Mathematical Model By Using Mixture Weibull Distribution For Finding The Combination Of Gad65 And Gaba For Modulation Of Spasticity

[1],S.Lakshmi , [2,]P.Gomathi Sundari

[1,]*Head and Associate Professor of Mathematics, K.N.Govt.Arts College for Women,Thanjavur -613005, TamilNadu, India.*

[2,]*Assistant Professor of Mathematics, Rajah Serfoji Government College, Thanjavur-613005, TamilNadu, India.*

Abstract

Loss of GABA-mediated pre-synaptic inhibition after spinal injury plays a key role in the progressive increase in spinal reflexes and the appearance of spasticity. Clinical studies show that the use of baclofen (GABAB receptor agonist), while effective in modulating spasticity is associated with major side effects such as general sedation and progressive tolerance development. The present study was to assess if a combined therapy composed of spinal segmentspecific upregulation of GAD65 (glutamate decarboxylase) gene once combined with systemic treatment with tiagabine (GABA uptake inhibitor) will lead to an antispasticity effect and whether such an effect will only be present in GAD65 gene over-expressing spinal segments. Here we use the mixture distribution produced from the combination of two or more Weibull distributions which has a number of parameters. A mixture distribution is even more useful because multiple causes of failure can be simultaneously modeled. Also these functions were represented by graphs that showed this variation. The estimation of parameters were effected by the different values of the mixing parameter and the results have been discussed from the corresponding mathematical figures.

Keywords: *Weibull distribution, Mixture Weibull distribution, Mixing parameter, GABA, GAD65, Tiagabine*

AMS Classification: 60 G_{xx}, 62 H_{xx}, 62P_{xx}

I. MATHEMATICAL MODELS

The mixture Weibull distribution produced from the combination has five or more parameters. These are; the shape parameters, scale parameters, location parameters, in addition to the mixing parameter (*w*). This type of distribution is even more useful because multiple causes of failure can be simultaneously modelled. Different values of the mixing parameter were used to obtain the estimation of the parameters and to find the probability density function of the mixture Weibull distribution.

1.1 MIXTURE MODELS

When we have n-fold mixture model that involves n sub-populations, then

$$F(x) = \sum_{i=1}^{n} w_i F(x_i) \quad \text{with} \quad w_i > 0, \quad \text{and} \quad \sum_{i=1}^{n} w_i = 1$$

where w_i is a mixing parameter and $F_i(x)$, $i=1,2,...,n$ are distribution functions either with two or three parameter Weibull distributions. The models involve two or more distributions with one or more being Weibull distribution. The distributions involved are called sub-populations or components, and the model is called finite Weibull mixture model. In the literature the Weibull mixture model has been referred by many other names [5,6], such as additive-mixed Weibull distributions, bimodal-mixed Weibull (for a two fold mixture), mixed-mode Weibull distribution, Weibull distribution of the mixed type, multi modal Weibull distribution, and so forth.

II. MIXTURE WEIBULL DISTRIBUTION

The probability density function (pdf) of a 2-parameter Weibull distribution is,

$$f(x:\alpha,\beta)=\frac{\alpha(x)^{\alpha-1}}{\beta^{\alpha}}\exp\left(-\left(\frac{x}{\beta}\right)^{\alpha}\right) \quad \text{for} \quad 0 \le x < \infty \quad (1)$$

and for a 3-parameter Weibull distribution is

$$f(x:\alpha,\beta,\gamma)=\frac{\alpha(x-\gamma)^{\alpha-1}}{\beta^{\alpha}}\exp\left(-\left(\frac{(x-\gamma}{\beta}\right)^{\alpha}\right) \quad \text{for} \quad \gamma \le x < \infty \quad (2)$$

where α, β, and γ are the shape, scale and location parameters respectively.
A mixture distribution is a distribution made of combining two or more component distributions. The probability density function of this mixture distribution can be shown as;

$$f(x)=w_1 f_1(x)+.....+w_n f_n(x) \qquad w_i>0, \text{ and } \sum_{i=1}^{n} w_i=1$$

where w_i is the mixing parameter which represents the proportion of mixing of the component distributions. The function $f_i(x)$ is the probability density function of the component distribution *i*. While *n* is the number of component distributions being mixed [7]. In this paper we will consider a simple case of only two component distributions, and the mixing parameter for each component will be defined simply as *w* and (1-*w*).

The probability density function of the mixture Weibull distribution of the two distributions in (1) and (2) is as follows;

$$f(x)=\frac{\alpha_1(x)^{\alpha_1-1}}{\beta_1^{\alpha}}\exp\left(-\left(\frac{x}{\beta_1}\right)^{\alpha_1}\right)+(1-w)\frac{\alpha_2(x-\gamma_2)^{\alpha_2-1}}{\beta_2^{\alpha_2}}\exp\left(-\left(\frac{(x-\gamma_2}{\beta_2}\right)^{\alpha_2}\right) \quad (3)$$

where, $\alpha_1, \alpha_2, \beta_1, \beta_2 > 0 \le \gamma \le x$ and $0 < w < 1$
Here *w* is the mixing parameter.

As a result, when the two sub-populations are given by equation (1), the model is characterized by five parameters, the shape and scale parameters for the two sub-populations and the mixing parameter *w*; with $0 < w < 1$

The probability density function and failure rate of the two-fold Weibull mixture are given by;

$$f(x) = wf_1(x) + (1-w) f_2(x)$$

and $$h(x)=\sum_{i=1}^{n} w_i(x)h_i(x)$$

where $$w_i(x)=\frac{w_i R_i(x)}{\sum_{i=1}^{n} w_i R_i(x)} \quad \text{and} \quad \sum_{i=1}^{n} w_i(x)=1$$

and $$R_i(x) = 1 - F_i(x)$$

$R_i(x)$ is the reliability function, Hence

$$h(x)=\frac{wR_1(x)}{wR_1(x)+(1-w)R_2(x)}h_1(x)+\frac{(1-w)R_2(x)}{wR_1(x)+(1-w)R_2(x)}h_2(x)$$

[7] who assumed that only the mixing proportions are unknown for the two component case;

$$f(x) = wf_1(x) + (1-w) f_2(x)$$

By integrating a family of equations of the form;

$$F(x) = wF_1(x) + (1-w) F_2(x)$$

which leads to the following

$$w=\frac{F(x)-F_2(x)}{F_1(x)-F_2(x)}$$

This gives necessary and sufficient conditions on F_1 and F_2 for the uniform attainment of the Cramer-Rao bound on the variance of *w*.

III. APPLICATIONS

We first quantified the loss of GABA-ergic neurons and boutonlike terminals in laminae VII and IX of lumbar spinal cord sections taken from spastic and sham-operated control animals. In comparison with control animals spastic animals had 50% less GABA-ergic neurons (average 39.0 ± 13.4 vs. 19.5 ± 4.2 per section; $p < 0.01$; Fig. 1A, B) in lamina VII. In those same sections, GABA-ergic contact with a-motoneurons was also assessed, revealing a significant reduction in spastic animals (Fig. 1D-white arrows): 19.4 ± 0.9 GABA/Syn-immunoreactive (IR) boutons contacting each motoneuron soma in control tissue compared to 10.1 ± 0.8 in animals with spasticity ($p < 0.01$; Fig. 1C, D). We next examined the number of GAD65 or GAD67 bouton-like structures that contacted VGluT1-IR primary afferent terminals in lamina IX (i.e. the site of GABAergic presynaptic Ia afferent inhibition). In control animals 26.7 ± 4.2% of primary afferent terminals had clear GAD65 contact, compared to only 5.3% ± 1.2% in ischemia-injured tissue ($p < 0.001$; Fig. 1E, F).

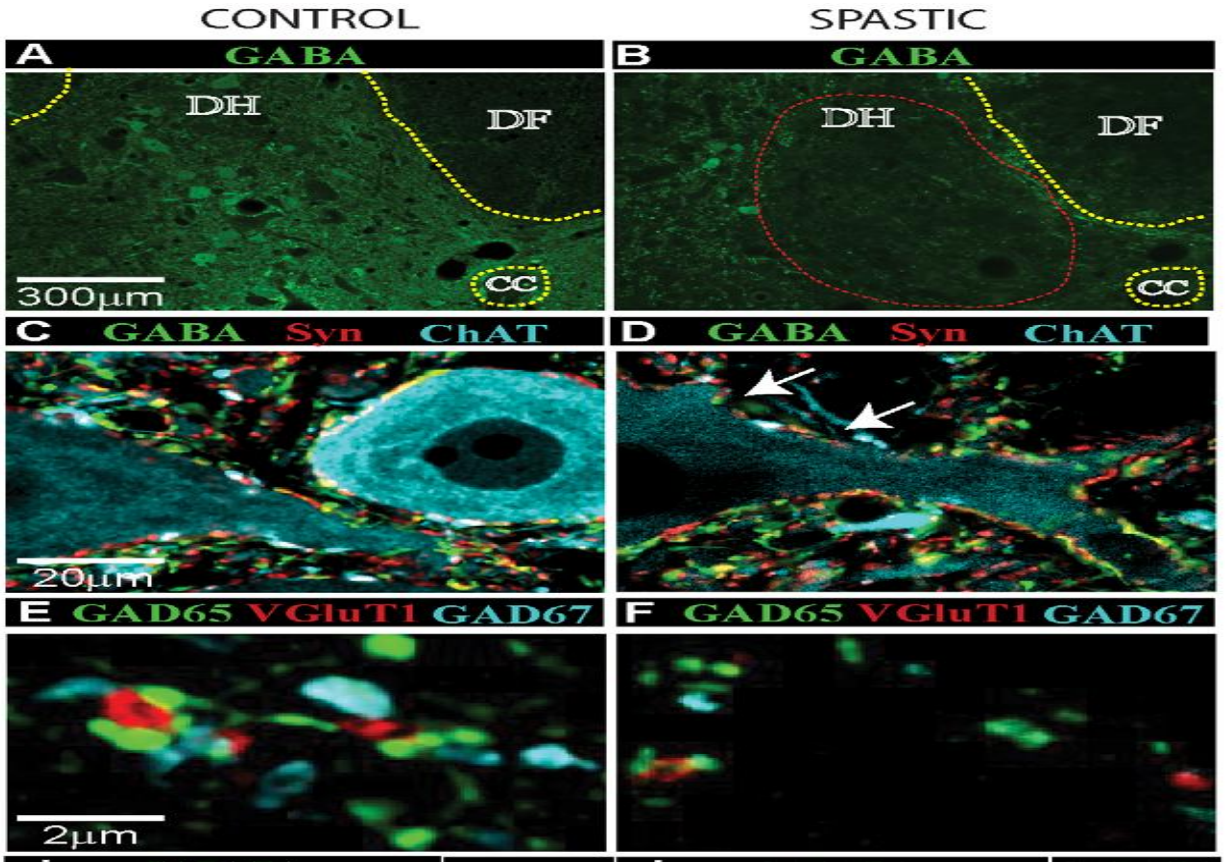


Figure 1. Loss of segmental inhibitory GABA-ergic interneurons and increased expression of GABA B R1+R2 receptor in amotoneurons after transient spinal cord ischemia is associated with the development of chronic muscle spasticity. (A, B) Transverse spinal cord sections taken from L2–L5 segments in control (A) or spinal ischemia-induced-spastic rat (B) at 24 h after intrathecal colchicine injection and stained for GABA. Note an apparent loss of GABA-ergic interneurons in the intermediate zone in spastic rat (B; red circle). (C–F) Loss of GABAergic interneurons corresponds with loss of GABA-IR

We tested if spinal GAD65 overexpression will lead to increased local GABA release and if such a release will have a similar anti-spastic effect once combined with systemic tiagabine (1, 4, 10, 20 or 40 mg/kg) treatment. Spastic animals received a total of 20 bilateral injections of HIV1-CMV-GAD65-GFP (n=6) or HIV1- CMV-GFP (n=6; control) lentivirus targeted into ischemia-injured L2–L5 spinal segments and underwent spasticity assessments 7–21 days after virus delivery. In control HIV1-CMV-GFP-injected spastic animals, systemic administration of tiagabine (40 mg/kg,i.p.) was without effect (Fig. 2A). In contrast, in HIV1-CMVGAD65- GFP-injected rats, treatment with tiagabine led to a potent and significant anti-spasticity effect. The peak effect was seen at 25 min after tiagabine administration and returned back to baseline by 60 min (Fig. 2A; $p > 0.01$). Dose response analysis for tiagabine showed that doses ≥4 mg/kg provided significant ($p > 0.01$) anti-spasticity effect at 15–25 min after tiagabine injection. No detectable effect on upper limb motor

function was seen after tiagabine treatment and all animals showed continuing ability to move their upper limbs and grab food pellets if offered.

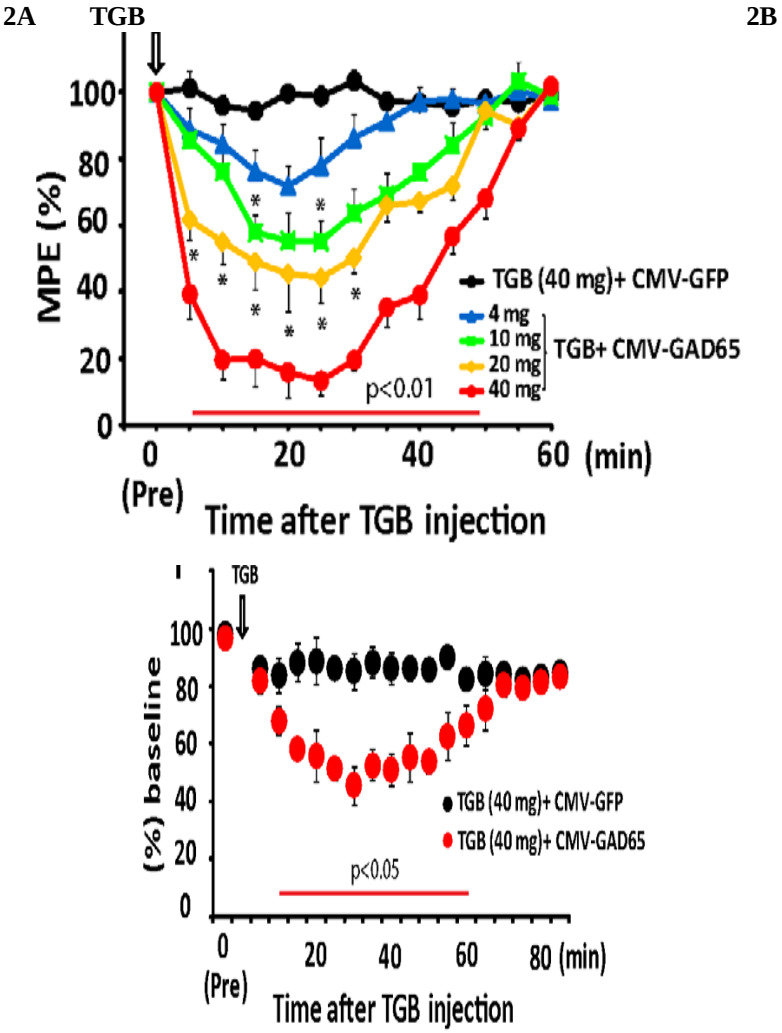


Figure 2A. Effective suppression of spasticity after combined therapy with systemic tiagabine and intrathecal injection of GABA or spinal parenchymal GAD65 gene delivery.

Time-course of anti-spastic effect after tiagabine treatment expressed as % of maximum possible effect in measured ankle resistance in HIV1-CMV-GFP or HIV1-CMV-GAD65-GFP lentivirus-injected animals (* P< 0.01; one-way analysis of variance-ANOVA, Bonferroni's posthoc test; MPE-maximum positive effect). **Figure 2B** Time-course of changes in H-wave amplitudes before and up to 90 min after tiagabine administration (red line-P,0.05; unpaired t test).

In separate experimental sessions, changes in H-reflex amplitudes evoked by high frequency stimulation was tested in ketamine-sedated animals. In spastic animals previously injected spinally with control lentivirus (HIV1-CMV-GFP; n = 6) no change in H-reflex amplitudes were seen up to 90 min after tiagabine injections (Fig. 2B). In animals receiving spinal injections of HIV1-CMV-GAD65-GFP lentivirus (n= 6) a significant (p,0.05) reduction of the H-wave amplitude was measured between 20–45 min after tiagabine injection and returned back to baseline by 65 min (Fig. 2B). Similar significant suppression of H-reflex activity in spastic patients after intrathecal baclofen treatment was reported [2].

The professionals believe, that the ability of such combined therapy in which systemically administered drugs (such as tiagabine) is effective in regulating the activity of the therapeutic product (GABA) in remote GAD65 gene-overexpressig sites can potentially have a significant clinical implications. **First**, the identity of specific spinal segments innervating the affected spastic muscle groups can be neurologically mapped, lateralized and selected for the segment/site-specific GAD65 gene delivery.

Second, extensive clinical data show a potent anti-spastic effect after intrathecal baclofen delivery and this effect is independent on the spinal or supraspinal origin of spasticity [1]. Thus, it is likely that spinal segmental GAD65 upregulation once combined with systemic GABA uptake inhibitor treatment will have a similar therapeutic effect in spasticity of supraspinal and spinal origin.

Third, comparable site-specific delivery of GAD65-encoding vectors targeting functionally/electrophysiologically-defined brain epileptic foci can be performed. Previous data from other laboratories have confirmed an improvement in the parkinsonian behavioural phenotype and neuronal rescue after AAV-CBAGAD65 delivery into the subthalamic nucleus in 6-OHDA– lesioned rats [2]. We speculate that proposed combination treatments can lead to a more pronounced anti-epileptic effect with less side effects such as general sedation.

Fourth, the serum half-life of tiagabine in human patients is between 5–8 hrs and therefore comparable duration of the antispasticty effect can be expected in human patients once combined with spinal parenchymal GAD65 gene delivery [3,4].

MATHEMATICAL RESULTS

Figure 2A :

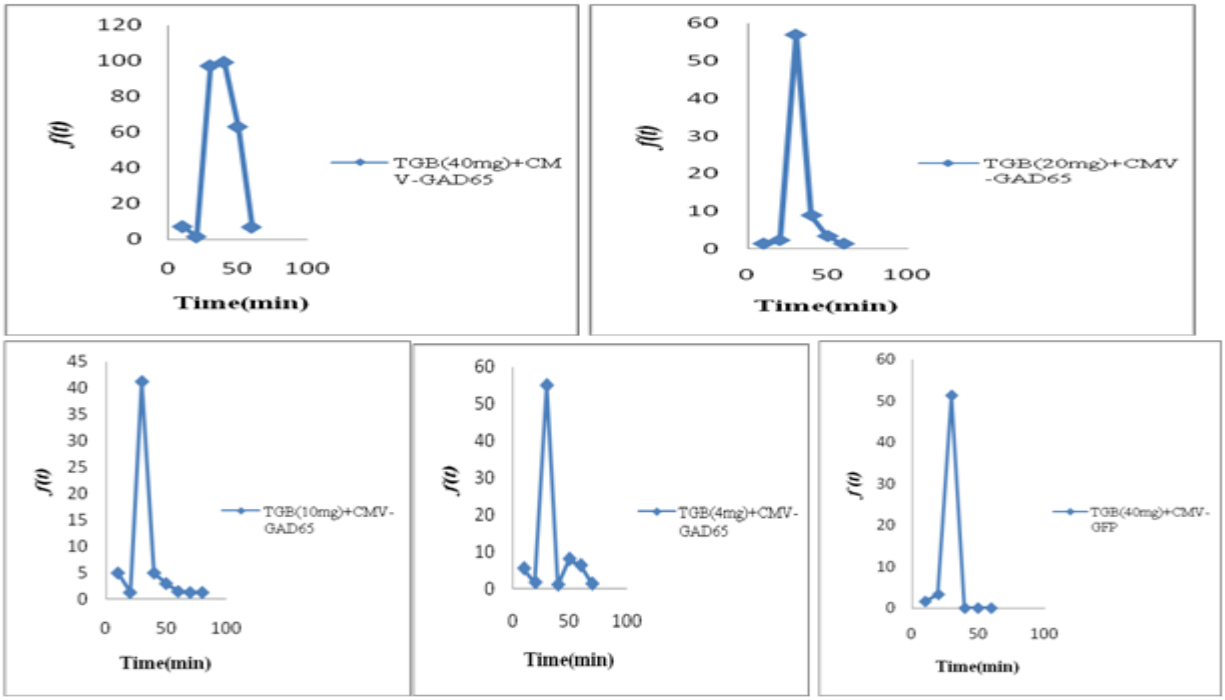
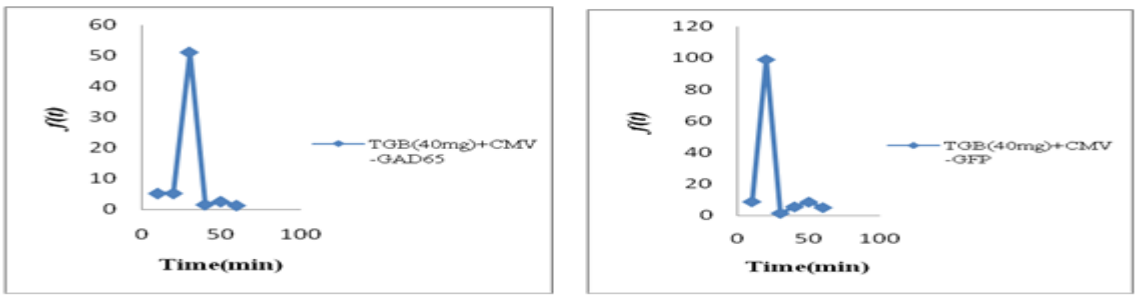


Figure 2B :



The function f(t) reaches the peak in the time interval (20, 30) and sudden decrease in the interval (30, 40). When the 10 mg Tiagabine injection given mixture probability density function f(t) reaches the peak at time t=30 minutes and sudden decrease to time axis at t=40 minutes . Similarly for the 3 case when 20 gm Tiagabine injection is given. The last case when the Tiagabine injection 40 mg is given mixture probability density function f(t) reaches the peak at time t=30 and is constant over there till t=40 and suddenly decreases to the baseline level when time t=60.

IV. CONCLUSION

These data show that treatment with orally bioavailable GABA-mimetic drugs if combined with spinal-segment-specific GAD65 gene over expression can represent a novel and highly effective anti-spasticity treatment which is associated with minimal side effects and is restricted to GAD65-gene over-expressing spinal segments. We also found the mixture probability density function. We graphed these functions and saw the variation on the curves when different values are given for the mixture probability density function. The function f(t) reaches the peak in the time interval (20, 30) and sudden decrease in the interval (30, 40). When the 10 mg Tiagabine injection given mixture probability density function f(t) reaches the peak at time t=30 minutes and sudden decrease to time axis at t=40 minutes . Similarly for the 3 case when 20 gm Tiagabine injection is given. The last case when the Tiagabine injection 40 mg is given mixture probability density function f(t) reaches the peak at time t=30 and is constant over there till t=40 and suddenly decreases to the baseline level when time t=60.

REFERENCES

[1] Remy-Neris O, Barbeau H, Daniel O, Boiteau F, Bussel B (1999) Effects of intrathecal clonidine injection on spinal reflexes andhuman locomotion in incomplete paraplegic subjects. Exp Brain Res 129: 433–440.
[2] Lazorthes Y, Sallerin-Caute B, Verdie JC, Bastide R, Carillo JP (1990) Chronicintrathecal baclofen administration for control ofsevere spasticity. J Neurosurg 72: 393–402.
[3] Gustavson LE, Mengel HB (1995) Pharmacokinetics of tiagabine, a gammaaminobutyric acid-uptake inhibitor, in healthysubjects after single and multiple doses. Epilepsia 36: 605–611.
[4] Dalby NO (2000) GABA-level increasing and anticonvulsant effects of three different GABA uptake inhibitors.Neuropharmacology 39: 2399–2407.
[5] Ahmad, K.E. and AbdulRahman, A.M. (1994) Upgrading a Nonlinear Discriminate Function Estimated from a Mixture of twoWeibull Distributions, Mathematics and Computer Modelling, 18, 41-51.
[6] Murthy, D.N.P, Xie, M. and Jiag, R. (2004), Weibull Models, John Wiley & Sons, Inc. Hoboken, New Jersey.
[7] Talis, G.M. and Light, R. (1968). The Use of Fractional Moments for Estimating the
[8] Parameters of Mixed Exponential Distribution. Technometrics, 10, 161-75.