SCIENTIFIC ARTICLE

# Pharmacokinetic and clinical effects of two bupivacaine concentrations on axillary brachial plexus block

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Abstract

*Introduction:* The risk of systemic bupivacaine toxicity is a persistent problem, which makes its pharmacokinetic study fundamental for regional anesthesia safety. There is little evidence of its influence on plasma peak at different concentrations. The present study compares two bupivacaine concentrations to establish how the concentration affects this drug plasma peak in axillary brachial plexus block. Postoperative latency and analgesia were also compared.

*Methods:* 30 patients were randomized. In the 0.25% Group, 0.25% bupivacaine (10 mL) was injected per nerve. In the 0.5% Group, 0.5% bupivacaine (5 mL) was injected per nerve. Peripheral blood samples were collected during the first 2 h after the blockade. For sample analyses, high performance liquid chromatography mass spectrometry was used.

*Results:* Plasma peak occurred 45 min after the blockade, with no difference between groups at the assessed time-points. Plasma peak was $933.97 \pm 328.03$ ng.mL$^{-1}$ (mean $\pm$ SD) in 0.25% Group and $1022.79 \pm 253.81$ ng.mL$^{-1}$ in 0.5% Group ($p = 0.414$). Latency was lower in 0.5% Group than in 0.25% Group ($10.67 \pm 3.71 \times 17.33$ min $\pm 5.30$, respectively, $p = 0.004$). No patient had pain within the first 4 h after the blockade.

*Conclusion:* For axillary brachial plexus block, there was no difference in bupivacaine plasma peak despite the use of different concentrations with the same local anesthetic mass. The concentration inversely influenced latency.

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**PALAVRAS-CHAVE**
Bupivacaína;
Plexo braquial;
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**Efeitos farmacocinéticos e clínicos de duas concentrações de bupivacaína no bloqueio do plexo braquial via axilar**

**Resumo**

*Introdução:* O risco de intoxicação sistêmica pelo uso da bupivacaína é um problema persistentee torna seu estudo farmacocinético fundamental para a segurança da anestesia regional. São escassas as evidências sobre a influência de diferentes concentrações no pico plasmático desse fármaco. O presente estudo compara duas concentrações de bupivacaína para estabelecer como a concentração afeta o pico plasmático desse fármaco no bloqueio do plexo braquial via axilar. Também se compararam latência e analgesia pós-operatória.

*Métodos:* Foram randomizados 30 pacientes. No Grupo 0,25%, injetaram-se 10 mL de bupivacaína 0,25% por nervo. No Grupo 0,5%, injetaram-se 5 mL de bupivacaína 0,5% por nervo. Amostras de sangue periférico foram colhidas durante as duas primeiras horas após o bloqueio. Para análise das amostras, usou-se a cromatografia líquida de alta frequência acoplada ao espectrômetro de massas.

*Resultados:* O pico plasmático ocorreu 45 minutos após o bloqueio, sem diferença entre os grupos nos tempos avaliados. O pico plasmático (média ± DP) foi $933,97 \pm 328,03$ ng.mL$^{-1}$ no Grupo 0,25% e $1.022,79 \pm 253,81$ ng.mL$^{-1}$ no Grupo 0,5% ($p = 0,414$). O Grupo 0,5% apresentou menor latência com relação ao Grupo 0,25% ($10,67 \pm 3,71 \times 17,33$ min $\pm 5,30$; respectivamente; $p = 0,004$). Nenhum paciente apresentou dor nas primeiras quatro horas após o bloqueio.

*Conclusão:* Para o bloqueio do plexo braquial via axilar, não foi detectada diferença no pico plasmático de bupivacaína apesar do uso de diferentes concentrações, com a mesma massa de anestésico local. A concentração influenciou inversamente a latência.

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## Introduction

The success of regional anesthesia (RA) is directly related to the evolution of knowledge and the development of local anesthetics (LA). Despite efforts to increase its safety, a persistent problem in clinical practice is local anesthetic systemic toxicity (LAST).[1,2] The rapid increase in LA plasma levels leads to devastating neurological and cardiac complications; LA systemic toxicity accounts for one-third of deaths or brain damage during regional anesthesia.[3,4] Regarding RA type, peripheral nerve block may require a greater LA volume, with a higher risk of LAST compared to epidural block.[1,5,6]

The increased use of ultrasound in clinical practice has reduced the occurrence of complications related to peripheral block, mainly due to the reduction of inadvertent vascular puncture.[7,8] Moreover, in order to improve the safety of regional anesthesia, anesthesia societies around the world recommend using the minimum dose required and stipulate the maximum LA dose to be used in peripheral blocks.[9] However, there is no consensus among the different societies about the maximum recommended dose of LA, which reveals a gap in understanding the pharmacokinetics of these drugs.[9] Furthermore, there are several factors that may influence LA plasma peak such as the infusion site vascularization and the tissue binding ability.[9]

Surprisingly, data on the effect of different LA concentrations on plasma levels are scarce and conflicting so far. However, understanding the pharmacokinetics of these chemical compounds is essential to prevent complications.[10–12]

This study was performed to evaluate this relationship, particularly regarding axillary brachial plexus block. Thus, the present study provides an analysis of the pharmacokinetic profiles of two bupivacaine concentrations obtained after axillary brachial plexus block. Despite the different concentrations used, the total mass of local anesthetic was maintained to evaluate the effect of these different concentrations on this drug plasma peak. As a secondary objective, latency and postoperative analgesia were evaluated in both groups.

## Material and method

A prospective and randomized clinical trial was performed at the operating room of the Hand and Upper Limb Surgery Department at a quaternary University Hospital. The anesthetic procedures were initiated in January 2014, after approval by the Ethics and Institutional Research Committee approval number 288,461. Inclusion criteria were: candidates for elective distal forearm and hand surgery, with brachial plexus block indication for anesthesia and analgesia, aged between 18 and 65 years, with physical status (ASA I or II) according to the American Society of Anesthesiologists, body mass index (BMI) less than 35 kg m$^{-2}$, and a signed written informed consent. Exclusion criteria were: cognitive impairment, infection at the puncture site, coagulopathy, history of bupivacaine allergy.

## Clinical methodology

After meeting the inclusion criteria, patients were randomized into groups to receive either 0.25% bupivacaine or 0.5% bupivacaine, according to computer-generated random numbers, which were considered as blocks of ten cases.

The 0.25% bupivacaine concentration was used because it is the lowest effective concentration for axillary brachial plexus block.[13] The 0.5% concentration was used because it is the highest bupivacaine concentration used in our service.

For greater accuracy of information, the patient received explanation to the differences between tactile and painful sensation. Routine monitoring for surgical procedure with electrocardioscopy, sphygmomanometer, and pulse oximetry was performed. Venous access was obtained in the upper limb contralateral to the surgical procedure, exclusively for blood sample collection.

The axillary brachial plexus block guided by ultrasound (S Series, Fujifilm Sonosite, Seattle, USA) was performed by a single experienced anesthetist, with the patient in the horizontal dorsal decubitus position, aiming at standardizing the blockade time, defined as the interval between the needle insertion and the end of LA injection. Thus, this time would not influence the latency of groups. Skin asepsis was made with a chlorhexidine-alcohol solution. After brachial plexus nerve visualization, LA was injected into each nerve identified in this pathway – namely, radial, ulnar, medial, and musculocutaneous. For the 0.25% Group, 10 mL of 0.25% bupivacaine (*Cristália Produtos Químicos*, São Paulo, Brazil) were injected into each nerve, totaling 40 mL per patient. For the 0.5% Group, 5 mL of 0.5% bupivacaine (*Cristália Produtos Químicos*, São Paulo, Brazil) were injected into each nerve, totaling 20 mL per patient.

An anesthesiologist, who was not present during the injection and was blinded to the concentration and volume of anesthetics used, evaluated the nerve blocks according to motor function, thermal sensitivity, and pain sensitivity.

In order to evaluate motor function, the modified Bromage scale was used (Table 1).[14,15] The assessed muscles were: finger flexors (median nerve), finger extensors (radial nerve), first finger adductor (ulnar nerve), and biceps (musculocutaneous nerve).

Thermal sensation assessment was made with gauze and alcohol, testing the sensitivity of the dermatomes innervated by the ulnar, median, radial, and musculocutaneous nerves.

The assessment of pain sensation at the upper limb was performed with the pinprick test (23G needle), testing the sensitivity of the ulnar, median, radial, and musculocutaneous dermatomes nerves.

This assessment happened every five minutes up to 30 min after the blockade. In this period, if surgical anesthesia had not been achieved, a supplemental injection of ultrasound-guided bupivacaine was performed, distal to the axilla, and the patient was excluded from the protocol.

Surgical anesthesia was defined as a motor scale less than or equal to Bromage two, with no sensation of cold and pinprick in the assessed dermatomes and also if there was no need for anesthetic supplementation during the procedure.

The end of the LA solution injection was considered as time zero (T0) to evaluate the blockade success rate.

Latency was defined as the time interval between T0 and the time at which the surgical anesthesia was achieved.

During surgical procedure, patients received midazolam (0.05 mg.kg$^{-1}$) for sedation. After surgical procedure, the patient was admitted to the post-anesthesia care unit, where he remained for four hours in order to assess the need for analgesic supplementation.

## Laboratory methodology

Venous blood samples were collected before the blockade, every 15 minutes (min) during the first hour and every 30 min in the second hour after the blockade, through an exclusive cannula. Initially, 5 mL were collected and scavenged to avoid any contamination from the previous collection. Subsequently, another 5 mL were collected and stored in two EDTA tubes. EDTA tubes were centrifuged at $3500 \times g$ for 10 min to obtain blood plasma. The obtained plasma was stored in cryogenic tubes in a freezer at a temperature of $-80\,^\circ$C until analysis.

A High Performance Liquid Chromatography – HPLC (Shimadzu, Kyoto, Japan) was used for the analysis, coupled to the mass spectrometer (Bruker Amazon, USA) with electrospray ionization source[16] and sequential mass spectrometry system (MS/MS).

After obtaining the precursor ion, a fragment from the precursor ion dissociation was obtained by the collision-induced dissociation process. HPLC–MS/MS data were monitored for internal standard (tryptophan) and bupivacaine standard. Positive mode analyzes were performed in the MS/MS mode with selection of molecular ions at $289\,m/z \Rightarrow 140.1\,m/z$, bupivacaine. The methodology was validated in accordance with the Food and Drug Administration international recommendations.[17]

## Statistical analysis

The aim of this study was to evaluate the difference in the maximum plasma levels achieved after ultrasound-guided axillary brachial plexus block with two different concentrations of bupivacaine and maintenance of the infused mass. To calculate the sample size needed to reveal this difference, the following assumptions were considered: (1) null hypothesis: H0: P0 25 max = P0 5 max; alternative hypothesis Ha: P0 25 max > P0 5 max, where P0 25 max = peak plasma achieved in the 0.25% Group and P 0.5% = peak plasma achieved in the 0.5% Group; (2) test for comparison of two independent sample means with a known standard deviation (Student's *t*-test); (3) significance level of 5% ($\alpha = 0.05$); (4) Sample power of 80% ($1 - \beta = 0.80$).

Considering the difference of about 40% reported in the literature for intramuscular injection, the sample size was calculated as 14 patients per group. Considering a loss rate of approximately 10% during collection and analysis, the sample number was increased to 32 patients. All analyzes were performed using SPSS (v 18.0) and Minitab (v 16).

Shapiro–Wilk test was used to verify if the analyzed data were normally distributed. For quantitative variables with normal distribution, the Student's *t*-test was used. ANOVA was used to assess the variance between

**Table 1**  Motor and sensory test.[14,15]

|  | Motor test | Local sensitive test |
|---|---|---|
| Median | Finger flexion | Thenar eminence |
| Radial | Wrist extension | Back of the hand |
| Ulnar | Abduction of the 5th finger | Hypothenar eminence |
| Musculocutaneous | Elbow flexion | Forearm lateral region |
| Score | Definition | |
| 4 | Full muscle strength in relevant muscle groups | |
| 3 | Reduced strength, but able to move against resistance | |
| 2 | Ability to move against gravity, but not against resistance | |
| 1 | Discrete movements (trembling) of muscle groups | |
| 0 | Lack of movement | |

Modified Bromage Scale.[14,15]

quantitative variables. The level of significance was 5% ($\alpha < 0.05$) and the tests with a descriptive level below 5% ($p < 0.05$) were considered significant.[18]

## Results

A total of 35 patients were selected for the study. Three patients were not included for not meeting the inclusion criteria while 32 patients were randomized. There was no difficulty in visualizing the axillary brachial plexus via ultrasound. Two patients, one from each group, were excluded from the protocol due to blockade failure; 30 patients completed the protocol up to sample analysis (Fig. 1). Demographic data of patients are shown in Table 2.

Blockade time was $192 \pm 28$ s for the 0.5% Group and $204 \pm 32$ s for the 0.25% Group ($p = 0.462$); it did not influence the latency time measurement. However, the blockade latency of the 0.5% Group was statistically lower than that of the 0.25% Group (Table 3). In addition, no difference was seen in the duration of surgical procedures (Table 3).

In all patients in whom surgical blockade was achieved, the surgical procedures were uneventful. During the blockade, no inadvertent intravascular puncture was observed, which could impair the outcome evaluation. In addition, no mild or severe symptoms of LA systemic toxicity were observed (e.g., tinnitus, perioral tingling, or seizures). Regarding postoperative analgesia, no patient reported pain up to four hours after the blockade. All patients were discharged on the same day of the procedure and there was no case of hospital readmission.

Regarding the pharmacokinetic study, the used doses of bupivacaine, in mg.kg$^{-1}$, were similar between groups and did not interfere with the results achieved (0.25% Group − $1.33 \pm 0.19$; 0.5% Group − $1.32 \pm 0.19$, $p = 0.826$). Plasma concentrations of bupivacaine after axillary brachial plexus block for 0.5% and 0.25% groups are shown in Table 4 and Fig. 2. There was no difference between plasma anesthetic concentrations at any time. The peak plasma for both groups occurred 45 min after the blockade. The mean maximum concentration of bupivacaine was $933.97 \pm 328.03$ ng.mL$^{-1}$ for 0.25% Group and $1022.79 \pm 253.81$ ng.mL$^{-1}$ for 0.5% Group.

**Table 2**  Demographics of patients.

|  | 0.25% Group | 0.5% Group | $p$ |
|---|---|---|---|
| $n$ | 15 | 15 | |
| Age (years) | 36.0 (10.3) | 35.7 (12.1) | 0.93[a] |
| BMI (kg m$^{-2}$) | 25.7 (3.9) | 26.2 (3.2) | 0.7[a] |
| Sex | | | |
| M/F | 10/5 | 11/4 | |
| ASA | | | |
| I/II | 7/8 | 6/9 | |

BMI, Body Mass Index.
[a] Student's $t$-test, age and BMI presented as mean (SD).

The different concentrations of LA used did not affect how fast the peak plasma was reached (Table 4 and Fig. 2).

## Discussion

LA plasma concentration depends on the dose administered, rate of systemic absorption, tissue distribution, and drug clearance. The decrease in LA systemic absorption increases its safety margin in clinical practice. LA dose is the variable most manipulated by the physician during clinical practice. The doctor can set the total dose to be administered and whether the drug is given in a more concentrated or more diluted solution. The present study aim was verify the relationship between the LA concentration and its peak plasma. Despite the difference in bupivacaine concentration between groups, the present study outcomes showed that the maximum plasma level achieved was similar.

These results are similar to those of a previous study that failed to find a difference in the maximum level of LA in epidural anesthesia when the mass was maintained constant.[12] Thus, this result increases the evidence that it is the total dose of LA that regulates the drug pharmacokinetics.[19,20]

One possible explanation would be that when using the higher concentration drug, the surface area available for absorption is smaller, which would result in a systemic absorption equal to that of the lower concentration drug but with a larger surface area available for absorption. It may
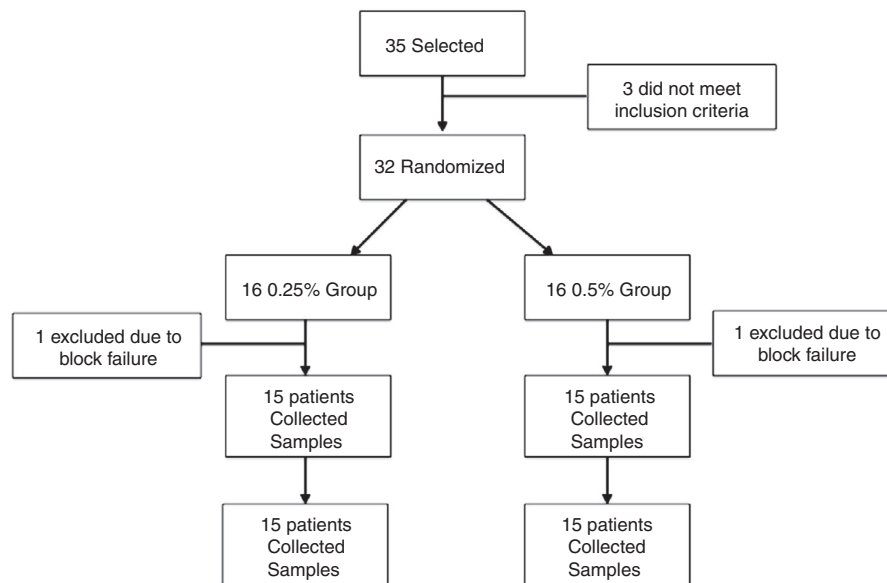
**Figure 1**  Clinical flow chart of the study.

**Table 3**  Blockade latency and surgical time per group.

| Technique variables | 0.25% Group (n = 15) | 0.5% Group (n = 15) | p |
|---|---|---|---|
| *Latency (min)* | | | 0.004 |
| Mean (standard deviation) | 17.3 (5.3) | 10.7 (3.7) | |
| Median | 20 | 10 | |
| *Duration of surgery (min)* | | | |
| Mean (standard deviation) | 88.6 (49.8) | 83.9 (44.9) | 0.41 |
| Median | 70 | 70 | |

Student's *t*-test.

**Table 4**  Comparison of plasma bupivacaine concentration at each time point after blockade.

| Time.min$^{-1}$ | Bupi 0.25% (ng.mL$^{-1}$) Mean (SD)[a] | 95% CI[a] | Bupi 0.5% (ng.mL$^{-1}$) Mean (SD)[a] | 95% CI[a] | p |
|---|---|---|---|---|---|
| 15 | 447.07 (162.37) | 364.91–529.24 | 525.22 (149.66) | 449.49–600.96 | 0.181 |
| 30 | 791.76 (251.32) | 664.58–918.95 | 826.59 (232.27) | 709.05–944.14 | 0.696 |
| 45 | 933.97 (328.03) | 767.97–1099.98 | 1022.79 (253.81) | 894.35–1151.24 | 0.414 |
| 60 | 631.37 (214.45) | 521.83–740.9 | 709.98 (191) | 613.32–806.6 | 0.301 |
| 90 | 614.85 (307.71) | 459.13–770.58 | 615.18 (196.33) | 515.83–714.54 | 0.997 |
| 120 | 410.95 (117.09) | 351.69–470.2 | 470.14 (122.8) | 408–532.29 | 0.187 |

[a] SD, standard deviation; CI, confidence interval; Bupi, bupivacaine.
Student's *t*-test.

be suggested that the surface area available for absorption plays an important role in the plasma peak of LA, neutralizes the effect of the difference in concentrations. This finding is demonstrated by the same peak plasma concentration and by the same pattern of plasma concentration over time in both groups, despite different concentrations. In addition, a possible difference in osmolarity between solutions could result in different peak plasma levels between groups. The osmolarity of the solutions used in this study were 293 mOsmoL.L$^{-1}$ for 0.25% bupivacaine and 239 mOsmoL.L$^{-1}$ for 0.5% bupivacaine. However, the osmolarity difference between groups did not influence the plasma peak.

In a previous study, Cohen et al. demonstrated that a portion of the higher concentration solutions could precipitate when injected into a tissue pH > 6.9. Solutions with lower concentrations did not show this precipitation.[10] The solutions used in the present study had the same pH. However, one limitation of the study was not to assess whether
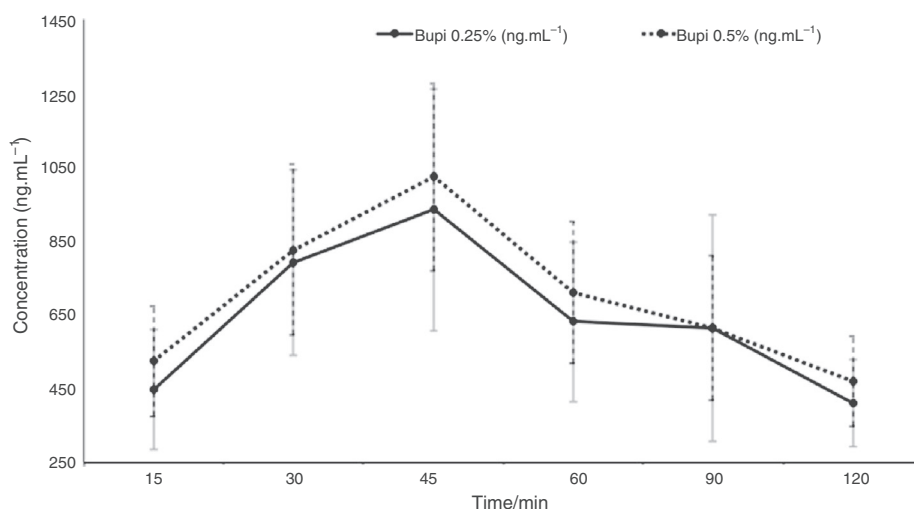
**Figure 2** Distribution of mean and standard deviation of bupivacaine plasma concentration per group at different time points after blockade.

this precipitation also occurred with bupivacaine at higher concentration in the axillary pathway, which would decrease the drug availability for absorption.

In addition, another interesting result of the present study was that blockade latency was lower for the 0.5% Group than for the 0.25% bupivacaine Group. A possible explanation for this result is that when the surface area available for exchange is limited, LA concentration becomes the main determinant of the latency period. A previous study has determined that the minimum LA volume required to involve the nerves in axillary brachial plexus block is about 3.5 mL.[21] In the present study, the lowest infusion volume was 5 mL per nerve. Therefore, the volume injected per nerve was greater than the minimum volume required to involve the entire nerve structure in both groups. Thus, the volume used was sufficient to encompass the entire neural exchange surface, with no difference between groups regarding this factor. In this case, the concentration probably becomes the most important factor related to the transmembrane passing through rate when the surface area is the same. This would explain the shorter latency period in the 0.5% bupivacaine group.

Motor and sensory blockade duration could not be evaluated due to the outpatient nature of the service. Despite this difference in blockade latency, postoperative analgesia up to four hours was the same between groups, showing that the use of different concentrations did not alter analgesia during this period.

Regarding LAST symptoms, a previous study demonstrated that following intravenous bupivacaine infusion, patients had mild toxicity symptoms (i.e., mouth tingling, dizziness, and tinnitus) with plasma concentrations ranging from 1000 to 2000 ng.mL$^{-1}$. Infusion was done at a rate of 10 mg.min$^{-1}$ up to a maximum dose of 150 mg or until the first symptoms appeared.[22] Although the plasma peak of bupivacaine seen in some patients in the present study also reached values between 1000 and 2000 ng.mL$^{-1}$ in both groups, this effect occurred after 45 min of perineural infusion and no patient had LAST symptoms. These results are similar to that of previous studies on the pharmacokine-

tics of bupivacaine during axillary brachial plexus block,[23,24] which found plasma peak levels within that interval without any of the patients showing symptoms of LA toxicity.[23] The rate at which the maximum plasma level is reached probably alters the systemic effects of toxicity. In addition, the time required to reach the plasma peak concentration in the present study was similar to that reported by other authors who described the pharmacokinetic profile of bupivacaine during axillary brachial plexus block. This finding emphasizes that an episode of LAST is more likely to occur during the first hour after the blockade, regardless of the concentration used. As all patients were sedated during the surgical procedure, any awareness of mild symptoms of LAST was limited.

## Conclusion

The aim of this study was to increase knowledge about the LA pharmacokinetics to increase the safety of RA. This study shows that there is no difference in peak plasma levels of bupivacaine, despite the use of different concentrations and volumes, since the LA mass was constant. However, concentration played an important role in determining this blockade latency period, inversely influencing the latency period.

## Funding

## Conflicts of interest

The authors declare no conflicts of interest.

## Acknowledgements

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