11 Sakamoto N, Terai M, Takenaka T, Maeno H. Inhibition of cyclic AMP phosphodiesterase by 2,6-dimethyl-4-(3-nitropheyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-(2-(*N*-benzyl-*N*-methylamino)) ethyl ester 5-methyl ester hydrochloride (YC-93), a potent vasodilator. *Biochem Pharmacol* 1978; **27** : 1269

12 Wen YY, Hu P, Chen MQ, Zhang SF. Effect of nifedipine on blood pressure, calmodulin and cyclic nucleotides in plasma and aorta from spontaneously hypertensive rats. *Acta Acad Med Sin* 1987; **9** : 113

中国药理学报 *Acta Pharmacologica Sinica* 1990 Sep; **11** (5) : 454-457

Latin square *vs* twin crossover design for mouse blood glucose assay to estimate insulin potency

LIN Zhi-Gong, ZHOU Hai-Jun (*National Institute for the Control of Pharmaceutical and Biological Products, Beijing 100050, China*)

ABSTRACT According to parallel line analysis, a Latin square design was used for estimating insulin potency in mouse blood glucose assay. Four to six groups of 4 × 4 Latin squares were used to estimate 80 %, 100% and 120% standard preparations and the recovery rates were 95-106 %. Meanwhile, in comparison with twin crossover design, 13 batches of variant preparations including some beyond expiry dates were checked by Latin square design. The results showed that the potencies were about the same in two designs. The average fiducial limit rates were still less than 25% but 40% or so of animal numbers were saved. Therefore, Latin square design is a precise and accurate assay for the estimation of insulin.

KEY WORDS research design; biological assay; blood glucose; insulin

Although twin crossover design is better than (2,2), 2 doses of standard and 2 doses of preparation, or (3, 3) assay,[1] it must cut back interact effect in statistical analysis. Latin square design can get more information from fewer animals in order to compare the drug effect. The experimental design possesses more effect in statistical analysis. Each subject can be used four times. The mouse blood glucose is estimated by a sensitive glucose oxidase method, a small amount of blood sample can be taken to replicate the treatment and if the investigator is prepared to get his results, a Latin square is a satisfactory design[2]. This paper reports on the results of a Latin square design in estimation of insulin bioactivity in comparison with twin crossover design.

Received 1989 May 2 Accepted 1990 Apr 21

MATERIALS AND METHODS

Insulin 1) National biological standards, batch 780307, 27 IU / mg were prepared by our Institute; 2) International biological standards, batch 83 / 515, 26 IU / mg, were provided by WHO International Laboratory for Biological Standards; 3) preparations of insulin were provided by Shanghai Biochemical Pharmaceutical Factory.

Mice Mice of same sex weighing 22 ± 2g were used and 16, 20 or 24 mice in each test. Before the assay all the mice should be deprived of food and fed with water only.

Determination of glucose Content of glucose of 0.06 ml blood taken from the orbital venous sinus was measured by glucose oxidase reagent method [3].

Design Mice were allotted at random into 4-6 blocks. Each block was carried on

one Latin square according to Table 1. An approximation of two-fold dilutions with insulin 20–80 mU / ml was prepared,sc 0.25 ml each solutions. The injections were carried out as Square on occasions (Tab 1). Not less than 3 h Later each solution was administered between two occasions. Exactly 40 min after each injection a suitable blood sample was taken for determining the response.

Tab 1. *n* blocks 4 × 4 Latin square design.

Subject	Dose on occassion			
	1	2	3	4
I	SL	SH	TL	TH
II	SH	SL	TH	TL
III	TL	TH	SL	SH
IV	TH	TL	SH	SL

SL: Standard preparation low dose;
SH: Standard preparation hign dose;
TL: Test preparation low dose;
TH: Test preparation high dose

Statistical analysis calculated the result of the assay by a Latin square design for the (2,2) parallel line analysis[4]. There was a computer program for a Latin square design as statistical analysis above mentioned[5].

RESULTS

Recovery rates assay 80, 100 and 120 % content of insulin that is national standard used as test preparation was estimated by a Latin square design. The recovery rates were in the range of 95–106 % (Tab 2). The results showed that this method is reliable.

Tab 2. Recovery rate for Latin square design.

Content (%)	Assay (%)	Recovery rate (%)	Fiducial limit rate(%)
80	79.13	98.9	16.5
	79.15	98.9	16.4
	83.49	104.3	21.1
100	95.88	95.9	16.3
	100.76	100.8	17.7
	105.62	105.6	11.2
120	125.82	104.9	17.8
	117.64	98.0	21.8

Test preparations check Choose various batches and assumed potency preparation being examined by method as described above. Meanwhile, in comparison with twin crossover design, the results (Tab 3) showed that the estimated potencies of insulin were almost the

Tab 3. Estimated potencies of insulin using mouse blood glucose assay by Latin squares and twin crossover design.

Batch	AT (IU / ml)	Latin squares *n*	R	SM	FL%	Twin crossover *n*	R	SM	FL%
S7	27.8	24	1.0927	0.0380	17.7	40	1.1290	0.0352	16.6
	(IU / mg)	24	0.9760	0.0500	23.2	40	0.9863	0.0494	23.2
860717	40	16	1.0414	0.0282	13.2	40	1.0990	0.0327	15.0
		16	0.9474	0.0409	19.0	40	0.9420	0.0395	18.4
861001	40	24	1.0523	0.0337	15.6	40	0.9416	0.0319	14.8
		24	0.9327	0.0494	22.8				
HJ-T1	50	24	0.9975	0.0293	13.5	40	0.9780	0.0371	17.3
		20	1.0239	0.0331	15.4	40	1.0364	0.0233	10.8
3460905	40	16	1.0865	0.0291	13.7	40	0.9894	0.0213	9.9
		20	1.0402	0.0397	18.5				
21405	100	20	1.0598	0.0378	17.6	40	0.9334	0.0366	17.0
		24	1.0760	0.0385	17.9				
21408	100	20	1.0618	0.0604	28.6	40	1.1577	0.0529	24.6
		16	1.0952	0.0402	18.9				

AT: Assumed potency; *n*: Number of animal; R: Relative potency; SM: Standard error of mean: FL%: Fiducial limit rate

Tab 4 . Estimated potencies of insulin which exceed expiry using mouse blood glucose assay by Latin squares and twin crosover design (AT = 40 IU / ml).

Batch	Latin squares				Twin crossover			
	n	R	SM	FL%	*n*	R	SM	FL%
771206	32	0.8899	0.0438	20.0	40	0.8789	0.0469	22.6
	28	0.8812	0.0340	15.6				
770623	20	0.8104	0.0377	17.3	40	0.8297	0.0388	18.7
	16	0.8046	0.0461	21.2				
770915	20	0.8476	0.0485	22.3	40	0.7337	0.0448	22.0
770904	20	0.7969	0.0391	17.9	40	0.8542	0.0320	15.3
770519	20	0.8885	0.0430	19.0	40	0.8717	0.0394	18.9
A–54 / 1	20	0.8006	0.0452	20.7	40	0.7872	0.0451	22.1

same. Two designs don't differ in experimental error, but animal number in Latin square design was 60% less than that in twin crossover design.

Beyond expiry preparation check Choose some batches and the potencies were examined by Latin squares design and twin crossover design. The assay showed that the potencies were about the same in two designs, but were lower than assumed potencied to reflect biological activity (Tab 4).

Error analysis The experimental error of a Latin squre design was expressed by average fiducial limits rate (FL %). The frequencies were the number of estimates. The distributions of FL% in two designs were showed in Fig 1. Average fiducial limit rates were 18.3% ± 3.5 (11.2–28.6 %) and 18.0% ± 4.3 (9.9–24.6 %) in Latin squares and twin crossover designs respectively.

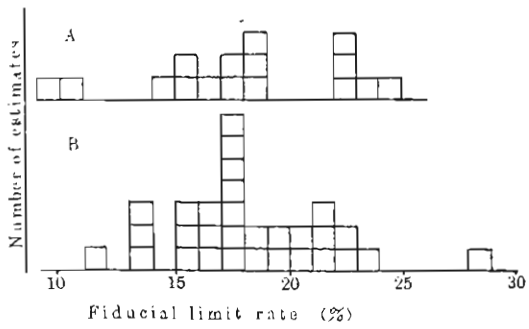

Fig 1. Distribution of fiducial limit rate (FL%) in (A) twin crossover and (B) Latin square designs.

DISCUSSION

It is possible to take repeatedly a few blood samples in mice estimated by glucose oxidate method for Latin square design. Each mice might get more data from Latin square design than that from twin crossover design. The experimental errors were decreased in Latin square design, but the potency was same as random or randomized block design.

As compared with twin crossover design, no correspondence with the interactions of difference between two occasions, more successful assay results were obtained in Latin square design. But Latin square design took more occasion and time. In the (2,2) assay of Latin square for estimating the potency of insulin, FL% might be less than 30% if there were 4–6 Latin square as usual. FL% might also be cut down by increasing Latin square numbers or combining calculation of the results[6] When observations are missing from Latin square design, a special procedure are required to prevent like a randomized block design[7].

Since Latin square design is one of the most perfect designs in twin crossover designs, the total dose on occations (SL, SH, TL, TH) could be arranged to each subjest. Therefore, the variation among subjects has been consciously decreased.

A (2,2) Latin square design might be used

in bioasssay of glucagon (the rabbit blood glucose assay), oxytocin (the rat uterus contraction assay), argipressin (the rat blood pressure assay) and so on. If each subject is to be used 6 times, an (3,3) assay using 6 litters of 6 animals might be arranged as the 6× 6 Latin square design. Fisher and Yates[8] have provided adequate tables of Latin squares.

REFERENCES

1 Ke RL, Shen GQ, Liu QL. Biological assay of insulin by blood glucose determination in mice. *Chin J Pharm Anal* 1984; 4 : 74
2 Finney DJ. *Statistical method in biological assay*. 3rd ed. London : Charles Griffin, 1978: 209 – 15
3 Lin ZG, Liu QL, Guo H, Ke RL. Bioassay of insulin by the estimation of blood sugar in mice; Determination of blood sugar by GOD method estimation. *Chin J Pharm Anal* 1985; 5 : 333
4 Lin ZG, Zhou HJ. Application of Latin square design in mouse insulin assay by blood sugar method. *Chin Pharmacol Bull* 1989; 5 : 115
5 Lin ZG, Zhou HJ. The computer program for Latin square design of statistical analysis. *J Biochem Pharm* 1988; (4) : 32
6 Pharmacopoeia Commission of the Ministry of Public Health of PRC. *Pharmacopoeia of the People's Republic of China*. Hong Kong: China Pharmaceutical Books Company, 1988 : A 73–5
7 周海钧、申蕴如, 主编. 生物检定统计方法. 第2版, 北京: 人民卫生出版社, 1988: 161–73
8 Fisher RA, Yates F. *Statistical table for biological, agricultural and medical research*. 6th ed. London: Oliver & Boyd, 1963 : 86–9

拉丁方和双交叉设计小鼠血糖法测定胰岛素效价的比较

林志共、周海钧 (中国药品生物制品检定所,北京 100050, 中国)

提要 根据平行线原理，应用拉丁方设计小鼠血糖法测定胰岛素效价. 4 至 6 组 4×4 拉丁方测定 80，100 和 120%含量的标准品，其回收率在 95–106%，测定 13 批不同制剂的胰岛素样品，包括超过有效期的检品，同时和双交叉设计比较，结果表明，两种设计效价测定几乎相同，FL%均能在 25%以下,但拉丁方设计能节约 40%左右的动物，是一种精确的测定胰岛素效价方法.

关键词 研究设计; 生物测定; 血糖; 胰岛素

中国药理学报 *Acta Pharmacologica Sinica* 1990 Sep; 11 (5) : 457–459

可乐定对实验性大鼠胃溃疡的作用

罗贯一、何 云、涂羚波 (江西大学生理教研室, 南昌 330047, 中国)

Effects of clonidine on experimental stomach ulcer in rats

LUO Guan-Yi, HE Yun, TU Ling-Bo
(*Department of Physiology, Jiangxi University, Nanchang 330047, China*)

ABSTRACT Clonidine 2 mg / kg ig inhibited the rat gastric ulcers induced by pyloric ligation, stress and indomethacin by 71%, 77% and 82%, respectively. Clonidine 2 mg / kg ig tended to accelerate the healing of gastric ulcer induced by acetic acid, and the healing rate was 61% . Clonidine decreased the secretion of gastric acid and pepsin, and increased the release of gastric barrier mucus. These actions may contribute to its protective effect against ulceration.

KEY WORDS clonidine; stomach ulcer; gastric acid; pepsin; gastric juice

提要 可乐定 2 mg / kg ig 可抑制大鼠幽门结扎、应激性及吲哚美辛诱发的胃溃疡，其抑制率分别为 71%，77%，82%，并可促进醋酸胃溃疡愈合，其愈合率为 61%. 可乐定还可减少胃酸和胃蛋白酶的分泌，增加胃壁结合粘液的分泌，这些作用可能与其抗溃疡效应有关.

关键词 可乐定; 胃溃疡; 胃酸; 胃蛋白酶; 胃液

Received 1990 Jan 8 Accepted 1990 May 9