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Original Article

Application of Outlier Robust Nonlinear Mixed Effect Estimation in Examining the Effect of Phenylephrine in Rat Corpus Cavernosum

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Abstract

Ignoring two main characteristics of the concentration-response data, correlation between observations and presence of outliers, may lead to misleading results. Therefore, the special method should be considered. The present study was designed to apply the outlier robust nonlinear mixed estimation for effects of phenylephrine on rat corpus cavernosum strips. In this study, eight different doses of phenylephrine in eight experimental groups were used. Each group consisted of eight rats. The concentration–response curves to phenylephrine (0.1µM to 300µM) were obtained by the cumulative addition of phenylephrine to the chamber. Because of the existence of an outlier to achieve robust estimations, M-estimation method and Huber function as a dispersion function were used. Cumulative administration of phenylephrine (0.1µM - 300µM) caused concentration-dependent contractions in strips of rat corpus cavernosum (-Log EC_{50} was 5 ± 0.31, 95% CI= 5.92 to 4.21). The contraction of corpus cavernosum started in the concentration of 0.3 µM and then gradually increased in a dose-dependent manner till it reached a plateau in 100 µM. To consider the clustering feature of concentration-response data, the 4pl regression with a random term has been used. To estimate parameters, because of existence of an outlier in dataset, the robust procedure has been applied. The contraction of corpus cavernosum started in the concentration of 0.3 µM and then gradually increased in a dose-dependent manner till it reached a plateau in 100 µM.

Key words: concentration-response curve, clustering data, model, outlier, phenylephrine, rat corpus cavernosum, robust.

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1. Introduction

Relaxation of corpus cavernosum smooth muscle is critical in inducing and maintaining penile erection [1]. Penile tumescence (erection) and de-tumescence are regulated by a complex neurophysiological process of relaxation and contraction of the corpus cavernosum [2, 3]. There is consensus among scientists that the neural control of erection via cavernosal nerve stimulation involves chemical mediators that are described as non-adrenergic non-cholinergic (NANC) transmitters [4-6]. Nitric oxide is considered to be the main NANC transmitter which mediates the relaxation of corpus cavernosum [6].

The four-parameter logistic [4pl] regression is a common model to analyze the concentration-response data which have special structure called clustered data [7]. The clustered structure is created by the repeating the measurements for the same individuals through time. In clustered data, observation within a cluster will typically exhibit positive correlation and this correlation must be considered in analysis [8].

In concentration-response studies, the existence of outliers appears as an unavoidable main problem. Thus, in order to reduce the disadvantages of outliers on statistical analysis, outliers must be identified and treated [9]. Outlier diagnosis methods are classified in two main categories, first of which is titled as intuitive method where outliers are diagnosed using graphs. In the second category, analyzing the residuals can be led for diagnosing outliers. The later approach is entitled as inferential method [10]. In presence of outliers the first solution comes to mind is omitting them and repeat the experiment to replace the omitted observations [11]. However, this procedure is not always possible; this process may cause waste of time, expense and energy. On the other hand, Outliers can greatly distort parameter estimates and subsequent standard errors. Consequently, inferences about the parameters are misleading. So instead of removing the outlier, an alternative approach is applying outlier robust statistical procedures. In these procedures, the influence of outliers in estimating the model parameters is adjusted [12]. As a result, the parameters estimates are more accurate.

The aim of this study is determining the effect of phenylephrine in rat corpus cavernosum to do this, outlier robust nonlinear mixed estimation is used since outlier exists.

2. Materials and Methods

The data used in this paper are part of a concentration–response study showing the contraction of corpus cavernosum induced by phenylephrine in the organ bath. In this study, eight different doses of phenylephrine in eight experimental groups were used. Each group consisted of eight rats. The concentration–response curves to phenylephrine (0.1μM to 300μM) were obtained by the cumulative addition of phenylephrine to the chamber. The male rats were killed by cervical dislocation. Penises were removed and promptly placed in a petri dish containing Krebs-bicarbonate solution [containing in mM: NaCl: 118.1, KCl: 4.7, KH2PO4: 1.0, MgSO4: 1.0, NaHCO3: 25.0, CaCl2:2.5 and glucose: 11.]; bubbled with a mixture of 95% O2 and 5% CO2. The glans penis and urethra were excised and the corpus cavernosum tissue was then dissected free from the tunica albuginea. The corpus cavernosum was separated by cutting the fibrous septum in penis. It was mounted in 25-ml organ chambers with one end tied to an electrode holder and the other to a wire connected to a force transducer [Letica Scientific Instruments, Barcelona, Spain] and was recorded using a PowerLab system [ADInstrument, Australia]. The chambers contained Krebs-bicarbonate solution [pH 7.4] at 37˚c equilibrated with 95% O2 and 5% CO2. The segments were allowed to equilibrate resting tension of 0.5 g for 60 min based on the previous study.

The 4pl regression model can be written as follows:

$$y_{ij} = A + \frac{D-A}{1+\left(\frac{x_{ij}}{C}\right)^{B}} + \epsilon_{ij}, \quad [1]$$

Where y_{ij} is the jth measured response of the subject exposed to x_{ij} dose, A is the upper asymptote parameter, D is the lower asymptote parameter, C is the ED50 parameter [the dose is required to elicit 50% response], and B is the rate parameter [13].

Two main characteristics of the available data (correlation between observations and presence of outliers) should be considered in the data analysis process. For accounting the cluster-to-cluster variability, a random term is added to the model1 for each cluster. This improved model has been applied to dose-response data by Williams as follows:

$$y_{ij} = (A + a_i) + \frac{D-(A+a_i)}{1+\left(\frac{x_{ij}}{C}\right)^{B}} + \epsilon_{ij} \quad [2]$$

Since the A, B, C, and D parameters are fixed effects, and the parameter a is a random term, model2 is a nonlinear mixed model.

For diagnosing the outliers, a plot of the response against the log dose was used. Because of the existence of an outlier to achieve robust estimations, M-estimation method and linearization technique were used [12]. Data were analysed using SAS9.2.

3. Results and Discussion

Eight different doses of phenylephrine in eight clusters were used in this study. 64 observations were measured. A plot of the response (the contraction of corpus cavernosum) against the logdose for each cluster is given in figure 1.

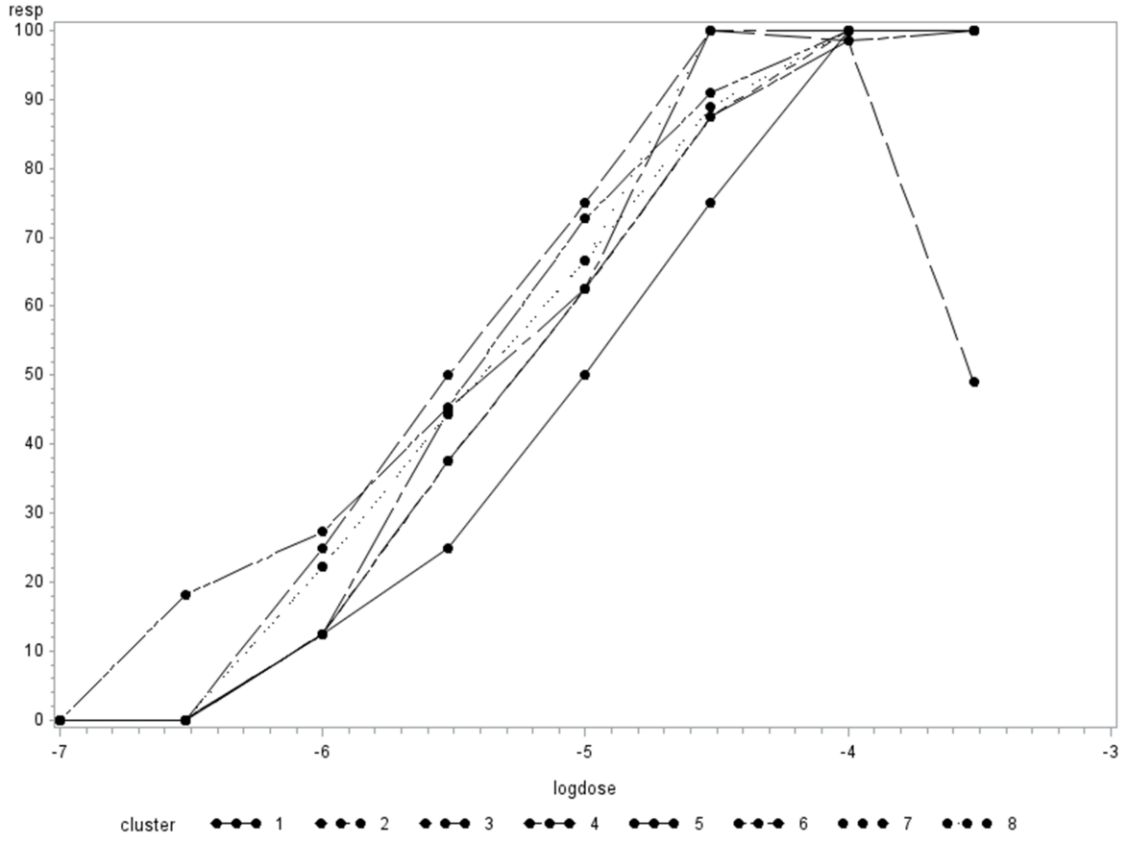

Figure 1. The percent of corpus cavernosum contraction against the logarithm of the dose in each cluster.

As shown in figure 1, there is an outlier in the eighth cluster, eighth dose, so robust estimation should be considered. However, in many studies this issue has been ignored and the common model has been applied. The 4pl with a random term parameters estimates, in columns (2) and (3) and the robust model parameters estimates in columns (4) and (5) have been displayed in table1.

For comparing the robust model and the common model, the Akaike Information Criterion (AIC) were obtained. The AIC statistic is 250.92 and 653.2 for robust model and common model which means robust model had better fit than the common model. Table1 shows the results of robust model for contraction of corpus cavernosum induced by phenylephrine in the organ bath. Cumulative administration of phenylephrine (0.1µM - 300µM) caused concentration-dependent contractions in strips of

Table1. Comparison of non-robust output and robust output.

	Non-robust model		Robust model	
Parameter[1]	**Estimate[2]**	**S.E[3]**	**Estimate[4]**	**S.E[5]**
A	90.00	26.15	100	**1.02**
B	0.70	0.03	0.94	**0.03**
C	4.659E-6	0.48	9.7008E-6	**0.31**
D	2	1.49	0	**1.18**
σ^2	30.98	3.05	1.3727E-9	**1.23**
σ_a^2	48.72	90.30	7.44	**1.44**

rat corpus cavernosum (-Log EC_{50} was 5 ± 0.31, 95% CI= 5.92 to 4.21).

The contraction of corpus cavernosum started in the concentration of 0.3 μM and then gradually increased in a dose-dependent manner till it reached a plateau in 100 μM.

As shown in figure 2, the parameter A estimate in the common model is much lower than this in robust model. This underestimating just happens because of the excessive influence of the outlier in the upper asymptote area. The Population Average (PA) in common model considerably underestimates the mean response in the upper asymptote.

In this paper, the effect of phenylephrine in rat Corpus cavernosum was examined. Cumulative administration of phenylephrine (0.1μM - 300μM) caused concentration-dependent contractions in strips of rat corpus cavernosum. To estimate the parameters, outlier robust nonlinear mixed model estimation was used since outlier exists.

Bozkurt et al. [7] demonstrated for the first time that nicotine potentiated the relaxation response induced by EFS in strips of rabbit corpus cavernosum tissues precontracted with phenylephrine. Bagcivan et al. [14] demonstrated that nicotine-induced concentration-dependent relaxation responses in corpus cavernosum strips in the rabbit when the tissues were pre-contracted with phenylephrine. Vural et al. [15] examined the effects of nicotine on neurogenic relaxation responses in the corpus cavernosum in rabbits including the role of nicotinic acetylcholine receptor subtypes.

It is necessary to discuss the appropriate statistical method to achieve correct results. Statistical analysis of the data can be misleading, if cluster-to-cluster variability is not included. Adding a random term to the 4pl can reduce the error variance. Thus, the estimation of parameters becomes more accurate [12]. In experimental studies especially the studies which are designed to examine the concentration-response relationships the presence of aberrant observations is unavoidable; if the aberrant data have been generated by a mistake in data entry or in the construction of the data set, then it may be possible to correct it. If it cannot be corrected, then it must be omitted [16].These observations will dominate the calculations, and can lead to inaccurate results [17]. This kind of aberrant observations is

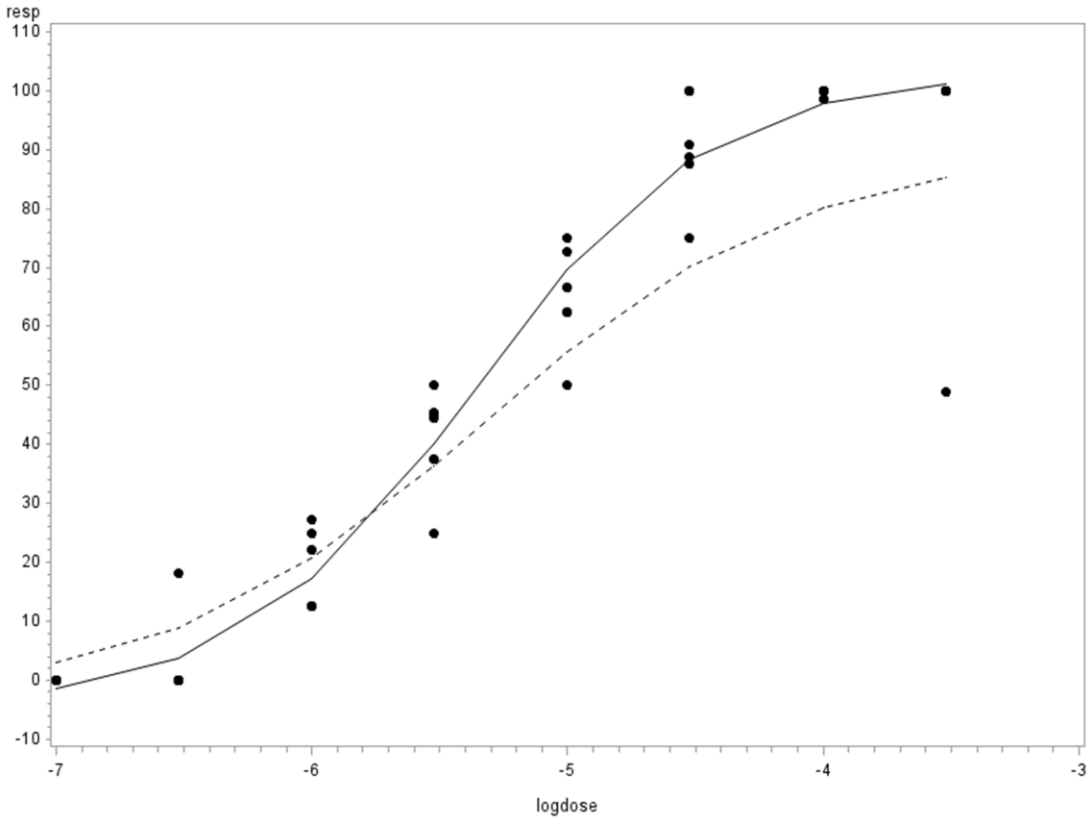

Figure 2. Comparison of non-robust PA curve and robust PA curve to concentration-response data.

called noise data. However, the outliers do not appear because of the mistakes. Hence, after determining the outliers it does not seem that the omission would be the best solution [16]. In this case it would be better to use outlier robust procedure to estimate the parameters [12]. In outlier robust statistical procedures, the influence of outliers in estimating parameters are adjusted. Depending on the model used for the analysis, several robust procedures can be used for solving the problem of outliers. Mancini et al. [18] and Muler and Yohia [19] proposed a robust M-estimator that assigns a much lower weight to the outliers than the Gaussian maximum likelihood estimators does. Pinheiro et al. [20] and Staudenmayer et al. [21] introduced robust estimation techniques in which both random effects and errors have multivariate Student-t distributions. Yeap and Davidian [22] proposed a two-stage approach for robust estimation in nonlinear mixed effects when outliers are present within and between individuals. Finally, the procedure which has been used in this article was proposed by Williams in 2015. He introduced a one-step approach by utilizing a robust version of the linearized Gaussian likelihood for the nonlinear mixed model [12].

As displayed in table1, the parameter *A* estimate based on two models is different. The outlier made a considerable difference in the estimations. Robust estimations are reliable because the robust model is not affected by outliers. The robust parameter estimations do not systematically underestimate the PA near the upper asymptote area. It is clear from figure 2 that the outlier has not influenced the estimates of the robust model as significantly as the estimates of the common model. Most researchers find that in the existence of the outliers, the Gaussian quasi-maximum likelihood estimators are very inaccurate. Accordingly, In the presence of the outliers four parameter logistic regression with a random term need to be robust using appropriate technique.

4. Conclusion

In this paper, in order to consider the clustering feature of concentration-response data, the 4pl regression with a random term has been used. To estimate the parameters of the model because of existence of an outlier in dataset, the robust procedure has been applied. As a final point, the contraction of corpus cavernosum started in the concentration of 0.3 μM and then gradually increased in a dose-dependent manner till it reached a plateau in 100 μM.

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