# COMPARATIVE RANDOMIZED, SINGLE DOSE, TWO-WAY CROSSOVER, OPEN-LABEL STUDY TO DETERMINE THE BIOEQUIVALENCE OF IRBESARTAN FORMULATION, IRBESARTAN GPO 150 MG TABLETS AND APROVEL® 150 MG TABLETS, AFTER ORAL ADMINISTRATION TO HEALTHY THAI VOLUNTEERS UNDER FASTING CONDITIONS

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**ABSTRACT:**

**Background:** Irbesartan is a vasodilator indicated for the treatment of hypertension with high efficacy and rapid absorption. A generic product, Irbesartan GPO has been developed with lower price, to provide an alternative choice for physicians and patients to access the lower price medicines with the same quality and safety as in the originals.

**Methods:** A comparative randomized, single dose, two-way crossover, open-label study to determine the bioequivalence of Irbesartan GPO 150 mg tablets and Aprovel® 150 mg tablets, after oral administration to healthy volunteers under fasting conditions, with 7 days washout period, was carried out. Blood samples were collected at predefined time points up to 72 hours. Plasma concentrations of irbesartan were analyzed using validated liquid chromatography tandem mass spectrometry (LC-MS/MS). Non-compartmental model was used for pharmacokinetic analysis.

**Results:** The mean values ± SD of pharmacokinetic parameters (test vs. reference) of area under the plasma concentration versus time curve from time zero to the last measurable concentration ($AUC_{0\text{-}tlast}$), the area under the plasma concentration versus time curve from time zero to the time infinity ($AUC_{0\text{-}\infty}$) and maximum plasma concentration ($C_{max}$) were 9507.632±2400.764 vs 9003.785±2136.219 ng.hr/mL, 9925.103±2378.850 vs 9469.099±2127.933 ng.hr/mL and 2373.466±585.390 vs 2373.054±584.536 ng/mL, respectively. The 90% Confidence Interval of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and $C_{max}$ were within the bioequivalence range of 80.00 - 125.00. Both products were well tolerated and there were no significant adverse drug reactions (ADRs).

**Conclusion:** Irbesartan GPO and Aprovel® were bioequivalent in terms of rate and extent of absorption.

**Keywords:** Irbesartan, Bioequivalence, Liquid chromatography tandem mass spectrometry, Pharmacokinetics

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## INTRODUCTION

Angiotensin II is a potent vasoconstrictor formed from angiotensin I in a reaction catalyzed by angiotensin-converting enzyme. Angiotensin II is an effecter hormone of the renin-angiotensin system and also stimulates aldosterone synthesis and secretion by adrenal cortex, cardiac contraction, renal resorption of sodium, activity of the sympathetic nervous system and smooth muscle cell

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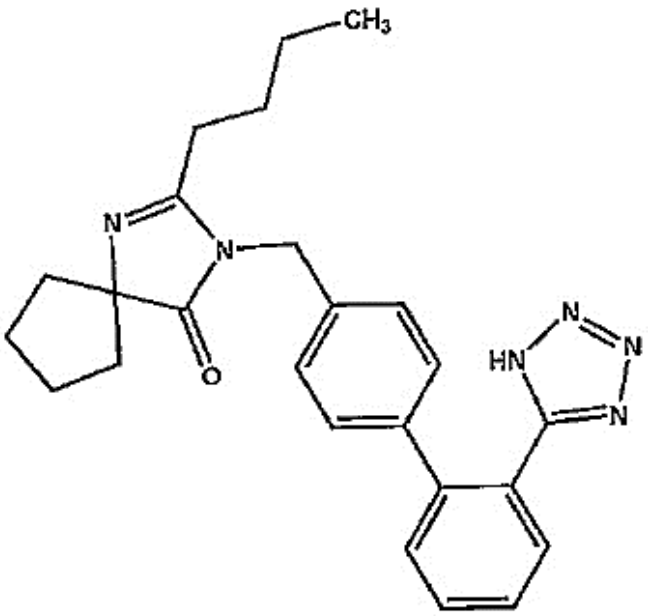


**Figure 1** Chemical structure of irbesartan.

growth. Irbesartan, a non-peptide compound, chemically described as a 2-butyl-3-[p-(o-1H-tetrazol-5-ylphenyl) benzyl]-1,3-diazaspiro[4.4] non-1-en-4one, is selective and noncompetitive [1] bind to the angiotensin II subtype1 (AT1) antagonist and no affinity for the AT2 receptor, α1-or α2-adrenoceptors or serotonergic receptors [1, 2] (Figure 1).

Irbesartan is rapidly and completely absorbed with an absolute bioavailability of 60 to 80 % [2, 3]. Its oral bioavailability is higher than losartan and valsartan [3]. Peak plasma concentration of irbesartan is attained within 1.5 to 2 hr after oral administration [3]. The bioavailability is not affected by food intake [2, 3]. A plasma protein binding is about 96% [2] and has a steady-state volume of distribution about 53 to 93 L [2].

The major route of metabolism is glucuronidation and oxidation with the cytochrome P450 isoform 2C9 [3]. Irbesartan and its metabolites are excreted by both biliary and renal [3]. Single or multiple oral doses of irbesartan 300 mg per day produced apparent total and renal clearance values of about 18 L/hr and 0.07 L/hr, respectively [2]. Elimination half-life is about 11 to 15 hrs, independent of dosage [2].

Since, there is no alter of the pharmacokinetics in patients with renal or hepatic impairment, therefore, no dosage adjustment of irbesartan is required in these patient populations [1, 3]. The drug is effective in the elderly also. Irbesartan can reduce blood pressure to a similar extent to enalapril and atenolol and to a significantly greater than losartan [1]. The combination of irbesartan and hydrochlorothiazide resulted in additive antihypertensive effects [1].

Irbesartan is available in 75, 150 and 300 mg tablets. Because of an expensive original product, the Government Pharmaceutical Organization have developed a generic Irbesartan GPO with lower price to be an alternative choice for physicians and patients to access the lower price medicines in same quality and safety as an originator.

## MATERIALS AND METHODS

### Study protocol

The clinical study protocol was reviewed and approved by The Ethics Committee, Faculty of Tropical Medicine, Mahidol University (Certificate of Ethical Approval No. MUTM 2014-014-01). The study was conducted following the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), Declaration of Helsinki and Standard Operation Procedures (SOPs) of Faculty of Tropical Medicine. The bioanalytical study and pharmacokinetic/statistical analysis were conducted as per Principles of Good Laboratory Practice and SOPs of Research and Development Institute, The Government Pharmaceutical Organization. Informed consent from each study participant was obtained before any study related procedures were initiated for each participant.

### Participants

The sample size computation was determined by considering the assumptions as T/R ratio 95-105%, Intra-subject variability for $C_{max}$ 22.5% (from the previous study of GPO), Significance level 5%,

**Table 1** The summary of validation results

| Information requested | | Data |
|---|---|---|
| Biological Matrix | | Human plasma |
| Anticoagulant (only human plasma) | | Sodium heparin |
| Selectivity | | No interference at the retention time and transition of irbesartan and internal standard. |
| Carry-over | | There was no significant carry-over observed at the retention time and transition of irbesartan and internal standard. |
| Linearity (Range) | | 19.953 to 6024.470 ng/mL |
| Coefficient of determination ($r^2$) | | Greater than 0.98 |
| Lower limit of quantification | | 19.953 ng/mL |
| Precision | Within-batch (Intra-day precision) | 2.0% to 7.4% |
| | Between-batch (Inter-day precision) | 3.9% to 9.2% |
| Accuracy | Within-batch (Intra-day precision) | 87.0% to 110.6% |
| | Between-batch (Inter-day precision) | 96.5% to 106.6% |
| Robustness and Ruggedness experiment | | Method is rugged and robust (up to 150 injections) |
| Average recovery of drug (%) | | LQC, MQC and HQC 68.8%, 70.8% and 70.8% |
| Average recovery of internal standard (%) | | 72.9% |
| Autosampler / Wet extract stability | | 149.0 hours (within 2 to 8°C) |
| Freeze and thaw stability | | 4 cycles |
| Bench top stability | | 17.0 hours (at room temperature) |
| Wet extract bench top stability | | 2.0 hours (at room temperature) |
| Reagents stability | | 9 days (at room temperature) |
| Mobile phase stability | | 9 days (at room temperature) |
| Long term stability of drug in matrix | | 155 days (at -65±10 °C) |

Power ≥ 80% and Bioequivalence limits 80.00-125.00%. Based on the above estimates, a sample size of 22 subjects would be sufficient to establish bioequivalence with adequate power. Considering dropouts and withdrawals, 28 subjects would be sufficient for this study.

Twenty-eight participants were selected randomly from healthy adult Thai male volunteers. The participants had an age range of 18-55 years with a BMI range of 18-25 kg/m$^2$. All participants were determined to be healthy by assessment of medical history, physical examination and laboratory examination such as complete blood count, hematocrit, hemoglobin, fasting blood sugar, blood urea nitrogen (BUN), serum creatinine, alkaline phosphatase, ALT, AST, total bilirubin, total protein, albumin, hepatitis B test, urine analysis and ECG.

The exclusion criteria included history of hypersensitivity to irbesartan and/or any of the excipients, history of medical symptoms (such as gastrointestinal, hepatic, renal, cardiovascular, diabetes mellitus and gallstone disease), clinically significant illness within 4 weeks before start the study, asthma, urticaria or other allergic reactions, alcohol dependence or drug abuse, smokers, intake xanthine products and any medication for 14 days preceding the study. In addition, the participants who participated in other clinical studies within 1 month prior to the start of the study or during the study were also excluded.

**Test and reference drugs**

Irbesartan GPO 150 mg film coated tablets (Batch No. S570001) manufactured by GPO on 06 January 2014 with expiry date of 06 January 2016 were used as the test product and Aprovel®150 mg film coated tablets (Batch No. 3A241) manufactured by Sanofi Winthrop Industrie, France in July 2013 with expiration date of June 2016 were used as the reference product.

**Study design**

This study was designed as a comparative randomized, single dose, two-way crossover, open-label study to determine the bioequivalence of irbesartan formulation, Irbesartan GPO 150 mg tablets and Aprovel® 150 mg tablets, after oral administration to healthy volunteers under fasting conditions.

After an overnight fast of at least 10.0 hrs, one tablet of irbesartan 150 mg of test or reference product was administered orally, while in a sitting position, to each participant with 240 mL of drinking water, at ambient temperature by the study protocol. Washout period was at least 7 days between treatments.

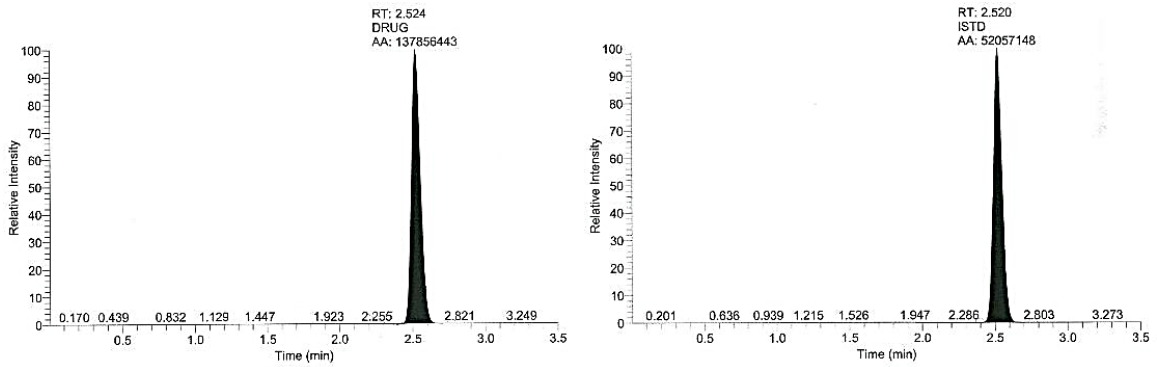


**Figure 2** Representative chromatograms of irbesartan (DRUG) and internal standard (ISTD)

Blood samples were collected through an indwelling intravenous cannula for 23 sampling times (0.000, 0.250, 0.500, 0.750, 1.000, 1.250, 1.500, 1.750, 2.000, 2.250, 2.500, 3.000, 3.500, 4.000, 5.000, 6.000, 8.000, 10.000, 12.000, 24.000, 36.000, 48.000 and 72.000 hrs). The blood samples were centrifuged at 3000±100 rcf for 5 minutes below 10°C to separate plasma. All plasma samples were transferred to pre-labeled polypropylene tubes and stored upright frozen at -65±10°C until analysis.

**Randomization and blinding**

The order of receiving the test and reference products for each participant during two periods of the study was determined according to a randomization schedule. The randomization schedule was generated with the SAS version 9.3 (SAS Institute Inc., USA) by pharmacokinetic and statistical investigator. The personnel involved in dispensing of study drug and verification of dispensed study drugs would be accountable for ensuring compliance to randomization schedule. The study personnel involved in the sample bioanalysis were kept blinded from the randomization treatment during analytical phase of the study.

**Drug analysis**

The plasma concentration of irbesartan in the study samples were determined by a validated LC-MS/MS method using Irbesartan-d4 as an internal standard. The analyte and internal standard were extracted from plasma using protein precipitation method which validated as per The US FDA guidance for industry, bioanalytical method validation and the Guideline on bioanalytical method validation of European Medicines Agency. The validation data and representative chromatograms of the analyte and internal standard were presented in Table 1 and Figure 2. The analytes and internal standard were monitored in the positive ion mode by applying ESI probe using the MRM transitions of m/z 429.285→207.090 for irbesartan and m/z 433.320→211.110 for internal standard. The chromatographic separation was obtained by using an ACE5 C18 150×4.6 mm column maintained at 40°C. The isocratic mobile phase was 0.1% formic acid solution (v/v): methanol (15:85) delivered at the flow rate 0.8 mL/minute.

**Pharmacokinetic and statistical analysis**

Efficacy was primarily assessed by the pharmacokinetic properties of the test and the reference products by estimation of irbesartan concentration in plasma. The primary pharmacokinetic parameters ($AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$ and $C_{max}$) and the secondary pharmacokinetic parameters ($t_{max}$, $\lambda_z$ and $t_{1/2}$) were calculated by non-compartmental model using Phoenix WinNonlin Software version 6.3 (Pharsight Corporation, USA). The primary pharmacokinetic parameters were transformed to ln scale before carrying out the statistical analysis. The statistical analysis was conducted by PROC GLM of SAS® version 9.3 (SAS Institute Inc., USA) for un-transformed and ln-transformed pharmacokinetic parameters. ANOVA model included sequence, formulation and period as fixed effects and subject as a random effect. An F-test was performed to determine the statistical significance of the effects involved in the model at a significance level of 5%. Bioequivalence of test and reference product was concluded, if the 90% confidence interval fell within the acceptance range of 80.00-125.00 % for ln-transformed pharmacokinetic parameters of irbesartan.

## RESULTS

**Participants**

Twenty-eight healthy adult Thai male volunteers were enrolled randomized and completed the study. Demographic data for all participants as age, weight, height and BMI were 27.7±7.4 year, 63.5±7.2 kg, 169.3±5.0 cm and 22.1±1.9 kg/m$^2$,

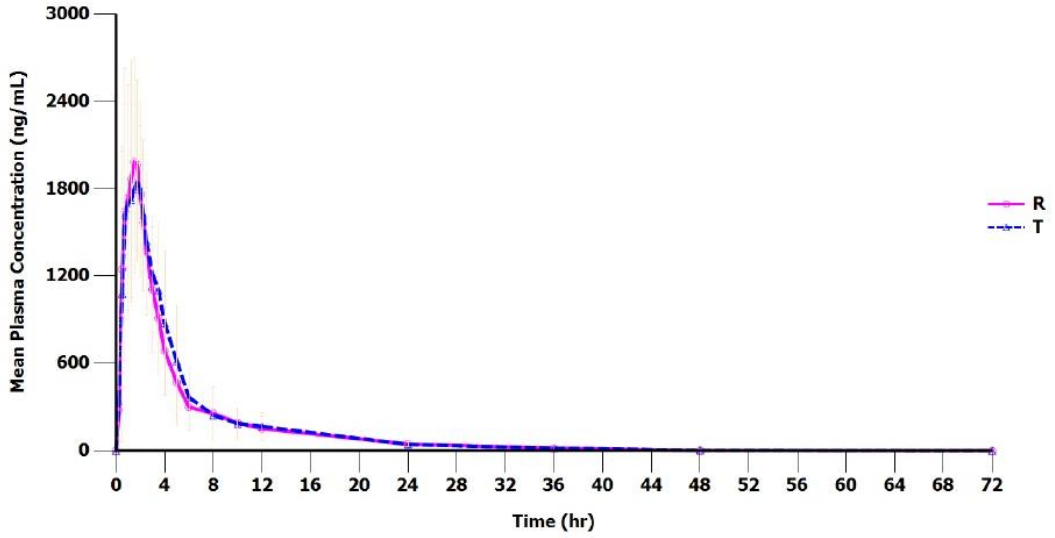


**Figure 3** Linear plot of mean (±SD) plasma concentration of irbesartan versus time curves after administration of test product [T] and reference product [R] in healthy Thai volunteers under fasting conditions

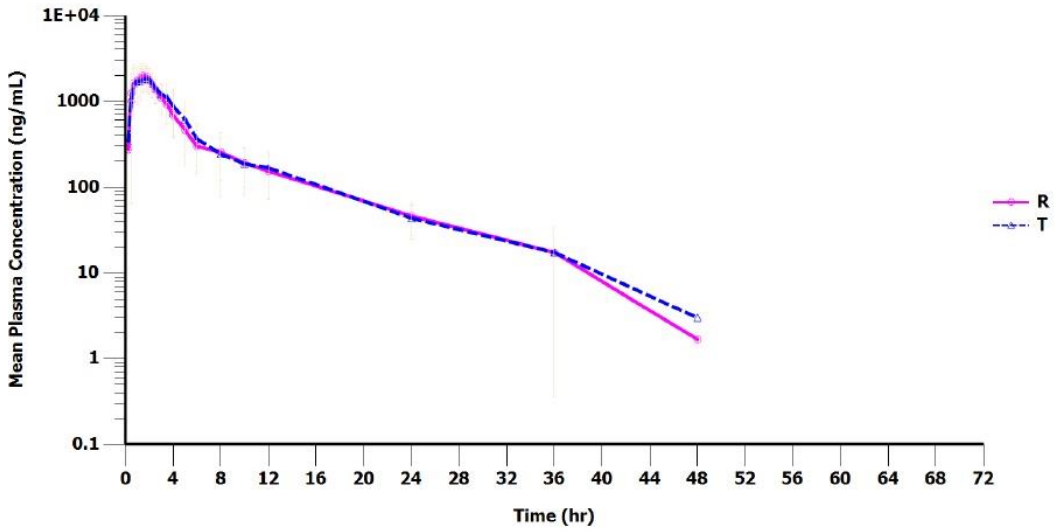


**Figure 4** Semi-logarithmic plot of mean (±SD) plasma concentration of irbesartan versus time curves after administration of test product [T] and reference product [R] in healthy Thai volunteers under fasting conditions

respectively. The demographic data for participants in treatment I (test product then reference product) as age, weight, height and BMI were 26.9±7.5 year, 62.9±5.5 kg, 169.3±5.0 cm and 21.9±1.4 kg/m$^2$, respectively and for participants in treatment II (reference product then test product) were 28.5±7.4 year, 64.1±8.8 kg, 169.3±5.3 cm and 22.3±2.3 kg/m$^2$, respectively. There was no participant withdrawn or dropped out. All data obtained from all participants were used for pharmacokinetic and statistical analysis. Both the test and the reference products were well tolerated since there was no clinically significant adverse event for participants in period I, during washout period and period II.

**Pharmacokinetic and bioequivalence analysis**

The linear and semi-logarithmic plots of mean plasma concentration versus time after administration of the test and reference products were presented in Figure 3 and 4, respectively. The pharmacokinetic parameters were presented in Table 2.

The extent of absorption for the test and the reference product reported as mean±SD of $AUC_{0\text{-tlast}}$ were 9507.632±2400.764 ng.hr/mL and 9003.785±2136.219 ng.hr/mL, respectively. $AUC_{0\text{-}\infty}$

**Table 2** Mean pharmacokinetic parameters of irbesartan of the test and reference product

| Parameters (units) | Test product (Mean±SD) | Reference product (Mean±SD) |
|---|---|---|
| $AUC_{0\text{-}tlast}$ (ng.hr/mL) | 9507.632±2400.764 | 9003.785±2136.219 |
| $AUC_{0\text{-}\infty}$ (ng.hr/mL) | 9925.103±2378.850 | 9469.099±2127.933 |
| $C_{max}$ (ng/mL) | 2373.466±585.390 | 2373.054±584.536 |
| $t_{max}$ (hr)* | 1.250 (0.500, 5.000) | 1.500 (0.750, 3.500) |
| $\lambda_z$ (1/hr) | 0.094±0.048 | 0.089±0.039 |
| $t_{1/2}$ (hr) | 9.340±4.929 | 9.753±5.241 |

*$t_{max}$ were represented in median (min, max) value.

**Table 3** Ratio of ln-transformed geometric least square mean and 90% confidence interval of irbesartan

| Parameters | Ratio of ln-transformed geometric least square mean | 90% CI |
|---|---|---|
| ln $AUC_{0\text{-}tlast}$ | 105.0 | 97.89-112.56 |
| ln $AUC_{0\text{-}\infty}$ | 104.3 | 97.95-110.99 |
| ln $C_{max}$ | 100.0 | 92.76-107.77 |

for the test product was 9925.103±2378.850 ng.hr/mL and for the reference product was 9469.099±2127.933 ng.hr/mL. The rate of absorption reported as $C_{max}$ was 2373.466±585.390 ng/mL and 2373.054±584.536 ng/mL for the test and the reference products, respectively. The median (min,max) of $t_{max}$ was 1.250 (0.500,5.000) hr for the test product and 1.500 (0.750,3.500) hr for the reference product. The mean value for $t_{1/2}$ for the test and the reference products were 9.340±4.929 hr and 9.753±5.241 hr, respectively. The least-squares means ratios and the 90% CIs of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and $C_{max}$ for the test to the reference product were 105.0 (97.89-112.56), 104.3 (97.95-110.99) and 100.0 (92.76-107.77) as presented in Table 3.

## DISCUSSION

The primary objective of this study is to assess the rate and extent of absorption of irbesartan from the test and the reference products. $C_{max}$ and $t_{max}$ are considered to estimate the rate of absorption, while AUC is considered to indicate the extent of absorption. From the previous published study of irbesartan 150 mg, the arithmetic mean value of $C_{max}$, $AUC_{0\text{-}\infty}$ and the median value of $t_{max}$ were about 1110 ng/mL, 5360 ng.hr/mL and 1.5 hr, respectively [4]. Besides, from the existing published study of irbesartan 300 mg, the arithmetic mean value of $C_{max}$ and $AUC_{0\text{-}\infty}$ were ranged from 2170 ng/mL to 3617 ng/mL and 12960 ng.hr/mL to 22494 ng.hr/mL, respectively [4-8]. The median value of $t_{max}$ observed ranged from 1.03 hr to 1.80 hr [4-8]. These results are in accordance with this study. The $t_{max}$ value shows that irbesartan is rapidly absorbed with peak concentrations are attained in less than 2 hrs after dosing. The $C_{max}$ and $AUC_{0\text{-}\infty}$ mean values of the test and the reference products were higher than the reported in previous published data. These can be suggested that may have a variation between ethnics.

The secondary objective is to evaluate the safety on the basis of clinical and laboratory examinations. Based on the study data, both products were well tolerated and no clinically significant or serious adverse events were observed in safety data.

Bioequivalence analysis was shown as the 90% CIs of the least-squares means ratios of the test to the reference product of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and $C_{max}$ were within the acceptance range of 80.00-125.00 %, indicating that the test and the reference product were bioequivalent.

## CONCLUSIONS

Overall, the study can be concluded that the test and the reference products established bioequivalence with respect to the rate and extent of absorption of irbesartan. Both formulations were well tolerated. Therefore, the test product could be safely administered to patients and is expected to produce the same therapeutic response to be used interchangeably with reference product.

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