Communications to the Editor

[Chem. Pharm. Bull. 23(12)2293—2296(1975)]

UDC 615.31.033.1 : 615.076.9

Simple Method for Repetitive Blood Sampling in a Self-Controlled Design in the Rat

A simple and reliable method was devised for investigating the effect of another drug on pharmacokinetic parameters of a drug in the rat. This consists of the cannulation technique repeated after a suitable interval in the same rat. The effect of phenobarbital on the elimination of diphenylhydantoin was presented as a typical example.

In an attempt to study drug interactions in the rat, a method for repetitive blood sampling repeated twice at a suitable interval in the same rat was devised. The kinetics of drug absorption, distribution, metabolism, and excretion may be modified by the prior or concurrent administration of another drug. To examine such pharmacokinetic interactions, pharmacokinetic parameters derived from the blood concentration-time curve after administration of a drug are compared with those after the concurrent administration of another drug with the drug. A self-controlled design is generally preferable for comparative studies, since there would be considerable inter-individual variations in pharmacokinetic parameters. The rat is not suitable for repetitive blood sampling repeated twice at an interval. Therefore, it is necessary to resort to larger animal species for this experimental design. In a drug interaction study using the rat, a statistical analysis may be performed on observed data and/or the derived pharmacokinetic parameters for the control and the treated group. However, a number of rats are usually needed in such a statistical study.

In this communication, we report a simple method for studying drug interaction in the rat, using a combination of diphenylhydantoin (DPH) and phenobarbital (PB) as a typical example. The method consists in repetitive blood sampling with transfusion repeated after an interval and retrieving the blood flow of the cannulated femoral artery and vein after the end of a first experiment. A rat, 200 to 280 g, is held on its back to a board and is anesthetized lightly with ether. The right or left femoral vein is exposed and then ligated just above the root of the superficial epigastric vein. A cannula (polyethylene tubing, 0.04 cm i.d., 0.06 cm o.d., 7 cm length), filled with heparin solution (200 units/ml) and closed with an arterial clamp at the other end, is inserted into the femoral vein, approximately 0.5 cm proximal to the ligature after careful venous puncture using a needle point. The cannula is introduced proximally up to 1.5—2.0 cm from the cut, and is secured by tying firmly around the vein. The ends of the tie are left uncut, and then again tied around the exposed tubing. The femoral artery on the same side is cannulated in a similar way.

A test drug is rapidly administered to the rat *via* the cannula inserted into the femoral vein two hours after the operation described above. An appropriate volume of blood sample is withdrawn into a small test tube at various intervals *via* the cannula inserted into the femoral artery. After each blood sampling, an equivalent volume of blood is transfused *via* the cannula inserted into the femoral vein. The blood for transfusion is taken from another rat of the same strain.

The ties securing the cannula inserted into one of the blood vessels are unfasten after the last transfusion, the cannula is extracted from the vessel, and then the ligature of the vessel is untied. A small amount of the rapidly polymerizing adhesive for surgical applications (Aron Alpha A "Sankyo") is used to close the cut. During this maneuver the both sides of the vessel to the cut are lifted up with the tips of a pair of fine curved-forceps to prevent bleeding from the cut. The blood flow in the vessel can be retrieved immediately, when the forceps are taken off and the blood vessel is lowered to its original position. The blood flow

of the other vessel is retrieved in a similar way. The subcutaneous tissue around the exposed vein and artery is reunited and the femoral skin incision is closed by discontinuous sutures. Then, the rat is individually housed and allowed free access to water and laboratory chow.

After predetermined days, the femoral vein and artery of the rat on the other side are cannulated in the same manner as described above. A cannula inserted into the cut of the femoral vein is used to administer the test drug and another drug which may possibly alter pharmacokinetic parameters of the test drug. Another cannula inserted from the same cut of the femoral vein is used to infuse another drug continuously. The cannula inserted into the femoral artery is used to withdraw blood samples. The order of the first control experiment and the second experiment to prove drug interactions may be exchanged in the above procedures. Instead of concurrent administration, drug interactions produced by enzyme induction may be shown by administering an inducer during the period between the two experiments.

The blood concentration-time curve of DPH after intravenous infusion was described in all rats by a biexponential equation ($\alpha > \beta$) of the form $C = Ae^{-\alpha t} + Be^{-\beta t}$. The estimated α values were larger than eight times the estimated β values, whereas the coefficients A and B were comparable values. Thus, the alteration of the elimination rate of DPH was estimated by a direct comparison of the slower disposition rate constant β, since the second term of the biexponential equation represented over nearly all the time course of blood DPH level. Table I lists the estimated β values and volumes of distribution after an intravenous bolus at repeated

Table I. Reproducibility of the Rate Constant β and Volume of Distribution of Diphenylhydantoin in the Same Rat after an Interval of One Week

Dose (mg/kg)	Rat	β (hr^{-1})			Volume of distribution (ml/kg)[b]		
		1st Exp.	2nd Exp.	Ratio[a]	1st Exp.	2nd Exp.	Ratio[a]
10	A	2.87	2.74	0.96	569	525	0.92
	B	1.82	1.82	1.00	493	499	1.01
	C	3.50	3.69	1.05	552	527	0.95
30	D	0.82	0.81	1.00	557	561	1.01
	E	0.49	0.50	1.02	440	512	1.16
	F	1.18	1.13	0.96	431	484	1.12

a) calculated as the second value divided by the first value.
b) calculated as a dose divided by a sum of A and B, which are the two coefficients of the biexponential equation obtained from a semilogarithmic plot of the blood concentration-time curve.

equal doses of 10 or 30 mg/kg DPH at an interval of one week. It is shown that satisfactorily reproducible β values and volumes of distribution for the two experiments were obtained. The individual β values at doses of 10 and 30 mg/kg varied 1.82 to 3.50 hr^{-1} and 0.49 to 1.18 hr^{-1}, respectively. However, no appreciable difference in β between the two experiments performed on different days was observed at the equal dose in the same rat.

It has been reported that the lowering of blood DPH level and shortening its biological half-life by chronic concurrent administration of PB in human and the rat arises as a result of induction of the drug-metabolizing enzymes (mixed oxigenases),[1,2] whereas acute concurrent administration of PB results in the competitive inhibition of DPH metabolism in the enzyme system.[2] This alteration in metabolic rate of DPH was examined quantitatively by comparing an estimated β value with the control β value in the same rat. The effect of PB pretreatment on the DPH disappearance rate was investigated by giving once a day over three days from the fourth day after a first control experiment. The second administration of an equal

1) P.L. Morselli, M. Rizzo, and S. Garattini, *Ann. N.Y. Acad. Sci.*, **179**, 88 (1971).
2) H. Kutt, *Ann. N.Y. Acad. Sci.*, **179**, 704 (1971).

DPH dose was given 24 hours after the last dose of PB. Approximately 10 blood samples (0.1—0.2 ml each) were taken at various times over 3 hours *via* a cannula inserted into the femoral artery. Blood concentrations of DPH and PB were determined by a minor modification of the ultraviolet spectrophotometric method of Saitoh, *et al.*[3] The effect of acute concurrent PB administration on the DPH disappearance rate was investigated by maintaining a constant PB level of approximately 25 μg/ml by using an intravenous bolus and a subsequent constant-rate infusion of PB on the seventh day after the first control experiment. An equal DPH dose to that in the first experiment was given after the constant PB concentration was attained. Blood samples were taken under the PB infusion and determined in the same manner as the pretreatment experiment. The results are shown in Table II. The repeated

Table II. Alteration in the Rate Constant β and Volume of Distribution of Diphenylhydantoin (DPH) after the Pretreatment and Concurrent Administration of Phenobarbital (PB)

DPH Dose (mg/kg)	Treatment	Rat	β (hr^{-1})			Volume of distribution (ml/kg)[a]		
			1st Exp.	2nd Exp.	Ratio[a]	1st Exp.	2nd Exp.	Ratio[a]
30	PB pretreatment[b] (75 mg/kg *i.p.*×3)	G	0.80	1.56	1.95	505	508	1.01
		H	0.74	2.28	3.09	618	634	1.03
		I	0.69	1.78	2.58	604	578	0.96
	Saline pretreatment[c] (1 ml/kg *i.p.*×3)	J	0.63	0.66	1.04	510	565	1.10
		K	0.53	0.53	1.00	583	634	1.08
		L	0.53	0.48	0.91	748	658	0.86
10	PB concurrent infusion[d]	M	1.77	0.88	0.49	525	400	0.76
		N	2.03	1.15	0.57	513	476	0.93
		O	2.68	1.39	0.52	569	519	0.91
	Saline concurrent infusion[e]	P	1.90	1.82	0.95	524	448	0.85
		Q	1.56	1.47	0.94	480	485	1.01
		R	2.41	2.50	1.04	526	532	1.01

a) the same as the legends of Table I
b) administered once a day for 3 days from the 4th day after the first control experiment
c) administered 0.9% NaCl 1 ml/kg in the same way as *b*)
d) administered in the second experiment by a constant intravenous infusion (1.05 mg/kg·hr) starting at 40 min after an intravenous bolus (21.5 mg/kg) to maintain a PB plateau level of approximately 25 μg/ml
The second DPH dose was given after the start of the intravenous infusion.
e) administered in the second experiment by a constant intravenous infusion (0.9% NaCl 0.12 ml/hr) starting at 40 min after an intravenous bolus (0.9% NaCl 1 ml/kg)

PB pretreatment increased the rate constant β of DPH at a dose of 30 mg/kg to about 2.5 times the control value. The blood PB concentration remained at a certain value in the range of 15—30 μg/ml throughout the second experiment. On the other hand, the concurrently administered PB decreased the rate constant β of DPH at a dose of 10 mg/kg to about one-half its control value.

The method of cannulations repeated after a suitable interval in the same rat presented here is simple and practical, and can be utilized to detect alterations of pharmacokinetic parameters of a drug unless significant intra-individual variations of the pharmacokinetic parameters are found at one time of administration and the next. It is desirable that the animal be permitted to recover for at least one week before initiating a second experiment. No appreciable difference in hematocrit value was observed between the first and second experiments. This technique in the rat has proved useful for studies on drug interacrions and details of these studies will be reported.

3) Y. Saitoh, K. Nishihara, F. Nakagawa, and T. Suzuki, *J. Pharm. Sci.*, **62**, 206 (1973).

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Received July 1, 1975

[Chem. Pharm. Bull. 23(12)3296—3298(1975)]

UDC 547.91.02 : 581.192

The Constituents of *Schizandra chinensis* BAILL. I. The Structures of Gomisin A, B and C

Three new schizandrin type lignans, gomisin A, B and C, were isolated from the fruits of *Schizandra chinensis* BAILL. (Schizandraceae) and their structures were elucidated to be I, II and III, respectively.

The fruits of *Schizandra chinensis* BAILL. (Schizandraceae) are used as an antitussive and tonic under the name of Kita-gomisi. Schizandrin, deoxyschizandrin, schizandrol and γ-schizandrin have been isolated from the unhydrolyzed fraction of the seed oil of this plant by Kochetkov, *et al.*[1)]

In this communication, we wish to report the structures of three new schizandrin type lignans, named gomisin A (I, yield 0.14%), B (II, 0.022%) and C (III, 0.004%), isolated from the fruits of the same plant. Their physical data are shown below.

Gomisin A (I) $C_{23}H_{28}O_7$ (M^+ 416.1868, Calcd. 416.1839), mp 88—89°, $[\alpha]_D$ +67.9°, ultraviolet (UV) λ_{max}^{EtOH} nm (log ε): 218 (4.88), 253 (4.30), 281 (sh 3.70) and 290 (sh 3.57), infrared (IR) ν_{max}^{KBr} cm^{-1}: 3500 (OH), nuclear magnetic resonance (NMR), δ in $CDCl_3$: 0.80 (3H, d, J= 7 Hz, >CH–C<u>H</u>$_3$), 1.25 (3H, s, HO–Ç–C<u>H</u>$_3$), 1.80 (1H, m, –C<u>H</u>), 1.85 (1H, s, OH), 2.44 (center) (2H, <u>ABX</u> octet, J_{AB}=14 Hz, J_{AX}=2 Hz, J_{BX}=6 Hz, –C<u>H</u>–C<u>H</u>$_2$–), 2.50 (center) (2H, AB q, J_{AB}=13.5 Hz, –CH_2–), 3.50 (3H, s), 3.80 (3H, s), 3.90 (6H, s) (4×OCH_3), 5.96 (2H, s, –OCH_2O–), 6.49 (1H, s, arom. H) and 6.62 (1H, s, arom. H).

Gomisin B (II), $C_{28}H_{34}O_9$ (M^+ 514), mp 95—97°, $[\alpha]_D$ −26.6°, UV λ_{max}^{EtOH} nm (log ε): 218 (4.98), 257 (sh 4.24) and 292 (sh 3.65), IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1715 (C=O), NMR, δ in $CDCl_3$: 1.13 (3H, d, J=7 Hz, –C<u>H</u>–C<u>H</u>$_3$), 1.33 (3H, s, HO–Ç–C<u>H</u>$_3$), 1.40 (3H, q, J=1.5 Hz, CH_3CH=C(C<u>H</u>$_3$)–), 1.60 (1H, s, OH), 1.86 (3H, d.q, J=7/1.5 Hz, C<u>H</u>$_3$CH=C(CH_3)–), 1.90 (1H, m, –C<u>H</u>), 2.0—2.45 (2H, m, –CH_2–), 3.57, 3.75, 3.85, 3.93 (each 3H, s, 4×OCH_3), 5.70 (1H, s, –C<u>H</u>–OCOR), 5.90 (2H, s, –OCH_2O–), 6.00 (1H, q,q, J=7/1.5 Hz, =C<u>H</u>), 6.48 (1H, s, arom. H) and 6.83 (1H, s, arom. H).

Gomisin C (III), $C_{30}H_{32}O_9$ (M^+ 536), mp 115—116°, $[\alpha]_D$ −186° UV λ_{max}^{EtOH} nm (log ε): 222 (4.91), 255 (sh 4.11) and 292 (sh 3.48), IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 1720 (C=O), 715 (arom. ring), NMR, δ in $CDCl_3$: 1.16 (3H, d, J=7 Hz, –C<u>H</u>–C<u>H</u>$_3$), 1.36 (3H, s, HO–Ç–C<u>H</u>$_3$), 1.65 (1H, s, OH), 1.65 (1H, m, –C<u>H</u>), 2.0—2.50 (2H, m, –CH_2–), 3.32 (3H, s), 3.60 (3H, s), 3.93 (6H, s)

1) *a*) N.K. Kochetkov, A. Khorlin, and O.S. Chizhov, *Zh. Obshch. Khim.*, **31**, 3454 (1961); *b*) N.K. Kochetkov, A. Khorlin, O.S. Chizhov, and V.I. Sheichenko, *Tetrahedron Letters*, **1961**, 730; *c*) N.K. Kochetkov, A. Khorlin, and O.S. Chizhov, *Tetrahedron Letters*, **1962**, 361; *d*) N.K. Kochetkov, A. Khorlin, and O.S. Chizhov, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, **1964**, 1036.