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Stability of a less-painful intravenous emulsion of clarithromycin

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Abstract

The chemical stability of a less-painful ready-to-use o/w emulsion for intravenous (i.v.) delivery of clarithromycin was investigated. The formulation contained 0.5% w/v drug, 2% w/v soybean oil, 5% w/v egg phosphatide, 0.6% w/v oleic acid, 0.3% hexanoic acid and 2.5% w/v glycerin in water. The change in the initial pH of emulsion (range: 6.5–8.0) did not significantly influence drug stability over a period of 30 days at 5 and 30°C. The increase in the initial pH of emulsion as well as its storage temperature gradually decreased the final pH over time. The emulsion prepared at pH 7.5 demonstrated a constant drop in pH over 9 months at 30°C at the rate of about 0.002 units drop in pH per day; however, it did not demonstrate significant loss of drug. Actual as well as projections using accelerated stability studies indicated the shelf-life (i.e., time to retain 90% of the label claim, $t_{90\%}$) of the emulsion formulation, at 25°C, to be ≥ 21 months. Overall, the results suggest that the development of a less-painful ready-to-use o/w emulsion of clarithromycin is feasible.

Keywords: Clarithromycin; Stability; Intravenous emulsion

1. Introduction

Clarithromycin is a new macrolide antibiotic with a methoxy group (-OCH_3) attached to the C_6 position of erythromycin which makes it more acid stable than erythromycin (Kohno et al., 1989, Nakagawa et al., 1992). It has been found to be more efficacious than erythromycin against streptococci and *Mycobacterium tuberculosis* (Fuji and Nishimura, 1988). It has also been found to be efficacious in the treatment of experimental *Treponema pallidum* and Lyme's disease (Alder et al., 1993). Clarithromycin is currently marketed in tablet form by Abbott Laboratories as Biaxin®. It is administered intravenously (i.v.) as the lactobionate salt, which is water soluble at pH ≤ 5. However, in common with other macrolide antibiotics, clarithromycin lactobionate has the potential for pain upon parenteral administration (Gupta et al., 1994). Hence, an o/w emulsion formulation was developed with the goal of reducing pain upon injection (Lovell et al., 1994). Due to limited solubility of this compound in routine aqueous as well as non-aqueous media, lipophilic counterions (oleic acid and hexanoic acid) were used to improve the solubility of drug in the oil phase. Egg phosphatide and glycerin

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were used for emulsion stabilization and tonicity adjustment, respectively. The final composition of the emulsion was 0.5% w/v drug, 2% w/v soybean oil, 5% w/v egg phosphatide, 0.6% w/v oleic acid, 0.3% hexanoic acid and 2.5% w/v glycerin in water.

In order to support clinical trials, studies were conducted to examine the stability of a clarithromycin emulsion and hence to select a formula with an acceptable shelf-life. Overall, the results suggest that an emulsion formulation of clarithromycin can be stored at room temperature for about 2 years without major loss of drug concentration.

2. Experimental

2.1. Materials and equipment

Clarithromycin base, soybean oil, sodium hydroxide and glycerin USP, were obtained from Abbott Laboratories and were reagent grade or USP code materials. Egg phosphatides (low electrolyte) were obtained from Pfansteihl Laboratories (Waukegan, IL). Hexanoic acid and oleic acid were purchased from Sigma. Chloroform, methanol and dibasic sodium phosphate were procured from Fisher and disposable 0.2 μm nylon filters from Nalgene were used.

A Silverson® homogenizer, model L2R, was used to obtain crude emulsions. A Microfluidics microfluidizer® or Manton-Gaulen M-15 homogenizer was used to obtain final formulations. A Wescor 5500 vapor pressure osmometer and a laser particle sizer, NICOMP, were used to determine osmolarity and particle size distribution of the formulations, respectively. A Waters pump, model 5907, a Waters 712 WISP automatic sample injector, a Spectraflow 783 programmable absorbance detector and a Spectra-Physics integrator were used for the HPLC analysis of drug.

2.2. Formulation development

The preparation of emulsions involved three steps: (a) dissolution of clarithromycin base in oil in presence of lipophilic counterions, (b) preparation of an aqueous phase containing egg phosphatides, glycerin and sodium hydroxide, and (c) incorporation of oil phase into aqueous phase to obtain an o/w emulsion.

Typically, the aqueous phase was homogenized to thoroughly disperse its components. Then the oil phase was incorporated into it with the aid of Silverson homogenizer. After stirring for 15 minutes, the crude emulsion was transferred to a Microfluidics microfluidizer to make 15 passes at 10000–15000 psi with cool water circulating around the equipment to maintain the temperature of emulsion at ≤ 40°C. Thereafter, the pH of the formulation was adjusted to a desired value (6.5–8.0) using 10% w/v aqueous sodium hydroxide solution and its volume made up with distilled water. The formulations were sterilized using a 0.22 μm Nalgene® nylon filter and 5-ml aliquots packaged in 10 ml type I glass bottles using teflon-coated stoppers.

2.3. Drug analysis

A reverse-phase HPLC method was employed for drug analysis. The mobile phase composition was 55% v/v 0.01 M potassium phosphate buffer pH 3.5, 40% v/v acetonitrile and 5% v/v methanol. A 50×4.6 mm ODS2 3 μm 'Little Champ' HPLC column (Regis) was used. The detection wavelength was 214 nm. The injection volume and mobile phase flow rate were set to 25 μl and 1 ml/min, respectively. Under these conditions, the retention time for clarithromycin was about 6.2 min, and its standard curve between the range of 10–500 μg/ml was linear with $r^2 \geq 0.990$.

The drug concentration in emulsion formulations was determined by diluting 0.5 ml sample to 10 ml with HPLC mobile phase, sonicating for 5 min, followed by centrifugation at 10000 rpm for 5 min. The clear supernatant was then directly injected on to the HPLC column to determine the drug concentration.

2.4. Stability studies

Formulations were stored in dark at 5, 30, 40, 50 and 60°C. Representative samples were re-

moved periodically and monitored for drug concentration by HPLC, pH and particle size. The accelerated temperature stability data was used to determine zero-order degradation rate constants (K_0) and establish Arrhenius plots, and thus predict degradation rate constant at room temperature. The data predicted at 5 and 30°C were compared with the actually measured data at the same temperatures. Each sample was also visually observed for product homogeneity, with particular attention to creaming, coalescence and oil separation.

3. Results and discussion

Relatively poor solubility of drug in oils prompted the use of lipophilic counterions in the formulation. In addition, this step was anticipated to minimize drug partitioning from oil into aqueous phase during and following its emulsification, and hence reduce pain upon i.v. administration.

The changes in physical stability of emulsion may be manifested by change in particle size of oil droplets, creaming, coalescence and phase separation (Hansrani et al., 1983; Santos Magalhaes et al., 1991). On the other hand, the changes in its chemical stability may be manifested in terms of release of free fatty acids and change in bulk pH (Washington and Davis, 1987; Kemps and Crommelin, 1988). These changes can occur during preparation of emulsion and/or during its storage. In view of promising biological results (e.g., possibility of pain reduction, and efficacy and toxicity comparable to that of clarithromycin lactobionate solution) (Gupta et al., 1991; Lovell et al., 1994), the clarithromycin o/w emulsion was evaluated for its long-term physical and chemical stability. No physical instability of emulsion was observed during or immediately after preparation. For example, no crystal growth was observed for several days after the preparation of emulsion.

3.1. Particle size

Fig. 1 displays the effect of storage condition on the particle size of the clarithromycin o/w emulsion initially adjusted to pH 7.5. The samples stored at 5 and 30°C did not demonstrate appreciable increase in particle size over the 165 day study period; however, at higher temperatures, the particle size increased by almost 20–60%. This phenomenon has been reported earlier by several workers (Pathak et al., 1990; Chaturvedi et al., 1992).

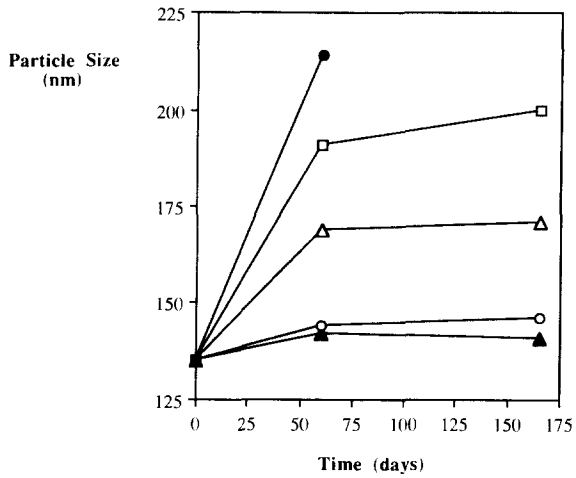

Fig. 1. Effect of storage temperature on the change in particle size of clarithromycin emulsion over 165 days. The initial pH of the emulsion was 7.5. (▲) 5°C, (○) 30°C, (△) 40°C, (□) 50°C, and (●) 60°C.

3.2. pH

In order to investigate the effect of pH of emulsion on its chemical stability, emulsions containing 5 mg/ml drug and fixed concentrations of other ingredients were adjusted to pH of 6.5, 7.0, 7.5 or 8.0. The emulsion adjusted to pH 6.5 cracked and separated into two phases by 2 weeks after storage at 60°C. Hence, no data could be collected from these samples after 2 weeks. Nonetheless, the fact that the emulsion adjusted to higher pH values (i.e., 7.0, 7.5 and 8.0) did not demonstrate similar a phenomenon at 60°C suggests that pH plays an important role in the stability of the clarithromycin o/w emulsion. The importance of the pH-stability relationship in emulsions has been reported earlier (Pathak et al., 1990).

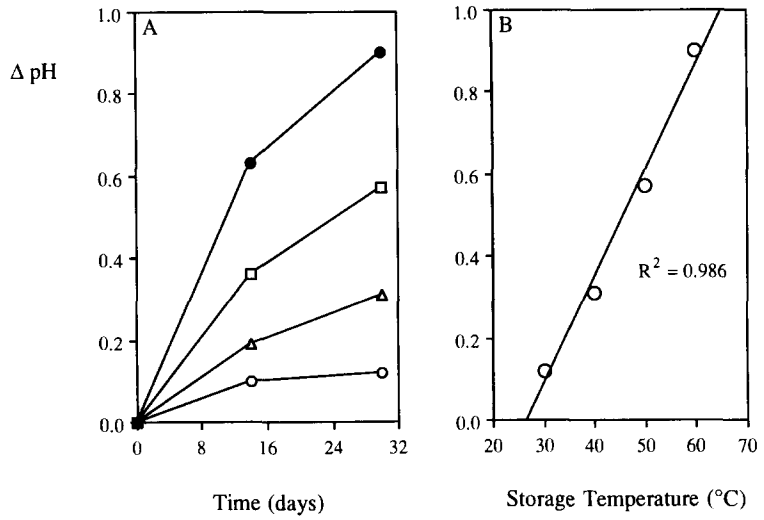

Fig. 2. (A) Effect of storage temperature and duration on the reduction in the pH of clarithromycin emulsion (ΔpH). The initial pH of the emulsion was 7.5. (○) 30°C, (△) 40°C, (□) 50°C, and (●) 60°C. (B) Plot of correlation between the storage temperature and reduction in the pH of clarithromycin emulsion (ΔpH) over 30 days. The initial pH of the emulsion was 7.5.

Several workers have reported a pH drop over time in phospholipid-based emulsions (Levy and Benita, 1991; Manning and Washington, 1992). Hence, the pH of various emulsions was monitored during the stability studies. Fig. 2A illustrates the effect of storage condition on the pH drop in the emulsion over 1 month. The emulsion was adjusted to pH 7.5 prior to storage at various temperatures. At 5°C, there was little change in the pH of the emulsion (data not shown); however, the increase in storage temperature appreciably effected the stability of the emulsion, caus-

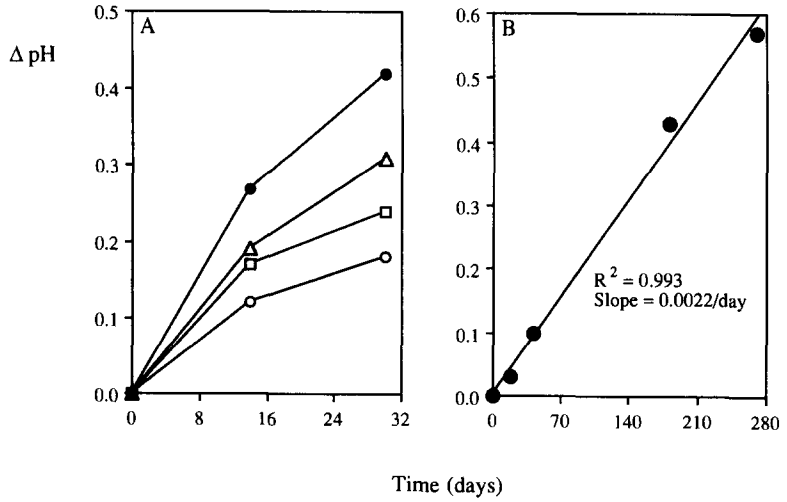

Fig. 3. (A) Effect of initial pH of clarithromycin emulsion on its pH reduction (ΔpH) as a function of time at 40°C. (○) pH 6.5, (□) pH 7.0, (△) pH 7.5, and (●) pH 8.0. (B) Effect of long-term storage of clarithromycin emulsion on its pH reduction (ΔpH) at 30°C. The initial pH of the emulsion was 7.5.

ing an increased drop in pH. This suggests possible pH instability if the formulation were to be autoclaved. When the pH drop over a period of 30 days was plotted against the storage temperature, it followed a linear relationship ($r^2 \geq 0.980$; see Fig. 2B). Similar trends were observed with emulsions initially adjusted to pH 6.5, 7.0 and 8.0.

The initial pH of the emulsion was also found to effect a pH drop as a function of storage time (see Fig. 3A). In fact, the lower the initial pH of the emulsion, the lower was its pH drop over a given time. Fig. 3B illustrates the pH stability of the clarithromycin o/w emulsion over a period of about 9 months at 30°C. The initial (zero-time) pH of the emulsion was 7.5. At 5°C, the drop in pH was minimal (≤ 0.20 pH units in 9 months). However, at 30°C, there was an appreciable linear drop in pH as a function of time ($r^2 = 0.993$), with pH drop rate approximating 0.0022 units/day. If the lower acceptable pH limit of a formulation is known, it is possible that this method may assist in predicting the time required to reach that limit.

The effect of storage conditions on the reduction in pH of emulsions can be explained in view of possible hydrolysis of phosphatides and/or oil resulting in liberation of free fatty acids. In fact, it has been suggested that the measurement of pH changes may provide an indication of the free fatty acid content of phospholipid-based emulsions (Herman and Groves, 1993). The hydrolysis of diacylphosphatidylcholines and diacylphosphatidylethanolamines to their corresponding monoacyl (lyso-) derivatives, followed by the degradation of lyso derivatives into corresponding glycerophosphoryl compounds, have been suggested as the key steps in the formation of free fatty acids. However, to a smaller extent, these can also be liberated by the hydrolysis of emulsified triglycerides into corresponding mono- and diglycerides (Herman and Groves, 1993). Implicitly, the higher the storage temperature, the more intense is the hydrolysis process and the release of acids, and hence, the greater is the extent of pH drop. This mechanism probably forms the basis of data illustrated in Fig. 2. With emulsions adjusted to comparatively higher pH (e.g., pH 8.0 vs 7.0), the alkali-catalyzed hydrolysis of oil, i.e., saponification, will occur relatively sooner thereby manifesting a pH drop in shorter time. Based on the same rationale, the emulsions adjusted to comparatively lower pH (e.g., pH 6.5 vs 7.5) would require longer time to undergo a similar degree of hydrolysis, and will therefore demonstrate less drop in pH over a given time than those adjusted to higher pH. This may explain the data displayed in Fig. 3A.

As mentioned earlier, the reduction in pH as well as some increase in their droplet particle size occurs in all fat emulsions, including drug-free nutritional emulsions like Liposyn® (unpublished data, Abbott Laboratories). Since the reduction in pH can have adverse effect of the stability, Liposyn® is adjusted to a comparatively high pH (≥ 8.0) prior to its autoclaving. This initial pH adjustment maintains the final pH of Liposyn® at ≥ 7.5 after autoclaving. With regards to the particle size, Liposyn® is passed through 5 μm filter, filled in bottles and then autoclaved. The last step increases the particle size of emulsion to some extent; however, the change is usually non-significant.

3.3. Drug concentration

Early work suggested that the initial pH of emulsion, in the range of 6.5–8.0, has minimal effect on the loss of drug in emulsion. For example, the emulsions adjusted to pH 6.5, 7.0, 7.5 and 8.0 were found to contain 95.8, 95.8, 94.9 and 93.9% drug after 7 days of storage at 60°C. Therefore, further work was pursued with emulsions adjusted to pH 7.5.

At 5°C, the emulsion did not demonstrate loss in drug concentration. However, the increase in the storage temperature caused appreciable losses in drug concentration in relatively short period of time. Fig. 4 illustrates the chemical stability of the clarithromycin o/w emulsion as a function of storage temperature. The resulting zero-order drug degradation rate constants (K_0) were used to construct an Arrhenius plot (see Fig. 5) and hence to predict the shelf-life (i.e., the time to retain 90% of the label claim, $t_{90\%}$) at 25 and 30°C (Martin et al., 1983).

Being a heterogeneous system, emulsion for-

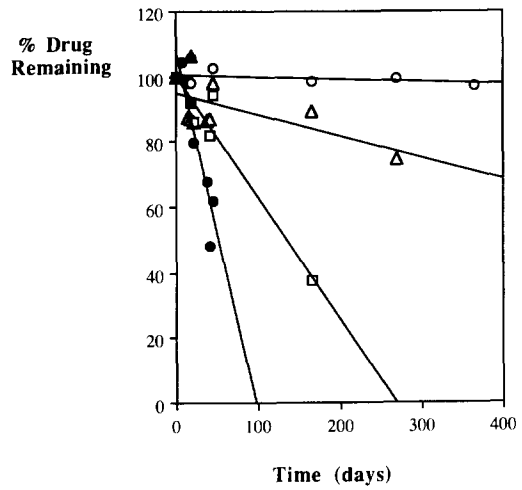

Fig. 4. Chemical stability of 5 mg/ml clarithromycin emulsion following storage at different temperatures for 42–365 days. (○) 30°C, (△) 40°C, (□) 50°C, and (●) 60°C.

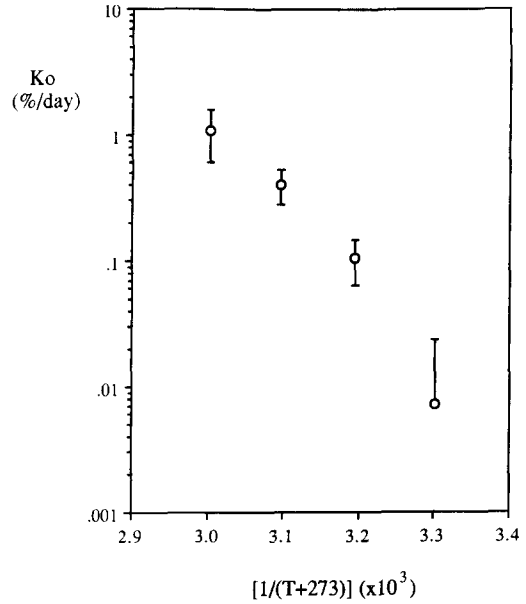

Fig. 5. Arrhenius plot of data at 30, 40, 50 and 60°C. T refers to storage temperature (in °C). Error bars indicate ±95% confidence interval limits for the respective degradation rates. For details, refer to Table 1.

mulations can undergo excessive drug degradation at elevated temperatures, perhaps at rates which are proportionately higher than those prevailing under ambient conditions. In order to investigate this issue, the degradation rates estimated at 30, 40, 50 and 60°C were subjected to Arrhenius treatment in three sets :

(a) Temperature-rate relationship with data collected at 40, 50 and 60°C. This was anticipated to provide a most conservative or worst-case estimate of the shelf-life of formulation;
(b) Temperature-rate relationship for data collected at 30, 40 and 50°C. This was anticipated to provide an optimistic or best-case estimate of the shelf-life of formulation; and
(c) Temperature-rate relationship with data collected at 30, 40, 50 and 60°C. The comparison of the goodness of fit of this relationship with those investigated above was anticipated to provide an idea of the temperature cut-off where the change in drug degradation mechanism occurs.

Table 1 summarizes the results of Arrhenius treatment of data in three sets according to the method explained above. As can be seen, a good linear relationship between log(degradation rate) and 1/temperature at 40, 50 and 60°C ($r^2 = 0.996$) suggests the existence of similar drug degradation mechanism(s) under these conditions. Reduction in the goodness of fit between log(degradation rate) and 1/temperature at 30,

Table 1
Summary of clarithromycin emulsion stability projections based on Arrhenius fit of data collected at different storage temperatures

Temperatures (°C)	R^2	Projected rate at 30°C (%/day)	Projected $t_{90\%}$ at 30°C (year)	Projected rate at 25°C (%/day)	Projected $t_{90\%}$ at 25°C (year)
40, 50 and 60	0.996	0.0301	0.91	0.0153	1.79
30, 40 and 50	0.971	0.0086	3.17	0.0029	9.49
30, 40, 50 and 60	0.958	0.0107	2.57	0.0042	6.48

Table 2
Comparison of actual vs projected stability of clarithromycin emulsion at 5°C

Determination criteria	Degradation rate (%/day)
Actual data over 365 days	0 (0.007) [a]
Projections	
(I) Using 40, 50 and 60°C data	0.0008 (0.0093)
(II) Using 30, 40 and 50°C data	0 (0.179)
(III Using 30, 40, 50 and 60°C data	0.0001 (0.0020)

[a] Data in parenthese refers to ±95% confidence interval.

40 and 50°C ($r^2 = 0.971$) suggests that the drug degradation mechanism at 30°C is probably different from that existing at 40 and 50°C. This is further evident with the log(degradation rate) vs 1/temperature relationship at 30, 40, 50 and 60°C ($r^2 = 0.958$). In fact, the degradation rate at 30°C projected using the data collected at 40, 50 and 60°C, i.e., 0.0301%/day, fell outside the 95% confidence range for the actual rate at 30°C, i.e., −0.0096 to 0.0238%/day, again indicating a change in degradation mechanism between 30 and ≥ 40°C storage temperatures.

Table 2 lists the actual as well as projected degradation rate of clarithromycin emulsion at 5°C. Actual analysis of emulsion samples over 1 year at 5°C yielded results within 98.8 and 101% of the label claim, with estimated zero drug degradation rate. Higher drug degradation rates at 5°C were projected in the absence of 30°C than that in the presence of 30°C data. Given the relatively large 95% confidence interval limits, the projected rates did not appear to be different; however, the results again suggest the possibility of overestimation of drug degradation rates at low storage temperatures (e.g., 5°C) using the data collected at higher storage temperatures (i.e., ≥ 40°C). Hence, the shelf-life of clarithromycin emulsion predicted using the data collected under accelerated conditions is likely to be an underestimate of the real-time shelf-life. This may explain the discrepancy between the projected shelf-life of 1.00–3.30 year and the actually monitored shelf-life, 3.86 year, at 30°C. Further stability studies at temperatures between 30 and 40°C are required to identify the critical temperature at which the change in drug degradation mechanism occurs.

It should be noted that at 25°C and pH 7.6, the $t_{90\%}$ of an aqueous solution of clarithromycin has been projected to be 0.56 years (unpublished data, Abbott Laboratories). Thus, it appears that the emulsion formulation improved the chemical stability of clarithromycin to an appreciable extent. Similar results have been reported for physostigmine o/w emulsion (Pathak et al., 1990).

It should be noted that prior to sealing, the vials containing emulsion were not flushed with nitrogen. It has been suggested that in emulsions oxygen preferentially dissolves in oil rather than in the aqueous phase, and the presence of oxygen has been shown to facilitate hydrolysis of phospholipid-based emulsions (Herman and Groves, 1993). Hence, it is possible that nitrogen purging of containers may further improve the stability of clarithromycin emulsion.

4. Conclusions

The clarithromycin o/w emulsion demonstrated changes in physical stability under ambient conditions. However, these changes (e.g., pH drop and increase in particle size) reflect a process which typically occurs in all fat emulsions. The results overall suggest that the development of clarithromycin emulsion with good stability at 25°C is feasible. The changes in the mechanism of drug degradation under accelerated conditions did not allow good estimation of the shelf-life of emulsion under ambient conditions. Hence, for future stability studies, real-time data may be more meaningful.

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