AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Estimation of Bioavailability of Biotechnologically Modified L-Lysine from *Corynebacterium Glutamicum* Relative to the Marketed Preparation of L-Lysine

Meenu Singh[1]*, Govind Mohan[1], D. Muralidhara Rao[2]
1. NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India.
2. Department of Biotechnology, S. K. University, Anantapur, Andhra Pradesh, India.

ABSTRACT

Bioavailability may be considered as one aspect of drug product quality that links the *in-vivo* performance of the drug product used in clinical trials with studies demonstrating evidence of safety and efficacy. In the present study, the bioavailability was performed for the qualitative evaluation of the L-lysine produced by modified *Corynebacterium glutamicum*. Thirty 3-week-old male Sprague-Dawley rats weighing 45-55 g were used. The animals were divided into three groups. The first group was kept on a protein free basal diet which was supplemented with the biotechnologically modified L-lysine from *Corynebacterium glutamicum*. The second group comprised of animals which were fed the same basal diet but supplemented with a marketed preparation of L-lysine. The third group was kept on a regular protein diet which served as a control. The animals were fed *ad libitum* for 13 days, during which weight gain and food consumption were recorded and food conversion efficiency (FCE; weight gained/weight food eaten) was calculated. Ratios of means were calculated using the (least-square means)LSM for untransformed and ln-transformed FCE. The bioavailability was estimated by measuring the pharmacological activity (weight gained) and compared with that of the marketed preparations of L-lysine under similar conditions. For the study reference and test samples,-s the 95% confidence interval fell within the range of 97.72 and 103.61, which was the indication of bio similarity for biological activity of both the samples.

Keywords: Bioavailability, L-lysine, *Corynebacterium glutamicum.*

*Corresponding Author Email: meenupharma@gmail.com
Received 23 June 2015, Accepted 30 June 2015

Please cite this article as: Singh M *et al*., Estimation of Bioavailability of Biotechnologically Modified L-Lysine from *Corynebacterium Glutamicum* Relative to the Marketed Preparation of L-Lysine. American Journal of PharmTech Research 2015.

INTRODUCTION

Direct and indirect methods are being used to assess drug bioavailability. The in-vivo bioavailability of a drug product is demonstrated by the rate and extent of drug absorption, as determined by comparison of measured parameters, viz. concentration of the active drug ingredient in the blood, cumulative urinary excretion rates, or pharmacological effects. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action. The design of the bioavailability study depends on the objectives of the study, the ability to analyze the drug (and metabolites) in biological fluids, the pharmacodynamics of the drug substance, the route of drug administration, and the nature of the drug product. Pharmacokinetic and/or pharmacodynamic parameters as well as clinical observations and in-vitro studies may be used to determine drug bioavailability from a drug product. In summary, pre-clinical studies are useful in determining the safety and efficacy of drug products. Bioavailability studies are used to define the effect of changes in the physicochemical properties of the drug substance and the effect of the drug product (dosage form) on the pharmacokinetics of the drug[1]. From a nutritional standpoint, lysine is a dietary indispensable amino acid that can only be derived from the diet and is often the first limiting amino acid for production in animals (pigs and poultry). Lysine can also be limiting in diets for humans, especially diets that are high in cereals and low in animal proteins. Lysine is a basic amino acid that possesses a reactive amino group on its side chain. This ε-amino group can undergo reactions with a wide variety of compounds that are present in foods and feedstuffs including reducing sugars, fats and their oxidation products, polyphenols, vitamins, food additives and other amino acids to produce modified lysine derivatives. Since lysine is the first limiting amino acid for growth in most pig and poultry diets, protecting lysine from damage during processing is important. Accurate data on the lysine content of diets and protein sources is also critical for efficient diet formulation. Furthermore, for human diets, accurate information on the lysine content is required for diets that are likely to be low in lysine (cereal-based diets) and also those diets for humans with a particular requirement for protein such as growing children, athletes or the elderly[2]. In the present study, the bioavailability of L-lysine prepared from modified *C. glutamicum*(Published in International Journal of Biological & Pharmaceutical Research, 2012; 3 (6): 758-761)[3] is determined and compared with the marketed preparation. This assay is also

providing a better assessment of the nutritional adequacy of human and companion animal diets in terms of dietary lysine leading to healthier foods for humans and companion animals.

MATERIALS AND METHOD

Study samples

The biotechnologically modified L-lysine from *Corynebacterium glutamicum* was tested and compared with the marketed preparation of L-lysine for digestible reactive lysine content using an *in-vivo* food conversion efficiency assay[4, 5].

Animals and experimental groups

Thirty male 3-week-old Sprague-Dawley rats were used; they weighed 45-55 g and were obtained from the Animal house of C.M.R. College of Pharmacy, Hyderabad, and A.P. They were fed for 2 days on a pre-experimental diet. This was the stock laboratory diet obtained from Hindustan Animal Feeds, Gujarat, India. After 2 days, the rats were weighed and allocated individually into cages in a room maintained at 22 ± 5 ^{o}C, with a 12 h light/dark cycle. Ethics approval for the animal trial was obtained from the Institutional Animal Ethics Committee, C.M.R. College of Pharmacy, Hyderabad, AP. The animals were divided into three groups. The first group was kept on a protein free basal diet which was supplemented with the biotechnologically modified L-lysine from *Corynebacterium glutamicum*. The second group comprised of animals which were fed the same basal diet but supplemented with a marketed preparation of L-lysine. The third group was kept on a regular protein diet which served as a control. The animals were treated in a one-way blocked, randomized design with ten rats per treatment (diet). Each of the ten horizontal rows of cages formed a single block containing one animal from each treatment. The upper block contained the heaviest rats and, going down through the blocks, the rats were progressively lighter. The blocks thus differed both in the weight of the rats at day 0 as well as the position of cages in the rack. Within each block, the treatments were allocated at random. All diets were powder diets and were fed *ad libitum*. The vitamin mixture was that of Peret et al. 1973[5]and the minerals (USP-XVII, as recommended by the Association of Official Analytical Chemists)[6,7]were purchased from Mission Viva care Limited, Gujarat.

Formulation of Diet

This diet was a modified version of the method of Mottu & Mauron[8]. The basal diet contained (g/kg): wheat gluten 100, zein 150, L-tryptophan 1.4, L-methionine 3.3, L-threonine 1.5, L-valine 1.8, L-histidine hydrochloride monohydrate 2.0, L-arginine 5.0, sucrose 250, arachis oil 50, cellulose 20, minerals 50, vitamins 12-5 and maize starch to 1000. The basal diet was

supplemented with the biotechnologically modified or a marketed preparation of L-lysine depending on the animal study group at a concentration of 3g/kg. The lysine adjustments were made at the expense of zein and maize starch to keep the diets iso nitrogenous. The control group was fed with standard protein diet available from Hindustan Animal Feeds, Gujarat, India.

Assay procedure

The animals were fed *ad libitum* for 13 days, weight gained and food consumption were recorded and food conversion efficiency (FCE; weight gained/weight food eaten) was calculated[9]. The summary statistics for the FCE was reported for both the test and reference products. The statistical measures were the arithmetic mean, standard deviation, geometric mean and the coefficient of variation for untransformed data. The inter-sample coefficients of variation for the (natural) ln-transformed data were reported.

Analysis of Variance

An analysis of variance (ANOVA) was performed on the untransformed and ln-transformed FCE. Each analysis of variance also included calculation of standard error associated with the differences of least-square means, adjusted differences between formulation means and the standard error.

Ratio Analysis

Ratios of means were calculated using the (least-square means)LSM for untransformed and ln-transformed FCE. Ratios of means were expressed as a percentage of the LSM for the reference treatment.

Power Test

The power (i.e. probability of detecting a 20% difference relative to the reference treatment LSM at the 5% significance level using a t-test under the null hypothesis was calculated for both untransformed and ln-transformed FCE.

Confidence Intervals

95% confidence intervals for the difference between treatments, least-square means (LSM) was calculated for both untransformed and ln-transformed FCE. The confidence intervals were expressed as a percentage relative to the LSM of the reference treatments. [9, 10]

RESULTS AND DISCUSSION

A total of 30 male Sprague - Dawley rats in three groups were weighed and placed individually in cages arranged in 10 rows with 3 cages in each row and were fed with standard diet for two days before the start of the study. The animals were assigned to the three groups with 10 animals in each

group. The distribution was randomized to reduce bias. The animals received the dose of l-lysine according to a randomized schedule i.e. test (T), control (C) and reference (R) treatments mentioned in the protocol. The animals had *ad libitum* access to powdered diet specific to their group. The food consumption was recorded daily by weighing the food provided and the leftover. After 13 days the animals were weighed again and the difference of weight was recorded and shown in Tables 1, 2, 3 and 4. Geometric Mean value for FCE was 0.2108 for reference R and 0.21078 for test T. On observing the above Geometric Mean values for FCE, we can say there was some variation between the test and reference L-lysine samples. The table 5 also showed the *p*-values for ANOVA, two one-sided tests for relative bioavailability, and ratio analysis for untransformed and ln-transformed FCE. The *p*-values were above the permissible limits. Criteria for untransformed and ln-transformeds are >0.05 (non-significant). Thus this showed that the variations in reference(R) and test (T) values were not proven to be statistically significant. The 95% confidence intervals were constructed for the ratios of the geometric LSM of ln-transformed FCE for the test T and reference R formulations of L-lysine. Acceptable relative bioavailability is to be concluded if the confidence intervals so constructed fall within the range of 90-115% for FCE. For the study reference and test samples, the 95% confidence interval fell within the range of 97.72 and 103.61. Here all the FCE confidence values fall within the acceptable range of 90.00 to 115.00%, so the test product is equivalent to reference product.The intra-sample variability for FCE was 0.0029.This indicates that the discrepancies between the sample readings of different animals within the experimental design did not differ significantly from each other (as shown in Figure 1). We can infer form this reading that the experimental data collection was done properly and there are no abnormal readings. All this data indicates that on the comparison of biological activity, the reference and test samples are bio similar. Also, both test and reference samples produced significantly higher biological activity compared to control, which indicates that these can be used as food supplements.

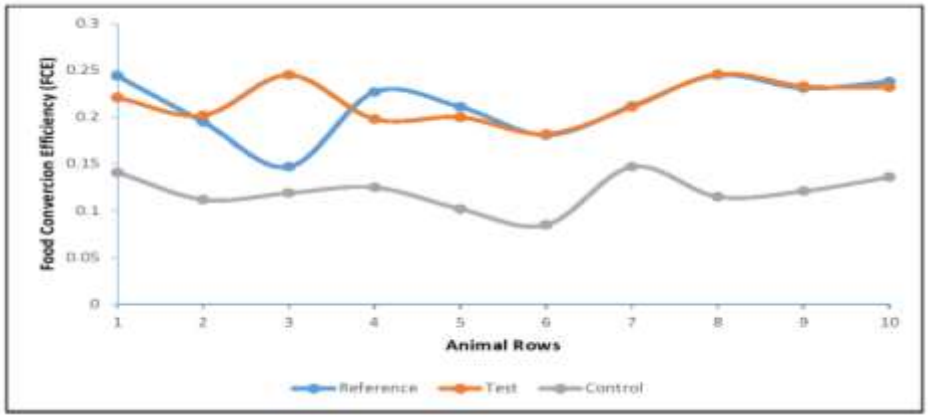

Figure 1: Comparison of Food conversion Efficiency (FCE) between Reference(R), Test(T) and Control(C)

Table1: Food conversion efficiency values for animals in the reference (r) group

Reference Group (R)	Initial Wt. (g)	Final Wt. (g)	Wt. Gain (g)	Food eaten (g)	Food Conversion Efficiency
1	56	79	23	94.3	0.244
2	65	90	25	128.2	0.195
3	54	75	21	142.9	0.147
4	72	99	27	118.9	0.227
5	78	100	22	104.3	0.211
6	60	79	19	105.0	0.181
7	58	82	24	113.2	0.212
8	64	82	18	73.5	0.245
9	77	97	20	86.6	0.231
10	76	97	21	88.2	0.238

Table 1, 2 and 3 depicts the food conversion efficiency of test (T), control (C) and reference (R) treatments estimated at the end of the study period. The table depicts the initial and final weights of the animals in their respective study groups. The weight gain was calculated by subtracting the initial weight from the final weight. The table also shows the recorded food eaten by the animals. This value is the cumulative food eaten over the period of the study.

Table 2: Food conversion efficiency values for animals in the Test (T) group

Test Group (T)	Initial Wt. (g)	Final Wt. (g)	Wt. Gain (g)	Food eaten (g)	Food Conversion Efficiency
1	61	81	20	90.5	0.221
2	62	86	24	118.8	0.202
3	58	85	27	110.2	0.245
4	62	86	24	121.2	0.198
5	55	74	19	95.0	0.2
6	53	76	23	126.4	0.182
7	67	94	27	128.0	0.211

8	58	74	16	65.0	0.246
9	63	81	18	77.3	0.233
10	65	86	21	90.5	0.232

Table 3: Food conversion efficiency values for animals in the control (C) group

Control Group (C)	Initial Wt. (g)	Final Wt. (g)	Wt. Gain (g)	Food eaten (g)	Food Conversion Efficiency
1	53	65	12	85.1	0.141
2	52	67	15	133.9	0.112
3	71	80	9	75.6	0.119
4	56	71	15	120.0	0.125
5	58	72	14	137.3	0.102
6	68	76	8	94.1	0.085
7	63	80	17	115.6	0.147
8	59	70	11	95.7	0.115
9	68	80	12	99.2	0.121
10	62	76	14	102.9	0.136

Table 4: Mean values of weight gain, food eaten and food conversion efficiency for the study groups

Group (N=10)	Wt. Gain (g)	Food eaten (g)	Food Conversion Efficiency
Reference	22±0.882	105.50±6.634	0.2131±0.010
Test	21.9±1.178	102.29±6.894	0.2170±0.007
Control	12.7±0.895	105.95±6.420	0.1203±0.006

Table 4 depicts the mean FCE, weight gain and food eaten data for test (T), control (C) and reference (R) treatments. From the table it is inferred that in this bioavailability study number of animals N =10; Mean weight gain for reference R was 22±0.882 g, for test T was 21.9±1.178 g and for control C was 12.7±0.895 g. Mean food eaten for reference R was 105.50±6.634 g, for test T was 102.29±6.894 g and for control C was 105.95±6.420 g. Mean food conversion efficiency (FCE) for reference R was 0.2131±0.010, for test T was 0.2170±0.007 and for control C was 0.1203±0.006.

Table 5: Relative bioavailability data comparison between Reference (R) & test (T) products

Measure		FCE
Reference product (R)	N	10
	Mean	0.2131
	SD	0.03133
	CV (%)	15%
	Geometric mean	0.2108
Test product (T)	N	10
	Mean	0.217
	SD	0.02175
	CV (%)	10%
	Geometric mean	0.21078

ANOVA p value		Ln-transformed	0.9912
		Un-transformed	0.7504
CV Inter sample		Inter sample	0.0029
95 % Confidence interval (T/R)	Ln-transformed	Lower	96.51
		Upper	104.4
		Power (%)	79.14
	Un-transformed	Lower	97.72
		Upper	103.61
		Power (%)	87.17

Table 5 depicts the relative bioavailability data comparison between Reference (R) and test (T) products. Standard Deviation (SD) for FCE was 0.03133 for reference R and 0.02175 for test T; here we have found that there were minimal variations in standard deviation values between test and reference product so the test drug was equivalent with the reference. Coefficient of Variation (CV %) for FCE was 15% for reference R and 10% for test T. From the above parameters we can calculate the relative bioavailability along with the following parameters will also give bio equivalence statement.

CONCLUSION

The relative bioavailability testing of the biotechnologically modified L-lysine from *Corynebacterium glutamicum* compared to marketed preparations of L-lysine indicated that both these samples are bio similar when tested for biological activity. The *p*-values for ANOVA testing are 0.9912 and 0.7504 for ln-transformed and untransformed data respectively. These values are above the permissible limits. Criteria for untransformed and ln-transformed are >0.05 (non-significant). Thus this shows that the variations in reference(R) and test (T) values are statistically insignificant. A 95% confidence interval testing revealed that the confidence limits were 97.72% and 103.61% for untransformed data. Acceptable relative bioavailability is to be concluded if the confidence intervals so constructed fall within the range of 90-115% for FCE. As all the confidence values fall within the given range, it can be concluded that the quality of biotechnologically modified L-lysine from *Corynebacterium glutamicum* is similar to the marketed preparations of L-lysine. The bioavailability testing results indicates that the L-lysine produced by the above fermentation techniques is qualitatively acceptable and produces similar biological response as the marketed preparations of L-lysine.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmacology, CMR College of Pharmacy, Hyderabad, India for providing Laboratory facilities for this research work.

REFERENCES

1. Schumacher GE. Use of bioavailability data by practitioners. I: pitfalls in interpreting the data. Am J Hosp Pharm 1975; 32(8): 639–42.
2. Hurrell RF, Carpenter KJ. The estimation of available lysine in foodstuffs after Maillard reactions. Progress in Food and Nutrition Sci 1981; 5: 159-176.
3. Ramesh M, Meenu S, Adarsh G, Abdul MR, Satyanarayana SV, Muralidhara DR. Over expression of aspartokinase gene in *Corynebacterium glutamicum* for higher production levels of L-lysine. Int J Biol Pharm Res 2012; 3 (6) 758-761.
4. Henrik K, Nielsen PA, Finot, Hurrell RF. Reactions of proteins with oxidizing lipids. 2. Influence on protein quality and on the bioavailability of lysine, methionine, cysteine and tryptophan as measured in rat assays. British Journal of Nutrition 1985; 53: 75-86.
5. Peret J, Macaire F, Chanez M. Effects of quantity and quality of dietary protein and variation in certain enzyme activities on glucose metabolism in the rat. J of Nutrition. 1973; 103: 866-874.
6. Henrik K, Nielsen, Loliger J, Hurrell RF. Reactions of proteins with oxidizing lipids 1. Analytical measurements of lipid oxidation and of amino acid losses in a whey protein-methyl linolenate model system. British J Nutrition. 1985; 53: 61-73.
7. W. Horwitz. Official Methods of Analysis of the Association of Official Analytical Chemists. 13th ed., Folkestone, United Kingdom: Association of Official Analytical Chemists; 1980: 774.
8. Mottu F, Mauron J. The differential determination of lysine in heated milk II. Comparison of the in vitro methods with the biological evaluation. J Sci Fd Agric 1967; 18: 57-62.
9. Peyret JL, Bayan N, Jolif G, Gulik KT, Mathieu L, Schechter E et al. Characterization of the cspB gene encoding PS2, an ordered surface layer protein in *Corynebacterium glutamicum.* Mol Microbiol 1993; 9: 97-109.
10. Westlake WJ. Symmetrical confidence intervals for bioequivalence trials. Biometrics 1976; 32: 741-4