	0.64	0.42	0.64	0.47	0.28	1.00	0.52	0.28	0.51 ± 0.23
						Product II							
0–1	2.53	1.43	2.54	—	2.49	—	0.05	0.45	0.09	6.13	0.05	2.48	1.82 ± 1.88
1–2	6.75	5.98	3.72	0.83	4.92	2.75	1.88	3.09	5.24	4.80	1.69	3.66	3.78 ± 1.82
2–3	1.53	1.49	1.16	0.92	1.56	2.40	1.83	2.26	1.07	2.52	2.69	1.97	1.78 ± 0.59
3–4	1.02	1.01	0.70	0.71	0.89	0.69	—	1.42	0.41	1.23	1.90	1.18	1.01 ± 0.41
4–6	0.91	0.98	0.74	1.13	0.93	0.69	1.80	1.69	1.90	1.01	2.39	0.98	1.26 ± 0.54
6–8	0.46	0.42	0.42	0.49	0.56	0.56	0.43	0.71	0.44	0.44	0.95	0.52	0.53 ± 0.16
8–10	0.29	0.30	0.25	—	0.34	0.36	0.32	0.10	0.69	0.26	0.37	0.32	0.33 ± 0.14
10–24	0.49	0.61	0.70	0.77	0.60	0.75	0.94	0.75	0.13	0.59	0.75	0.78	0.66 ± 0.20

Collection of Samples—Blood samples were taken by direct venipuncture at 0, 0.5, 1, 1.5 2, 2.5, 3, 4, 5, 6, 7, 8, 10, and 24 h and placed in heparinized tubes[3]. Following centrifugation, the plasma were stored in the deep-freeze until analyzed. A predose 24-h urine collection was made; after treatment complete urine samples were collected over the intervals of 0–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–10, and 10–24 h. The volume of each sample was recorded; aliquots were stored in the deep-freeze for electrolyte and drug assay. Urinary sodium was measured by flame photometry.

Furosemide Assay—Furosemide concentrations in plasma and urine were fluorometrically determined following HPLC separation. Urine was directly chromatographed after centrifugation. Following an initial 5-mL ether extraction of 0.25 mL of plasma plus 0.25 mL of phosphate buffer (pH 7.0) (with the ether layer discarded), the plasma was acidified with 50 μL of 5 M HCl and furosemide was extracted into 5 mL of ether. The ether layer was separated and evaporated under nitrogen; the residue was taken up with 0.1 mL of pH 7.0 phosphate buffer. An internal standard (100 ng of the phenyl analogue of furosemide) was simultaneously taken through the plasma procedure.

The chromatographic apparatus consisted of an automatic sampler[4], a solvent delivery pump[5], and a fluorometric detector with automatic overload reset[6]. The extracts were separated on a 12-cm × 4.3-mm column packed with 5-μm reverse-phase irregular organically modified silica[7]. The eluant was methanol–0.1 M H_3PO_4 (53:47). The excitation wavelength was 233 nm, and the emission wavelength was 370 nm (sharp-cut filter, with additional blue filter[8]).

The method was found to be linear over the concentration ranges of 10–1000 ng/mL for plasma and 0.5–10 μg/mL for urine. Assay precision for the plasma method was ± (7.7 + 0.039 C_p) ng/mL, where C_p is the plasma concentration; for urine, precision was ± (0.5 + 0.013 C_u) μg/mL, where C_u is urine concentration.

Pharmacokinetic Analysis—Initial estimates of furosemide elimination half-lives were calculated, using urinary data, by linear regression analysis of the logarithm of rate of urinary furosemide excretion against time (2). These estimates were employed as the starting values in an iterative least-squares curve-fitting program developed for a minicomputer[9], which generated a series of fitted coefficients and exponential constants. Most profiles fitted a model

[3] Li-Heparin; Walter Sarstedt Ltd., Leicester, U.K.
[4] WISP 710A; Waters Associates, Milford, Mass.
[5] M 6000A; Waters Associates, Milford, Mass.
[6] SF 970; Kratos Schoeffel Instruments, Westwood, N.J.
[7] LichroSorb RP-18; H. Knauer GmbH, 1000 Berlin 37, W. Germany.
[8] CS 5-61; Corning Optical Products Dept., Corning, N.Y.
[9] HP 1000 MX(E); Hewlett-Packard Ltd., Winnersh, Wokingham, Berks, U.K.

Table III—Results of Curve-Fitting [a]

	Subject											
	1	2	3	4	5	6	7	8	9	10	11	12
						Product I						
A, ng/mL	9677	9508	2840	7113	19340	5030	9867	24380	2865	15970	2393	6570
B, ng/mL	235.3	289.5	254.7	226.5	1045	758.2	112.5	143.4	308.7	480.8	718.8	171.6
C, ng/mL	10590	10490	3102	8710	20480	5790	10980	26200	5136	22280	4117	7627
α, h^{-1}	1.216	1.018	1.374	1.073	1.678	1.153	1.073	1.188	0.959	1.159	0.847	1.149
β, h^{-1}	0.355	0.271	0.350	0.223	0.569	0.374	0.294	0.174	0.201	0.238	0.343	0.218
γ, h^{-1}	1.974	1.678	13.42	1.896	1.968	4.957	1.469	1.458	1.623	1.755	1.981	2.257
r	0.985	0.991	0.995	0.913	0.983	0.998	0.915	0.933	0.956	0.993	0.908	0.903
						Product II						
A, ng/mL	9599	7312	2111	9137	16530	87980	77.32	3986	200.8	11190	—	11220
B, ng/mL	138.1	2138	399.7	1402	178.1	3504	3761	567.5	1229	402.3	7318	159.4
C, ng/mL	10350	10150	2515	10540	16840	91400	4957	5233	1466	12730	7776	12270
α, h^{-1}	1.069	1.633	1.249	1.089	1.225	3.380	1.003	0.817	0.356	1.285	—	1.134
β, h^{-1}	0.171	0.646	0.348	0.498	0.204	0.576	0.135	0.293	0.327	0.279	0.551	0.199
γ, h^{-1}	1.458	1.928	2.885	1.022	1.405	3.070	1.524	1.164	0.431	1.844	0.700	1.525
r	0.913	0.903	0.998	0.959	0.992	0.954	0.949	0.957	0.851	0.959	0.901	0.975

[a] $C_p = Ae^{-\alpha t} + Be^{-\beta t} - Ce^{-\gamma t}$.

Table IV—Urine Output, mL

Time, h	Subject												Mean ± *SD*
	1	2	3	4	5	6	7	8	9	10	11	12	
							Product I						
Predose (24 h)	685	1130	672	2650	1222	2560	1300	1320	1252	2000	1220	1283	1441 ± 638
0-1	885	785	790	343	684	662	116	376		143	258	447	457 ± 298
1-2	821	573	533	650	413	315	950	605	387	695	1141	595	640 ± 238
2-3	237	207	108	242	378	159	362	122	384	658	426	201	290 ± 158
3-4	67	48	37	92	26	105	44	45		243	107	59	73 ± 62
4-6	60	—	26	46	45	48	81	—	233	113	111	46	67 ± 63
6-8	58	140	84	127	54	67	105	218	219	96	148	92	117 ± 56
8-10	52	82	54	82	57	67	79	120	109	123	108	84	85 ± 25
10-24	360	420	475	380	450	400	410	550	1470	615	410	1200	595 ± 358
							Product II						
Predose (24 h)	830	1136	725	1310	1060	2000	1453	1040	1702	1508	1250	1620	1303 ± 373
0-1	360	319	452	—	450	—	75	176	255	905	55	433	290 ± 259
1-2	905	649	500	148	638	780	527	538	633	596	410	477	567 ± 189
2-3	168	134	100	186	142	278	428	255	293	430	544	296	271 ± 138
3-4	73	52	40	256	42	54	—	82	128	151	276	85	103 ± 86
4-6	68	65	55	254	51	62	238	85	198	115	256	61	126 ± 85
6-8	64	80	69	106	62	77	177	155	98	97	148	88	102 ± 38
8-10	78	78	72		60	95	144	38	246	110	119	85	94 ± 61
10-24	390	630	880	790	350	410	645	510	1090	650	595	440	615 ± 220

with two elimination phases following absorption. The half-lives were calculated from each declining exponential term; the areas under the plasma level time curve to 10 h ($AUC_{0\ 10\ h}$) and to infinite time ($AUC_{0\ \infty}$) for each subject were calculated from the fitted equation by integration. Maximum plasma concentration (C_{max}) and time to maximum concentration (t_{max}) were taken directly from the observed data.

Statistical Analysis—Analysis of variance (ANOVA) was used to test for significant subject, treatment, or week differences (3) for each pharmacokinetic or pharmacodynamic parameter. Missing data were estimated using Yates' method (4). Before analysis, a normality test was carried out (5). If necessary, the results were logarithmically transformed to normalize the distribution of data and to stabilize the variance. The 95% confidence limits of Shirley (6) were applied to the mean treatment differences for each parameter. This gave a measure of precision and established whether the limits were symmetrical about a mean distribution of zero. The results for generic furosemide (product II) were expressed as a percentage of those for brand-name furosemide (product I).

The split-plot analysis of Westlake (7) was also used. This examined the data in two ways: (*a*) giving a measurement of quantitative differences in overall mean plasma concentrations for each treatment, and (*b*) giving an evaluation of the relative shapes of the plasma concentration-time profiles of the two treatments.

RESULTS

Table I shows the individual plasma levels of furosemide associated with each treatment. Mean plasma concentration with time is plotted in Fig. 1. Table II shows the amount of furosemide in each urine collection. The results of pharmacokinetic curve-fitting appear in Table III. Table IV gives the individual urine volumes, and Table V gives the individual urinary sodium data. The key pharmacokinetic and pharmacodynamic results are summarized in Table VI, and a summary of statistical analyses with 95% confidence limits is shown in Table VII.

Pharmacokinetics—For one individual (subject 9) following product II, the curve-fit was unacceptable (Table III, $r = 0.851$). In this case, derived pharmacokinetic values ($t_{1/2}$ and AUC terms) were estimated by Yates' method (5).

Following product I administration, the mean maximum plasma furosemide concentration (C_{max}) was 1658 ng/mL (range 550–2950 ng/mL). Following product II administration, the values were significantly lower ($p < 0.01$) with a mean C_{max} of 947 ng/mL (range 210–1800 ng/mL). There was no significant difference in time to maximum concentration (t_{max}), although there was a trend toward later values following product II treatment (*i.e.*, an asymmetrical confidence limit).

The overall bioavailability judged from AUC values followed the same trend as that shown by C_{max}, with a highly significant ($p < 0.01$) difference between treatments in values both to 10 h and to infinite time. Product I gave a mean $AUC_{0-\infty}$ of 3066 ng·h/mL (range 1362–5223 ng·h/mL), whereas product II gave a mean $AUC_{0-\infty}$ of 2098 ng·h/mL (range 891–3252 ng·h/mL). Figure 2 shows the individual $AUC_{0-\infty}$ values diagrammatically, and illustrates that despite some interindividual variability in plasma level profiles, subjects tended to keep a similar rank order of bioavailability following each preparation of furosemide.

Urinary elimination of unchanged furosemide accounted for between 17–50% of the administered dose. Again, a significant difference ($p = 0.02$) was observed between formulations in respect of the cumulative 0-24-h urinary furosemide, with product I giving a mean value of 13.6 mg (range 8.0–19.6 mg) and product II giving a mean value of 10.8 mg (range 4.9–17.0 mg).

Table V—Urinary Sodium Output, mmol

Time, h	Subject 1	2	3	4	5	6	7	8	9	10	11	12	Mean ± *SD*
						Product I							
Predose (24 h)	199	113	108	305	141	269	156	119	144	230	195	103	174 ± 67
0–1	137	86	99	46	82	60	20	36	—	17	40	58	57 ± 39
1–2	111	57	72	85	52	35	138	67	45	45	154	83	82 ± 36
2–3	37	23	15	31	49	14	52	13	50	79	62	30	38 ± 21
3–4	11	4	4	10	2	6	4	4	—	27	15	8	8 ± 7
4–6	11	—	3	3	3	1	10	—	41	7	19	5	9 ± 12
6–8	16	11	10	8	6	3	16	22	37	5	32	12	15 ± 11
8–10	9	6	7	5	7	4	12	14	20	9	24	12	11 ± 6
10–24	59	13	71	25	59	40	62	50	29	55	68	60	49 ± 18
						Product II							
Predose (24 h)	100	91	83	144	80	240	189	125	60	241	206	105	139 ± 64
0–1	50	33	52	—	45	—	17	17	8	122	12	63	35 ± 35
1–2	127	81	55	19	77	66	79	62	66	86	55	64	70 ± 25
2–3	25	17	11	25	18	29	68	33	13	60	73	49	35 ± 22
3–4	11	6	2	18	5	5	—	9	5	20	30	13	10 ± 9
4–6	10	6	2	24	4	5	45	11	24	8	35	8	15 ± 14
6–8	9	8	3	16	7	7	42	23	9	9	25	18	15 ± 11
8–10	13	9	5	—	7	7	31	3	22	12	20	15	12 ± 9
10–24	45	50	35	103	42	51	94	51	11	68	95	81	61 ± 28

Table VI—Summary of Kinetic and Dynamic Results

Parameter	Subject 1	2	3	4	5	6	7	8	9	10	11	12	Mean ± *SD*
						Product I							
C_{max}, ng/mL	1650	1650	1610	1810	1630	2950	1060	1870	550	1380	1500	2240	1658 ± 585
t_{max}, h	1.0	1.5	0.5	1.0	1.0	0.5	1.5	1.0	1.5	1.5	1.5	1.0	1.1 ± 0.4
$t_{1/2,\alpha}$, h	0.55	0.68	0.51	0.65	0.41	0.60	0.65	0.58	0.72	0.60	0.82	0.60	0.6 ± 0.1
$t_{1/2,\beta}$, h	1.95	2.56	1.98	3.11	1.22	1.85	2.36	3.99	3.45	2.91	2.02	3.19	2.55 ± 0.70
AUC_{0-10h}, ng·h/mL	2933	4085	2542	2922	2951	5175	2084	3222	1127	2889	2771	3033	2977 ± 981
$AUC_{0-\infty}$, ng·h/mL	2943	4159	2564	3037	2964	5223	2102	3373	1362	3099	2845	3125	3066 ± 959
0–24-h urinary furosemide, mg	19.6	15.6	14.2	11.2	17.2	12.1	12.3	11.7	8.0	11.4	16.5	13.4	13.6 ± 3.2
Urine volume, mL													
0–4 h	2010	1613	1468	1327	1501	1241	1472	1148	771	1739	1932	1302	1460 ± 343
0–24 h	2540	2255	2107	1962	2107	1823	2147	2036	2802	2685	2709	2724	2325 ± 345
Sodium output, mmol													
0–4 h	296	170	190	172	185	115	214	120	95	168	271	179	181 ± 59
0–24 h	391	200	281	213	260	163	314	206	222	244	414	268	264 ± 76
						Product II							
C_{max}, ng/mL	1800	1350	880	210	1010	1340	420	770	240	1580	620	1140	947 ± 518
t_{max}, h	1.0	1.5	0.5	1.5	1.0	1.5	2.0	1.5	5.0	1.0	2.0	1.0	1.6 ± 1.2
$t_{1/2,\alpha}$, h	0.65	0.43	0.56	0.64	0.57	0.21	0.69	0.85	1.95	0.54	—	0.61	0.70 ± 0.44
$t_{1/2,\beta}$, h	4.05	1.07	1.99	1.39	3.39	1.20	5.14	2.37	2.12	2.49	1.26	3.48	2.50 ± 1.28
AUC_{0-10h}, ng·h/mL	2537	2519	1932	871	2268	2327	911	2204	705	3158	2113	2532	2006 ± 772
$AUC_{0-\infty}$, ng·h/mL	2687	2525	1968	891	2382	2346	1070	2321	918	3252	2167	2646	2098 ± 756
0–24-h urinary furosemide, mg	14.0	12.2	10.2	4.9	12.3	8.2	7.3	10.5	10.0	17.0	10.8	11.9	10.8 ± 3.1
Urine volume, mL													
0–4 h	1506	1154	1092	590	1272	1112	1030	1051	1309	2082	1285	1291	1231 ± 349
0–24 h	2106	2007	2168	1740	1795	1756	2234	1839	2941	3054	2403	1965	2167 ± 438
Sodium output, mmol													
0–4 h	213	137	120	62	145	100	164	121	92	288	170	189	150 ± 61
0–24 h	290	210	165	205	205	170	376	209	158	385	345	311	252 ± 84

Split-plot analysis showed highly significant ($p < 0.01$) differences between formulations both in the overall mean plasma values and in the treatment–time shape of the curves.

Pharmacodynamics—The missing values in the urinary tables reflect the fact that not all subjects were able to micturate on demand. Prior to statistical evaluation of pharmacodynamic data, estimates of the "missing" values were made by assuming that the urine eventually collected was actually produced at a constant rate over both "observed" and "missing" time periods.

The pharmacodynamic measurements presented for analysis in Tables VI and VII are those from the 0–4- and 0–24-h urine collection periods; the former covered the period of maximum diuresis (Tables IV and V) and was considered to be the most appropriate measure of meaningful pharmacodynamic effect. The mean 0–4-h urine volume following product I was 1460 mL (range 771–2010 mL); the equivalent mean result for product II was 1231 mL (range 590–2082 mL). The mean 0–4-h urine sodium output following product I was 181 mmol (range 95–296 mmol), and the mean 0–4-h urine sodium output following generic furosemide was 150 mmol (range 62–288 mmol). Most subjects showed less diuresis following product II; however, for the 0–4-h urine volume this trend was clearly reversed in subjects 9 and 10. With respect to urinary sodium, subject 10 again reversed the general trend toward diminished natriuresis following product II; subject 9 showed little difference between treatments in this respect.

Statistical analysis of these pharmacodynamic results (Table VII), in contrast with the corresponding analysis of pharmacokinetic data, did not show any significant differences between the effects of the two furosemide preparations. However, the 95% confidence intervals, especially for 0–4-h data, were asymmetrical with product II giving between −33% and 2% ($p = 0.07$) of the 0–4-h urine output of product I and between −38% and 4% ($p = 0.08$) of the 0–4-h sodium output of product I, reflecting the general trend for the generic formulation to show a diminished effect.

DISCUSSION

This study has shown statistically significant differences in the bioavailability of two furosemide formulations, the generic tablet giving up to 66% lower C_{max} values, up to 52% lower $AUC_{0-\infty}$ values, and up to 37% less urinary elimination of furosemide over 24 h. Although not statistically significant, there was also a trend toward later t_{max} values following the generic treatment. However, simultaneous analysis of drug effect did not give correspondingly significant differences in urine output and sodium excretion. Nevertheless,

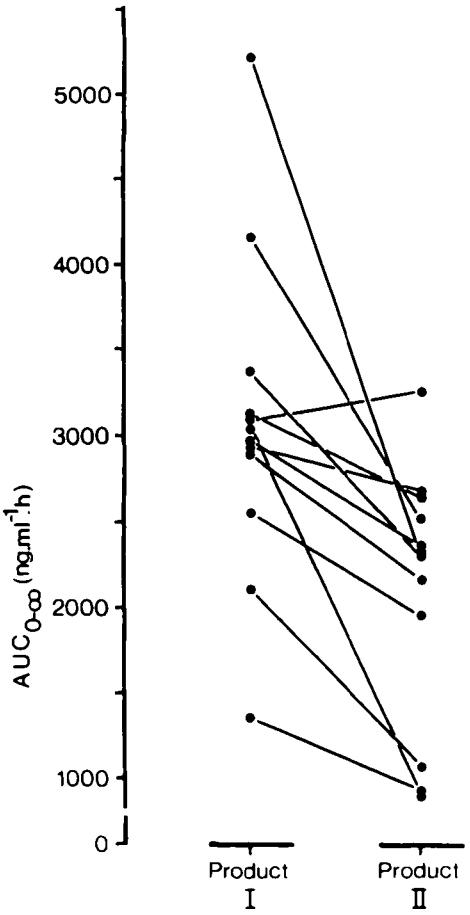

Figure 2—*Comparative bioavailability of the two furosemide formulations.*

pharmacodynamic differences between formulations over 0–4-h collection periods were significant at the 10% level, and the 95% confidence intervals were clearly asymmetrical; therefore, biological equivalence of these two formulations cannot be assumed.

The analysis of pharmacodynamic effects has concentrated on 0–4-h data rather than the complete collection interval of the experiment, since following acute diuresis and natriuresis in normal subjects, homeostatic mechanisms can result in compensatory "rebound" conservation of salt and water by 24 h. This tendency is shown by the diminished significance of 0–24-h statistical analysis in comparison with that for 0–4 h, especially with respect to urinary sodium excretion. The inherent logarithmic shape of the classical dose–response curve in pharmacology makes any pharmacodynamic measurement a less sensitive measure of formulation differences, since a given percentage change in drug concentration will give a smaller percentage change in drug effect: a linear change on the latter scale corresponds to a logarithmic change on the former scale.

This study supports the clinical observations of diuretic ineffectiveness which led to the withdrawal of this generic furosemide product. Since it has been reported that the absorption of furosemide is impaired in the presence of edema (8), it is possible that unequivalent furosemide formulations may show proportionately greater pharmacokinetic and pharmacodynamic differences in patients with cardiac failure than in normal subjects.

Kelly *et al.* (9) have previously shown that the bioavailability of brand-name furosemide tablets was similar to that of an aqueous solution. A comparative bioavailability study from Sweden (10) which included pharmacokinetic and pharmacodynamic observations revealed no significant differences between brand-name furosemide tablets and a generic tablet; however, a study from Israel (11) demonstrated statistically significant pharmacokinetic and pharmacodynamic differences between two other furosemide products. A preliminary observation from investigators in New Zealand (12) suggested that there may be clinically important differences in the response to brand-name and generic furosemide tablets, although a later more formal study (13) reported that the two furosemide products in question were in fact bioequivalent. However, it should be noted that this last study consisted only of urinary pharmacodynamic measurements, and our present investigation has shown that this may not be a sufficiently sensitive technique. Investigators in Australia (14) reported bioequivalence of yet another generic furosemide product basing their conclusions on measurements of urine volume and urine electrolytes.

Table VII—Summary of Statistical Results

Parameter	Overall Differences (ANOVA)[a] Subject Treatment Week 1	2	3	95% Confidence Interval (Generic as % of Standard)
C_{max}	NS	**	NS	−66% to −21%
t_{max}	NS	NS	NS	− 8% to +82%
$t_{1/2\alpha}$	NS	NS	NS	−18% to +21%
$t_{1/2\beta}$	NS	NS	•	−24% to +37%
$AUC_{0-10\ h}$	*	**	NS	−50% to −14%
$AUC_{0-\infty}$	•	**	NS	−52% to −15%
0–24 h urinary furosemide	NS	*	NS	−37% to − 5%
Urine volume				
0–4 h	NS	NS	NS	−33% to + 2%
0–24 h	NS	NS	NS	−15% to + 2%
Sodium output				
0–4 h	*	NS	NS	−38% to + 4%
0–24 h	**	NS	*	−19% to +10%

[a] Key: (NS) = not significant; (*) = significant at the 5% level; (**) = significant at the 1% level.

Furosemide can be used in clinical circumstances where a prompt diuresis is essential. While it is clear from the above discussion that some generic formulations are equivalent to brand-name tablets, it is also evident that other products may not be adequate. Alternative furosemide formulations should be carefully evaluated, preferably by means of full pharmacokinetic and pharmacodynamic investigations in humans.

In the absence of greater detail, it is difficult to comment on any pharmaceutical factors which may have contributed to inequivalence of these furosemide formulations. However, calcium and phosphate were detected in product II and not in product I following the application of standard National Formulary methods[10]. Residue analysis also showed differences in the amounts of inorganic excipients (4% for product I and 37% for product II)[10]. These observations appear to be in keeping with the findings of Rubinstein (15) who reported that the bioavailability of experimental batches of furosemide was considerably influenced by the choice of excipient. He also found a poor correlation between dissolution rate and drug bioavailability.

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