ORIGINAL ARTICLE

Reuse of laboratory animals in experiments: A statistical approach

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ABSTRACT

Background: Large number of animals are used in the screening of drug experiments in search of an active lead compound. Oral glucose tolerance test (OGTT) studies are most popular to identify the anti-hyperglycemic compounds. In this study we have explored the possibility: 'can the animals used as controls in OGTT experiments be reused again'.

Materials and Methods: Fifty Sprague-Dawley strains of albino rats, which were used once as untreated control group in anti-hyperglycemic screening studies, were divided into 5 groups. Standard protocol of OGTT studies was followed. Blood as sample has been used for glucose profiling. Data was analysed using 'within error one way analysis of variance' followed by Newman Keuls test for individual comparisons. The level of significance 0.05 was used to define p-value.

Results: The daily blood glucose level was not significantly different between groups. However, the glucose level of 10 week old animals were significantly higher ($P<0.01$) than the fresh animals group.

Conclusion: Results in this study revealed that the reuse of animals should not be preferred in OGTT experiments.

Keywords: Diabetes mellitus, oral glucose tolerance test, re-use animals, Sprague- Dawley rats.

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INTRODUCTION

Development of new drugs is a time-consuming and expensive process which requires a fairly large number of animals to obtain results.[1] In antidiabetic drug screening, one group in the experiment is held as untreated control group, known as a parallel control group in the study design. Oral glucose tolerance test (OGTT) is one such experimental study in which a very large number of small animals are regularly screened in search of anti-hyperglycemic lead compound. Sprague Dawley rats are the most commonly used vertebrate species because of their size, low cost, easy to handle and fast reproduction rate and are considered the best model for such studies.[2] Normally five to six compounds are screened at a time, the activities of which are compared with untreated control group. All groups have an equal number of animals. This results in requirement of 30-36 rats per experiment. A large number of such experiments are carried out in our institute, from which untreated animals remain in an appreciable number. They are healthy and active animals which cannot be sacrificed ethically. Can they be reused?

Scientist and Institutional Animal Care and Use Committee (IACUC) members are making sincere efforts on reducing the number of animals in experiments. There are several options to overcome the problem of non-availability of animals for research but those may not work always.[3,4] The other option could be to reuse the animals.[5] But the viability of reuse should be scientifically tested. A lot of animals are used in the regular screening of antidiabetic compounds. All of them are sacrificed after ones used in such experiments. It was our curiosity to know if the number can be cut by reusing animals again for OGTT studies. In this paper we have tried to answer the query "Can the requirement of large number of animals be reduced in anti-hyperglycemic screening of compounds by reusing the animals"?

MATERIALS AND METHODS

Animals: Fifty Sprague-Dawley strains of albino rats bred in the animal house of Central Drug Research Institute, Lucknow, India were used in this study. The rats were housed in an air-conditioned room. The Animals were fed with the pellet diet and water *ad-libitum*. The following general room conditions 24-28°C temperature, 60-70% relative humidity, 6 to 10 air changes per hour and 12 hour day and night cycle were maintained throughout the study. All ethical manners for use of animals in scientific research were carefully adopted. The 'reuse of animals' refers to the untreated control group used previously once in anti-hyperglycemic screening study. Ten animals belonging to each of the following five groups were used in the study;

Group 1: Six week old fresh animals

Group 2: Ten week old animals – used once earlier as control (without treatment)

Group 3: Ten week old animals – used earlier as Glybenclamide (standard drug) treated group

Group 4: Twelve week old animals – used earlier as control (without treatment)

Group 5: Twelve week old animals – used earlier as Glybenclamide (standard drug) treated group

Observations (Static): The experiment started on day 0 at 10 a.m. by measuring body weight and serum glucose of the animals. The body weight and serum glucose level were recorded daily at 10 a.m. for six consecutive days in addition to day 0. Table 1 and table 2 showed the body weight and serum glucose levels of animals, respectively. The animals were put on fasting at 6 p.m. on day 6. On day 7 oral glucose tolerance test (OGTT) was done. Animals in each cage were fed with 150 gm of pellets and 250 ml of water daily. The amount of water and food consumed was recorded daily at 10 a.m. by measuring the left over quantity in each cage. After measurement the water and pellets were replaced.

Oral Glucose Tolerance Test methodology: The animals on which blood glucose level was measured were deprived of food for 16 hrs. Fasting blood glucose level of each rat was checked by glucostrips (Roche) at 10 am. This observation was considered as 0 min (fasting) glucose level. Rats were then primed with Glucose (3.0 gm/kg orally). After 30 minutes post glucose load the blood glucose levels was subsequently observed at 30, 60, 90 and 120 minutes[6]. Food but not water was withheld from the cages during the course of experimentation.

Statistical analysis: The observations of blood glucose level and body weight were expressed as mean ± S.D. The observations were repeated measures because the same rat was used on different occasions. These two variables were analysed using 'within error one way analysis of variance'. The individual comparison was done by Newman Keuls test. The assessment of OGTT activity was expressed as percent change in AUC with respect to fresh animal. The probability for level of significance used was 0.05.

RESULTS

The summary of body weight has been shown in table 1. The repeated measures analysis followed by individual comparison show a highly significant difference ($P<0.01$) of body weight of used animals when compared with the body weight of fresh animals on all days (day 0-day 6) of the study.

The summary of the blood glucose level has been shown in the table 2. The repeated measures analysis found that on day 0, the glucose level of ten week old animals used once as control (group 2) and ten week old animals treated with Glybenclamide (group 3) were significantly higher ($P<0.01$) than Six week old fresh animals (group 1), while the blood glucose level of twelve week old animals used as

Table 1: Body weight of different groups of Sprague Dawley rats from day zero to six

Groups		Body Weight (gms.)						
		0 Day	1 Day	2 Day	3 Day	4 Day	5 Day	6 Day
Group 1	Mean	92.2	94.4	97.8	101.60	104.00	107.60	110.40
	SD	6.97	6.98	6.96	6.8	6.6	8.14	8.32
Group 2	Mean	176.30	178.20	181.20	184.80	186.20	187.60	188.80
	SD	22.16	21.89	22.59	23.36	22.50	21.85	23.27
Group 3	Mean	162.00	164.60	167.90	167.60	168.30	169.40	173.00
	SD	15.84	15.71	14.92	16.14	18.82	20.84	20.76
Group 4	Mean	211.1	212.90	215.10	219.20	222.30	222.30	225.40
	SD	41.60	40.22	38.59	39.16	38.79	38.00	37.24
Group 5	Mean	207.50	211.40	214.10	216.50	218.90	220.40	223.60
	SD	29.86	30.63	30.50	30.59	30.31	30.34	30.30

Table 2: Blood glucose profile of different groups of Sprague Dawley rats from day zero to six

Groups		Blood Glucose (mg/dl)						
		0 Day	1 Day	2 Day	3 Day	4 Day	5 Day	6 Day
Group 1	Mean	67.20	93.90	86.30	86.60	92.10	85.30	97.40
	SD	9.60	8.33	5.25	18.51	12.16	10.80	13.19
Group 2	Mean	89.80	94.10	86.50	99.10	89.10	89.50	99.60
	SD	8.82	5.82	10.35	10.14	5.92	9.61	11.05
Group 3	Mean	85.2	94.80	89.60	88.20	86.20	81.60	86.90
	SD	4.78	7.70	6.98	15.75	7.55	15.71	17.39
Group 4	Mean	72.60	97.10	99.70	92.90	102.70	88.40	109.20
	SD	10.50	6.87	18.15	8.44	12.45	13.37	13.38
Group 5	Mean	78.70	91.80	93.60	94.90	97.20	90.20	101.80
	SD	9.71	9.43	12.49	10.45	2.78	11.94	10.53

control (group 4) and twelve week old animals treated with Glybenclamide (group 5) were similar to group 1 ($p>0.05$). The serum glucose levels of all groups were not significantly different ($P>0.05$) than six week old fresh animals (group 1), from day 1 to day 5. The average food intake profile of animals has been shown in figure 1.

All animals from group 2 to group 5 have serum glucose

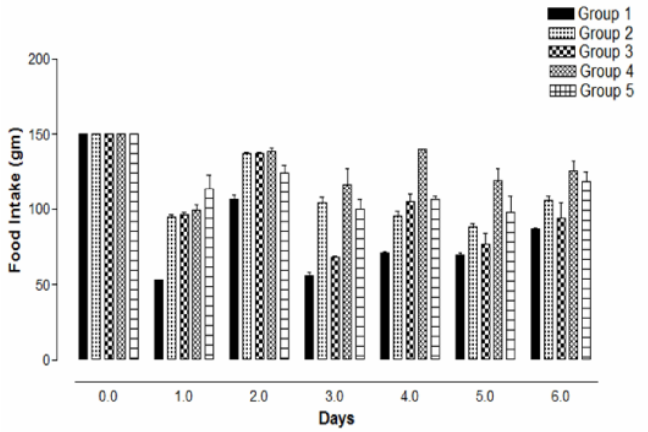

Figure 1: Measurement of daily food intake of normoglycemic rats from day zero to day six

level below the 10% lowering as cutoff in the AUC with the exception of one animal. The initial glucose level of all these animals was lower than 60 mg/dl. However, the initial glucose level of fresh animals was more than 60 mg/dl which is required level for OGTT experiments.

The OGTT profile of each group has been shown in figure 2. The anti-hyperglycemic activity was measured by AUC of serum glucose levels from T0 to T120 Minutes. The one way analysis of variance results show a significant difference in anti-hyperglycemic activity by the animals of different categories.

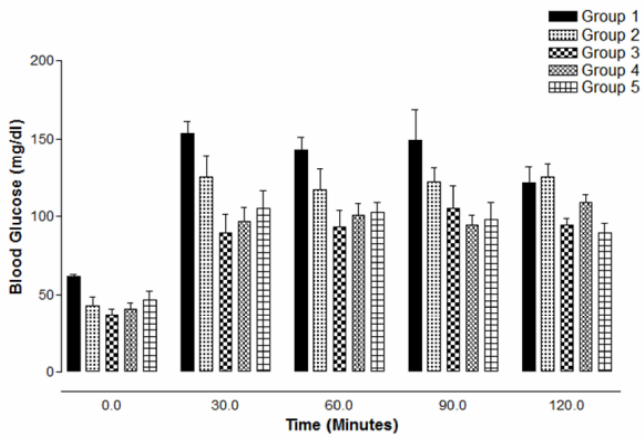

Figure 2: Oral glucose tolerance test of normoglycemic rats on the day six

Three groups, the ten week old animals treated with Glybenclamide (Group 3), twelve week old animals used as control (Group 4) and twelve week old animals treated with Glybenclamide (Group 5) showed significantly lower glucose profile ($p<0.01$) than the fresh animals group. The ten week old animals used once as control (Group 2) was not significantly different ($p>0.05$) from the fresh animals group.

DISCUSSION

A large number of animals are required in the screening experiments while looking for an active lead compound. Oral glucose tolerance test (OGTT) studies are popular to identify the anti-hyperglycemic compound. Diabetes mellitus is a chronic disease characterized by persistent hyperglycemia due to absolute or relative deficiency of circulating insulin levels. Compounds are regularly screened in search of anti-hyperglycemic activity through OGTT studies conducted on animals.

The results show a significant variation in the serum glucose levels of the animals of different groups. This effect may be

due to the biochemical changes that took place within the body once they were a part of experiment. Our experiments included two classes of animals (i) untreated control and (ii) treated with standard drug. It was expected that at the serum glucose profile of ten week and twelve week old animals used once as control would behave similar to the fresh animals.

Though the glucose lowering in the ten week old animals was statistically not significant but it has shown 15.5 % lowering in the glucose level. This is a fairly high reduction. The significant reduction of serum glucose in the other three groups i.e. the ten week old animals treated with Glybenclamide (Group 3), twelve week old animals used as control (Group 4) and twelve week old animals treated with Glybenclamide (Group 5) indicate changes in the metabolic process of animals once they are used. A similar reason could be attributed to ten week old animals used once. A reason to this effect was also observed when the quantity of food intake by the animals decreased after day 2. In other words, animals once used might have gone into stress. The overall stress experienced once by the animals may have affected their metabolic activity. A reason of animals' stress could be the six days long interval of stay in cages in addition to overnight fasting before conducting OGTT experiment especially for those who have experienced the pains before.

We started the experiment with the null hypothesis that there is no harm in using the animals used once and the serum glucose profile would restore after 2 weeks of stay in animal house. Since our findings have rejected this null hypothesis so we conclude that the Sprague Dawley rats once used in the OGTT studies cannot and should not be reused for the same.

It is important to note here that harmful effects of testing compounds remain unknown in case of new drug development studies. There is every likelihood about the permanent changes occurred in body systems. It may be because drug circulates in the body, but safety studies are not carried out usually at the stage of early drug development. In a very natural way, due to additionally, increased functional demand, regenerative growth takes place in a number of organs after the organs are either damaged, removed, or cease to function.[7] This may be the result of compensatory hypertrophy, compensatory hyperplasia or both.[8] With regards to drug development, surgical studies on one part of organ out of two may not affect much if animals are reused once they have recovered after surgery.

CONCLUSION

Rats once used for OGTT studies cannot be used for similar studies of drug screening. As-far-as screening for new drugs is concerned; it is less likely to reduce the number of rats in OGTT studies. However, these animals may be useful for other researches to be explored.

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ABBREVIATIONS

b.w.: body weight; SEM: Standard Error Median; AUC: Area Under the Curve; SD: Standard Deviation

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