Diese mit Radioisotopen markierte Untersuchung des Kardiologen Prof. Marchetti von 1971 der Universität Mailand und der Mitautoren aus der Pathologie und der Biochemie beweist die orale Resorption des Strophanthins über die Darmschleimhaut. Darüberhinaus eine lineare Korrelation zur verabfolgten Dosis, d.h. je mehr man gibt umso mehr kommt an. Diese Untersuchung bestätigt die Vielfache Feststellung anderer Autoren mit ähnlichen Fragestellungen.

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Blood Levels and Tissue Distribution of ^{3}H-Ouabain Administered per os

An experimental and clinical study

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Many investigations have been carried out on the metabolism of cardiac glycosides, using biological, colorimetric and polarographic methods (Hatcher and Brodie 1910; Richaud 1921; Knaffe-Lenz 1926; Hall 1932; Cattel and Gold 1938; Paff 1940; Lehman and Paff 1942; Gold *et al.* 1942; Friedman and Bine 1947; Hilton 1950; Magalini *et al.* 1955).

Although these studies were performed at a time when methods had not yet been fully developed for detecting very small amounts of cardiac glycosides in blood, urine and tissue, they enabled some important observations concerning the fate of cardiac drugs in the organism to be clarified. More recently most of these findings have been confirmed by using more sensitive methods employing labelled cardiac glycosides (Okita *et al.* 1955; Tubbs *et al.* 1964; Marchetti *et al.* 1967; Doherty 1968).

Most of these investigations deal with digoxin and only a small number with digitoxin and deslanoside C. The present knowledge of g-strophantin (ouabain) is very scanty especially as regards its absorption from the gastro-intestinal tract (De *et al.* 1961; Garbe and Nowak 1968; Forth *et al.* 1969; Greenberger *et al.* 1969, Lahrtz *et al.* 1968).

From the data available so far it seems that ouabain is absorbed from the intestinal tract even if to a lesser degree than digoxin, peruvoside and proscillaridin.

This study has been undertaken with a view to investigating the intestinal absorption of tritium-labelled ouabain more closely. Non-anaesthetized guinea-pigs were used in an attempt to obtain better physiological experimental conditions. The results were compared with those obtained in human subjects without heart disease.

Methods

The tritium-labelled ouabain was checked for purity by thin-layer chromatography (Kieselgel GF_{254} Merck, thickness 0.5 mm) and was eluted with chloroform/methanol/water = 65/35/5.

Twenty bands 1 cm wide were removed from the layer and counted with a Packard Model 3320 Tri-Carb Liquid Scintillation Spectrometer.

The purity of ^{3}H-ouabain was also confirmed by scanner radiochromatography and autoradiography on thin layers (Fig. 1).

In order to detect any possible exchange of ^{3}H between ^{3}H-ouabain and some constituents of the biological fluids or tissues, 0.3 ml of biological fluid (blood, urine, bile), 200 mg of intestine content, and 0.5 ml of homogenates of various organs and tissues (heart, liver, kidney, intestinal tract, spleen, muscle and skin) were mixed in test tubes with 1000 µg/kg of ^{3}H-ouabain having an activity of 100 000 dpm. The tubes were sealed with ground-glass stoppers and maintained at 37° C for 15 h, after which 5 ml of ethanol were added, the tubes were shaken and then centrifugated at 3000 rpm. for 5 min.

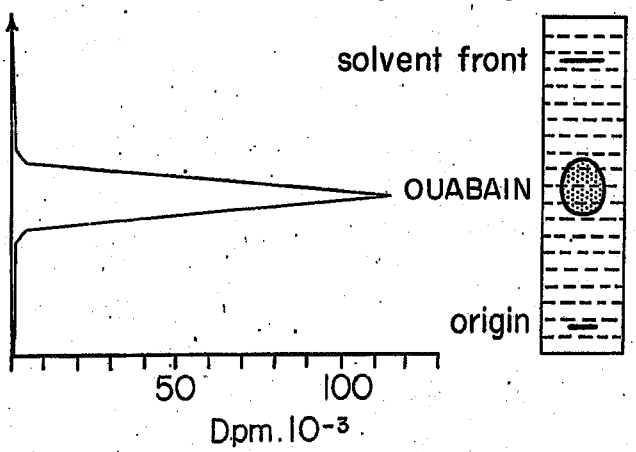

Fig. 1: Thin-layer chromatography (Kieselgel GF_{254}) showing purity of tritiated ouabain. Eluent is chloroform/methanol/water = 65/35/5. Twenty bands each 1 cm in width were removed and counted with a liquid scintillation spectrometer.

The clear supernatant was concentrated to about 0.5 ml at 37° C in a nitrogen stream; part of the supernatant was eluted on a thin layer plate with chloroform/methanol/water = 65/35/5.

Twenty bands 1 cm in width were removed from the thin layer, eluted and counted by the Tri Carb liquid scintillation counter. In agreement with the results of Cox *et al.* (1959) no labelled ouabain metabolites or ^3H bound with any other substances could be detected in any organ, tissue or biological fluid or even in the intestinal content.

Sixty-three guinea-pigs were starved for 24 h, divided into 3 groups and then given orally ouabain solution (50 µg/ml, specific activity of 2.84×10^8 dpm/ml) in amounts of 250, 500, 1000 µg/kg respectively. After being given ouabain the animals were placed in special individual metabolic cages. 21 animals were killed after 1 h, 21 after 5 h and 21 after 15 h, giving nine groups of 7 guinea-pigs.

Specimens of about 300 mg were drawn from the organs of the sacrificed animals, placed in a calibrated tube and weighed. The tubes were placed in a water bath at 40° C. The organs dissolved within about 3 h. One ml of the resulting solution was taken from each tube and placed in the count flasks, to which 15 ml of scintillation liquid (PPO 5 g, dimethyl POPOP 0.3 g, toluene to 1000 ml) were added.

Urine and bile were added directly to the scintillation liquid with 1 ml of Soluene TM 100 in order to allow the phases to mix. The flasks were placed in the Tricarb scintillation spectrometer and counted for 50 min. Gain was 50%.

After adding 15 000 dpm of ^3H-ouabain the counts were repeated for 2 min under the same analytical conditions in order to check the efficiency of the measurements in the organs (internal standard).

Background activities were obtained from parallel experiments on the organs of the five untreated guinea pigs; these ranged between 26 and 29 cpm (average 27 cpm) and were deducted from each count.

An Olivetti P 101 computer was used for data processing.

The distribution of absorbed ouabain in the different organs was calculated by assuming the blood volume equal to 10% and the skeletal muscular mass equal to 40% of body weight. The weight of the carcass (skin, hair, bones, and fat) was calculated from the difference between the weight of the organs, blood and muscle and total body weight. The amount of absorbed ouabain was obtained by subtracting the sum of all the radioactive values found in each organ from the total amount of radioactivity administered orally.

A clinical study was carried out on 21 patients without cardiac or digestive diseases, eight of the subjects being given 50 µg/kg ^3H-ouabain p. os, eight 100 µg/kg and five 150 µg/kg. A solution containing 1 mg/ml, having an activity of 133.776×10^8 dpm/ml was used. Blood and urine samples were collected from all the patients after 3, 5, 10 and 24 h and analysed using the same techniques employed in the guinea-pig experiments.

Results

1. Distribution of absorbed ouabain in guinea-pig organs

Table 1 shows the distribution of ouabain in the organs and biological fluids from the nine groups of guinea-pigs. Concentrations are expressed as mean values of specific activity in dpm/g or dpm/ml in each group. It will be seen that the amount of ouabain found in most of the tissues, blood and bile is similar, with the exception of the intestine, the kidneys and the urine, in which the glycoside concentration was found to be considerably higher.

2. Enteral absorption of ouabain in guinea-pigs

One hour after oral administration the total quantity of absorbed ouabain was about 3.5%; after 5 h it varied between 6% and 7%; after 15 h it had increased to between 9.4% and 9.7%. These results were the same with all three doses (Table 2).

Table 2 also shows the administered and absorbed doses expressed in dpm/kg, after 1, 5 and 15 h with all three doses (250, 500 and 1000 µg/kg).

Fig. 2 indicates the straight-lines of regression between the administered and absorbed amounts of ouabain during each experimental period (1, 5 and 15 h).

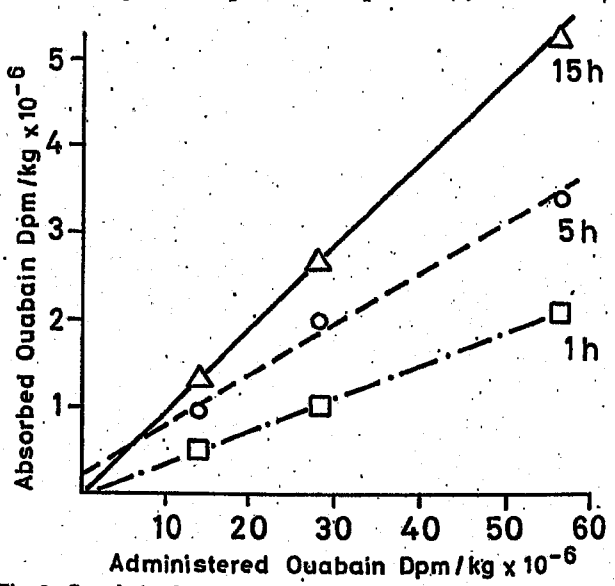

Fig. 2: Correlation between dose administered and quantity absorbed (in dpm/kg of body weight) in guinea-pigs killed 1, 5 and 15 h after receiving ouabain orally. Linear equations calculated by the straight-line regression method were: after 1 h: ^3H-ouabain absorbed (dpm/kg) = −40 494 + 0.0375 × ^3H-ouabain administered (dpm/kg); r = 1.0000; after 5 h: ^3H-ouabain absorbed (dpm/kg) = 226,000 + 0.0574 × ^3H-ouabain administered (dpm/kg); r = 0.9980; after 15 h: ^3H-ouabain absorbed (dpm/kg) = 27,000 + 0.0937 × ^3H-ouabain administered (dpm/kg); r = 1.0000.

Table 1: Specific activity in dpm/g or dpm/ml in organs, tissues and biological fluids of guinea-pigs treated orally with 3 doses (250, 500 and 1000 µg/kg of body weight) of tritiated ouabain. Twenty-one animals were killed after 1 h, twenty-one after 5 h and twenty-one after 15 h. Mean values ± standard error.

	Guinea pigs killed after 1 h			Guinea pigs killed after 5 h			Guinea pigs killed after 15 h		
Dose	250 µg/kg	500 µg/kg	1000 µg/kg	250 µg/kg	500 µg/kg	1000 µg/kg	250 µg/kg	500 µg/kg	1000 µg/kg
Number	7	7	7	7	7	7	7	7	7
Heart	204± 27	374± 59	547± 87	678± 17	1537± 126	2778± 40	592± 65	1222± 208	2858± 220
Liver	242± 30	428± 96	714± 130	892± 40	1978± 72	4469± 390	668± 75	1760± 20	4040± 270
Kidneys	925± 215	2586± 481	3395± 647	1599± 87	3260± 325	6815± 900	992± 64	3166± 340	6140± 665
Muscle	193± 28	357± 82	514± 37	788± 69	1716± 55	2755± 155	668± 22	1375± 68	2680± 190
Carcass	164± 34	246± 47	368± 57	632± 71	1220± 86	2511± 163	544± 25	1312± 157	2425± 270
Intestinal wall	7041±1213	12140±1845	37633±4299	3094± 337	6137±1074	8507±1230	3356± 704	5871±1313	14960±2960
Blood	289± 23	495± 54	708± 81	1069± 79	1577± 74	3782± 85	1018± 23	1515± 53	3805± 217
Urine	3075±2201	4687± 827	11548±4798	4416± 516	14528± 864	35035±7622	15875±5670	34379±8115	59140±9340
Bile	388± 103	588± 90	868± 131	1326± 21	2265± 41	4374± 426	1458± 89	2740± 307	8910± 620
Spleen	260± 28	355± 92	674± 45	371± 56	1123± 90	2824± 564	430± 116	1085± 72	1630± 208

Table 2: Percentage of intestinal absorption, activity administered and activity absorbed (in dpm/kg of body weight) in guinea-pigs treated orally with tritiated ouabain as described in Table 1. Mean values ± standard error.

	Guinea pigs killed after 1 h			Guinea pigs killed after 5 h			Guinea pigs killed after 15 h		
Dose	250 µg/kg	500 µg/kg	1000 µg/kg	250 µg/kg	500 µg/kg	1000 µg/kg	250 µg/kg	500 µg/kg	1000 µg/kg
Number	7	7	7	7	7	7	7	7	7
% of absorbed ouabain	3.54±0.28	3.55±0.19	3.66±0.30	6.81±0.38	6.86±0.23	6.06±0.30	9.71±0.59	9.42±0.49	9.40±0.63
Administered activity in dpm/kg	14 090 000	28 180 000	56 360 000	14 090 000	28 180 000	56 360 000	14 090 000	28 180 000	56 360 000
Absorbed activity in dpm/kg	490 500 ±36 950	1 003 000 ±53 350	2 065 000 ±169 700	968 200 ±54 415	1 941 500 ±64 293	3 430 000 ±171 468	1 345 286 ±94 717	2 671 500 ±144 500	5 307 500 ±358 000

The coefficient of linear correlation is practically 1, which shows that the quantity of absorbed ouabain is closely proportional to the doses administered.

3. Blood levels and urinary excretion of ouabain in non-cardiac human subjects

Table 3 shows the specific activities in dpm/ml found in the blood and urine of twenty-one patients.

Urinary elimination at each of the three doses given (50, 100 and 150 μg/kg) is illustrated in Fig. 3.

Ouabain could be detected in the urine three hours after administration and increased gradually in quantity until it reached 1%—1.5% of the total administered dose after 24 h.

Ouabain levels in blood and urine of humans were found to be practically comparable with those observed in guinea-pig organic fluids and tissues. It is thus likely that ouabain distribution in the body is quite uniform and that its blood levels reflect the mean body concentration fairly closely. On this assumption, the quantities of ouabain in the human body were calculated by multiplying body weight by the specific activity in dpm/ml found in the patients blood 5 h after administration. By adding this radioactivity value to that eliminated in the urine after 5 h, it was possible to calculate the total quantity of ouabain absorbed by the intestinal tract. This varied between 6.87% and 7.04% of the total doses administered, and was close to that observed in the guinea-pigs (Tab. 4, Fig. 4).

Discussion

The results obtained in guinea pig experiments show that about 3.5% of the total dose of ouabain administered per os accumulates in the blood, urine, organs and tissues within 1 h; this percentage rises to 6—7% after 5 h, and to 9.5% after 15 h. By increasing the doses of ouabain the amount which accumulates in the tissues and fluids increases proportionately; the percentage absorbed after 1,5 and 15 h therefore remains practically unchanged.

These results agree with data obtained by Forth *et al.* (1969), who carried out investigations with ^{3}H-ouabain on the isolated or *in situ* intestine of rats and guinea-pigs, whereas they do not agree with those of Lauterbach and Vogel, who could not find any absorption proportional to the administered doses for quantities exceeding about 50% of the lethal doses. It

Table 3: Specific activities of ^{3}H-ouabain (dpm/ml) in blood and urine found in patients orally given 50, 100 and 150 μg/kg after 3, 5, 10 and 24 h.

Patients			Administered ouabain	Blood				Urine									
				3 h	5 h	10 h	24 h	3 h		5 h		10 h		24 h			
Name	Weight	μg/kg	dpm/kg	dpm/ml	dpm/ml	dpm/ml	dpm/ml	ml	dpm/ml	Total dpm	ml	dpm/ml	Total dpm	ml	dpm/ml	Total dpm	Total dpm
B.C.	75	50	6 518 000	453	402	382	389	50	16 419	820 950	240	4 124	989 760	170	5 887	1 000 790	1 795 600
O.M.	57			498	470	412	405	120	3 485	418 200	150	2 015	301 950	80	10 175	813 840	1 797 300
G.G.	61			458	454	449	435	110	4 480	492 800	150	5 925	510 250	200	4 661	932 200	1 569 000
S.M.	62			586	586	488	440	160	8 630	1 380 800	20	24 400	488 000	120	16 952	2 034 240	1 757 280
C.G.	51			540	520	520	520	180	12 200	2 196 000	150	4 396	659 400	250	4 084	1 021 000	1 573 000
B.A.	107			474	420	568	568	65	12 920	839 800	80	15 080	1 206 400	260	6 540	1 648 400	1 890 400
M.T.	58			420	420	368	316	100	13 100	1 310 000	120	11 320	1 358 400	420	6 000	2 520 000	2 160 000
R.O.	51.5			420	570	316	262	160	7 880	1 260 800	45	18 140	816 300	155	7 260	1 125 300	1 120 000
Average ± standard error				456 ±25	428 ±28	384 ±20	367 ±22	118 ±16	9 889 ±1 616	1 089 918 ±203 907	117 ±24	10 425 ±2 915	791 307 ±151 052	206 ±37	7 669 ±1 492	1 386 971 ±217 860	1 707 572 ±106 788
M.G.	56	100	13 056 000	1291	1186	1185	1196	90	6 951	625 590	25	8 395	209 825	140	6 772	948 080	4 725	2 835 000
B.A.	65			922	843	758	764	530	5 016	2 658 480	140	4 531	634 340	650	3 597	2 286 110	1 874	2 511 150
F.A.	45			722	744	658	644	60	50 952	1 857 120	25	24 935	623 375	100	13 083	1 508 300	1 340	4 022	2 011 000
M.A.	64			1000	960	820	860	120	26 794	3 215 280	90	20 682	1 861 380	160	14 806	2 368 960	500	6 752	2 700 800
P.L.	65			920	740	800	640	290	9 946	2 874 260	70	20 090	1 406 300	190	12 266	2 330 540	400	5 894	2 686 860
B.P.	51			950	844	790	686	180	4 860	874 800	100	16 360	1 636 000	140	10 280	1 439 200	690	3 160	1 738 000
B.A.	57.5			790	736	704	696	200	9 560	1 912 000	55	20 480	1 126 400	230	11 500	2 645 000	550	6 380	3 828 000
Z.D.	66			842	790	684	580	220	5 500	1 210 000	25	9 420	235 500	175	14 660	2 565 500	600	9 560	6 405 200
Average ± standard error				950 ±61	855 ±55	800 ±59	758 ±70	211 ±55	12 447 ±3 707	1 903 441 ±338 265	66 ±15	15 612 ±2 597	966 646 ±223 726	220 ±60	10 870 ±1 397	1 983 961 ±229 450	668 ±101	5 046 ±866	3 089 502 ±521 699
B.L.	50	150	19 554 000	1768	1542	1356	1328	800	5 990	4 792 500	120	19 579	2 349 509	120	34 235	4 108 216	400	13 991	5 596 531
L.R.	60			1412	1376	1186	1158	175	28 527	4 957 000	5	26 232	131 162	240	13 140	3 159 946	400	5 468	2 187 245
A.M.	57.8			1360	1142	1086	1094	100	30 972	3 097 000	20	45 767	875 347	110	20 330	2 236 290	400	6 183	2 473 286
P.E.	61.7			1328	1260	1220	1170	220	20 506	4 511 295	110	23 868	2 625 000	250	14 824	3 706 090	1200	6 672	8 006 170
B.F.	66.2			1450	1274	1126	1150	160	29 175	4 668 000	80	33 205	2 656 445	200	18 785	3 757 000	700	7 552	5 286 154
Average ± standard error				1460 ±80	1519 ±68	1191 ±44	1180 ±40	291 ± 129	24 566 ±5 009	4 405 119 ±335 125	67 ±25	31 536 ±4 594	1 727 492 ±516 242	184 ±29	21 649 ±8 902	3 392 308 ±326 905	620 ±156	8 518 ±1 677	4 709 877 ±1 080 505

should however be noted that these data were obtained with an indirect biological method described by Hatcher (1910). Also, our results differ considerably from those obtained in three human subjects by Lahrtz *et al.*, who were not able to detect any radioactivity in the blood after oral administration of 1 μC/kg of ^{3}H-ouabain. But the amount of radioactivity administered by Lahrtz *et al.* was probably too small to give rise to any detectable quantities of ouabain in the blood. However, the finding in urine of about 0.5—2% of ^{3}H-ouabain demonstrates that even in the patients studied by Lahrtz *et al.* this drug was significantly absorbed.

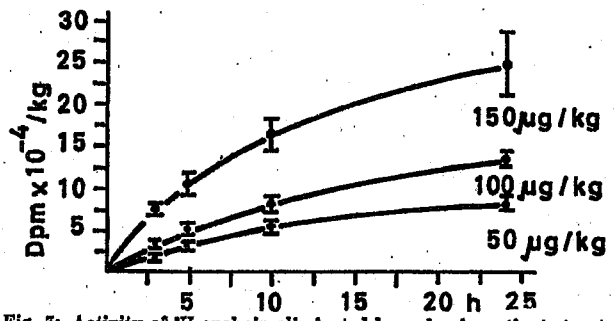

Fig. 3: Activity of ^{3}H-ouabain eliminated by urine in patients treated orally with 50, 100, 150 μg/kg.

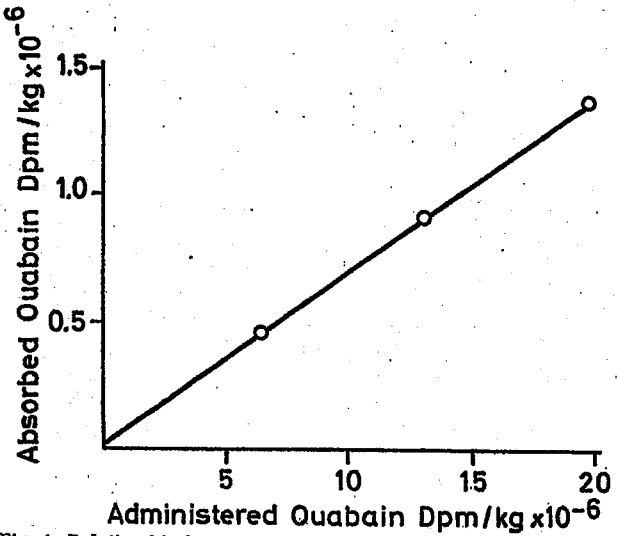

Fig. 4: Relationship between quantity of ouabain administered orally and amount absorbed in man. After 5 h: ^{3}H-ouabain absorbed (dpm/kg) = 17 706 + 0.0678 × ^{3}H-ouabain administered (dpm/kg); r = 0.9453.

^{3}H-oubain levels in the blood and tissues of the guinea-pig show that the drug becomes distributed throughout the body in quite a uniform way, the highest concentrations being found in the kidneys and urine. The high concentration observed in the intestinal wall can be explained if it is borne in mind that more than 90% of the ^{3}H-ouabain administered is present in the intestine contents. It may thus be deduced that ouabain can easily penetrate the intestinal epithelial cells but that its transfer from the cells to the intestinal vessels is definitely limited.

The results described here indicate that with ouabain as with other cardiac glycosides (digoxin — Doherty 1968; deslanoside C — Marchetti *et al.* 1967) it is not possible to demonstrate any preferential accumulation in the myocardium, but only a higher concentration in the kidneys. It may be concluded that the amount of ^{3}H-ouabain in the tissues is mainly related to enteral absorption, a stage which can be considered to be the "rate limiting step" in the kinetics of the drug when administered orally.

Summary

Tissue distribution, urinary clearance and per cent intestinal absorption of tritiated g-strophantin (ouabain) were investigated by the liquid scintillation technique in guinea-pigs after oral administration of 250, 500 and 1000 μg/kg respectively.

The results showed that ouabain is distributed quite uniformly in all tissues, its level being higher in the intestinal wall and the kidneys. The highest concentration was observed in the urine.

The intestinal absorption of ouabain amounted to 3.5, 6—7% and 9.5%, 1, 5 and 15 h respectively after administration of all doses. There was a linear correlation between the doses administered and the quantity absorbed. Data relating to blood and urine levels in twenty-one human subjects are in agreement with the results reported above.

Table 4: Activity of ^{3}H-oubain administered, present in the body, eliminated by urine and % of intestinal absorption in patients after 5 h.

Patients		Administered ouabain		Ouabain eliminated by urine		Ouabain in the body		Absorbed ouabain		% of absorbed ouabain	
Name	Body Weight kg	μg/kg	dpm/kg	dpm/kg		dpm/kg		dpm/kg			
B.C.	73			24 800		402 000		426 800		6.55	
O.M.	57			12 620		470 000		482 620		7.40	
G.G.	61			16 400		434 000		450 400		6.91	
S.M.	62			30 200	30 690	587 500	428 062	617 700	458 752	9.48	7.04
C.G.	51	50	6 518 000	56 000	±5 460	320 000	±27 730	376 000	±25 610	5.77	±0.39
B.A.	107			19 100		421 000		440 100		6.75	
M.T.	58			46 000		420 000		466 000		7.15	
R.O.	51.3			40 400		370 000		410 400		6.30	
M.G.	56			14 900		1 186 000		1 200 900		9.21	
B.A.	65			50 700		843 000		893 700		6.85	
F.A.	45			56 100		741 000		797 100		6.11	
M.A.	64			79 400	48 862	960 000	855 000	1 039 400	903 862	7.98	6.93
P.L.	65	100	13 036 000	66 000	±7 540	741 000	±54 290	807 000	±51 780	6.19	±0.40
B.P.	51			49 200		843 000		892 200		6.84	
B.A.	57.5			52 800		738 000		790 800		6.07	
Z.D.	66			21 800		788 000		809 800		6.21	
B.L.	50			143 000		1 447 000		1 590 000		8.14	
B.F.	60			85 000		1 292 000		1 377 000		7.04	
P.E.	57.8	150	19 554 000	68 800	104 680	1 072 000	1 237 600	1 140 800	1 342 280	5.83	6.87
A.M.	61.7			115 800	±12 853	1 182 000	±62 900	1 297 800	±73 162	6.64	±0.38
L.R.	66.2			110 800		1 195 000		1 305 800		6.68	

Zusammenfassung

Blutspiegel und Verteilung im Gewebe von oral verabreichtem ^{3}H-Ouabain

Experimentelle und klinische Untersuchungen

Die Verteilung im Gewebe, die Ausscheidung mit dem Urin und der Prozentsatz der intestinalen Resorption von mit Tritium markiertem g-Strophanthin (Ouabain) wurden mit Hilfe der Liquid-Scintillations-Technik an Meerschweinchen untersucht nach oraler Gabe von 250, 500 bzw. 1000 μg/kg.

Es zeigte sich, daß Ouabain in allen Geweben ziemlich einheitlich verteilt war, nur in der Darmwand und in den Nieren war der Gehalt höher. Die höchste Konzentration wurde im Urin gefunden.

Die intestinale Resorption von Ouabain betrug 1, 5 bzw. 15 h nach Verabreichung 3,5, 6—7 bzw. 9,5%. Es bestand eine lineare Korrelation zwischen den verabreichten Dosen und der resorbierten Menge. Die bei 21 Probanden festgestellten Konzentrationen in Blut und Urin stimmen mit den beschriebenen Befunden überein.

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Die Wirkstoff-Freigabe bei magensaftresistent überzogenen Gelatinekapseln in vivo und in vitro

4. Mitteilung: Untersuchungen mit der pH-Endoradiosonde am Menschen

Von Th. Eckert, G. Cordes und R. Seidel

1. EINLEITUNG UND PROBLEMSTELLUNG

Die Erfassung der Wirkstoff-Freigabe aus den Arzneiformen ist die erste Phase jeder pharmakokinetischen Untersuchung. Die vorliegende Arbeit befaßt sich insbesondere mit der im allgemeinen nur schwer erfaßbaren Wirkstoff-Freigabe im Darm des Menschen.

Bei einer großen Zahl von Wirkstoffen ist es erforderlich, sie vor der peroralen Applikation mit einem magensaftresistenten Lack zu überziehen. Dies geschieht im wesentlichen aus 2 unterschiedlichen Gründen: einmal um säureempfindliche Substanzen vor der Salzsäure des Magens zu schützen, in anderen Fällen um Reizungen der Magenschleimhaut durch den Wirkstoff zu vermeiden. Im Hinblick auf die breite Anwendung dieser Verfahrensweise werden von den meisten Pharmakopöen Prüfungsvorschriften auf die Magensaftresistenz solcher Arzneiformen und ihrer Zerfallbarkeit in künstlichen Darmsäften angegeben. Es handelt sich jedoch dabei ausschließlich um in-vitro-Teste mit künstlichen Verdauungssäften, deren Aussagefähigkeit für die Wirkstoff-Freigabe in vivo nur sehr begrenzt ist.

Es ist das Ziel der vorliegenden Untersuchung, am Beispiel von Gelatine-Steckkapseln die Abhängigkeit der Wirkstoff-Freigabe im Darm des Menschen von der Menge des magensaftresistenten Überzugs zu ermitteln. Aus einer derartigen Untersuchung ergibt sich für die pharmazeutische Praxis die Möglichkeit, den Überzug nach Menge und Auftragsart so einzustellen, daß der Wirkstoff zeitlich gezielt nach einer bestimmten Verweilzeit im Darm freigesetzt wird. Zum anderen ergeben sich aus einem Vergleich der Wirkstoff-Freigabe in vivo mit in-vitro-Ergebnissen Korrelationen zwischen der Freigabe in vivo und der in vitro.

Alle zu dieser Frage bisher mit unterschiedlichen Methoden durchgeführten Untersuchungen [1—5] haben zu dem Ergebnis geführt, daß die Wirkstoff-Freigabe in vivo wesentlich längere Zeit in Anspruch nimmt, als dies bei den Versuchen in vitro der Fall war. Hier muß allerdings darauf hingewiesen werden, daß die Methoden zur Erfassung der Freigabe in vivo sehr unterschiedlich sind. Die röntgenographische Methode mit Hilfe einer $BaSO_4$-haltigen Arzneiform erlaubt — von der Strahlenbelastung abgesehen — nur eine zeitlich diskontinuierliche Beobachtung. In die Bestimmung der Blutspiegelwerte geht außer der Wirkstoff-Freigabe auch die Absorptionsgeschwindigkeit ein. Demgegenüber ist die im Rahmen dieser Arbeit angewendete Methode der pH-Endoradiosonde [6, 7, 8] zur selektiven Feststellung der Wirkstoff-Freigabe insbesondere auch im Darm sehr geeignet.

2. MAGENSAFTRESISTENT ÜBERZOGENE GELATINE-STECKKAPSELN

Die im folgenden beschriebenen Untersuchungen zur Wirkstoff-Freigabe in vivo und in vitro wurden mit magensaftresistent überzogenen Gelatine-Steckkapseln (Capsugel®, „Snap-fit" Nr. 1) durchgeführt, die pro Kapsel 250 mg feingepulvertes Glutaminsäure-HCl enthielten. Glutaminsäure-HCl wurde als „Wirkstoff" deshalb gewählt, weil es bei der Freisetzung der sauer reagierenden Substanz in dem annähernd neutralen Milieu des Darms zu einer milden Ansäuerung kommt, die von einer angekoppelten pH-Endoradiosonde angezeigt wird.

Die so gefüllten Gelatinekapseln waren zur Erzielung einer Magensaftresistenz nach einem besonderen Verfahren [9] im Wirbelbett chargenweise mit wechselnden Mengen Celluloseacetatphthalat (CAP) überzogen, nachdem zuvor die Kapseloberfläche zur Erzielung einer besseren Haftfähigkeit vorbehandelt worden war*).

Der Überzug hatte folgende Zusammensetzung:

Celluloseacetatphthalat	76,3%
Cera alba	5,9%
1,2-Propylenglycol	5,9%
1,5-Sorbitanmonooleat	11,9%

Durch eine spezielle Auftragetechnik wurde sichergestellt, daß der Lackfilm auf der Kapsel eine sehr gleichmäßige Beschaffenheit aufwies. Die gleichmäßige Stärke des Überzuges ist von entscheidender Bedeutung insbesondere für die im Rahmen dieser Arbeit angestrebten Aufstellung einer Korrelation zwischen der Überzugsmenge und der Zerfallszeit. Wenn der Lackauftrag mit einer anderen Technik durchgeführt würde, so müßte die erforderliche Menge an CAP neu festgelegt werden.

Die Dicke des Überzugs bei Kapseln mit einem Überzug von 2 mg CAP/cm² wurde mit dem Lichtschnitt-Mikroskop „Zeiss" geprüft. Die Messungen wurden bei einer Objektivvergrößerung 400× an verschiedenen Stellen der Kapseloberfläche in Abständen von jeweils 100 μ und bei verschiedenen Kapseln durchgeführt (Abb. 1).

Die Messungen ergaben eine scheinbare Dicke des Überzuges s' von 10,8 μ mit einer mittleren Standardabweichung ± 9,1%. Hieraus wurde mit Hilfe des Brechungsindex n die wahre Schichtdicke s berechnet.

$$s = s' \sqrt{2\,n^2 - 1}$$

$$s = 20{,}4\ \mu \pm 9{,}1\%$$

*) Hersteller: Sanol Arzneimittel Dr. Schwarz GmbH, Monheim.