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Does the Tacrolimus Trough Level Adequately Predict Drug Exposure in Patients Requiring a High Tacrolimus Dose?

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Background. Tacrolimus (Tac) has a narrow therapeutic range. Dosing is generally targeted at Tac trough levels (C_0), notwithstanding conflicting reports on the correlation between Tac C_0 and systemic exposure measured by the area-under-the-concentration-over-time curve (AUC). The Tac dose required to meet the target C_0 varies highly among patients. We hypothesized that patients requiring a relatively high Tac dose for a certain C_0 may show a higher AUC. **Methods.** We retrospectively analyzed data from 53 patients in which a 24-h Tac AUC_{24} estimation was performed at our center. Patients were divided into those taking a low (≤0.15 mg/kg) or high (>0.15 mg/kg) once-daily Tac dose. Multiple linear regression models were used to investigate if the association between C_0 and AUC_{24} changes according to dose level. **Results.** Despite the large difference in mean Tac dose between the low- and high-dose group (7 versus 17 mg/d), C_0 levels were similar. However, the mean AUC_{24} was substantially higher in the high-dose group (320 ± 96 h·µg/L versus 255 ± 81 h·µg/L, $P < 0.001$). This difference remained significant after adjusting for age and race. For a same C_0, every 0.01 mg/kg increase in Tac dose resulted in an AUC_{24} increase of 3.59 h·µg/L. **Conclusions.** This study challenges the general belief that C_0 levels are sufficiently reliable to estimate systemic drug exposure. We demonstrated that patients requiring a relatively high Tac dose to attain therapeutic C_0 levels have higher drug exposure and could therefore potentially be overdosed.

(Transplantation Direct 2023;9: e1439; doi: 10.1097/TXD.0000000000001439.)

The calcineurin-inhibitor tacrolimus (Tac) is a central component of the antirejection therapy of most solid organ transplant recipients.[1] It is marketed as immediate-release Tac tablets for twice-daily administration or as modified-release tablets for once-daily administration (TacQD). TacQD shows the same efficacy as Tac tablets for twice-daily administration in terms of renal function, patient and graft survival at 12 mo, and a similar safety profile.[2,3]

Tac is extensively metabolized by intestinal and hepatic cytochrome P450 3A (CYP3A5 and CYP3A4) and effluxed by P-glycoprotein expressed in the intestinal mucosa.[4,5] These presystemic processes contribute significantly to large variability in the rate and extent of drug absorption.[6,7] Tac oral bioavailability is the result of the gastrointestinal transit time influenced by the presence or absence of food, efflux transport, and intestinal and hepatic first-pass.

In addition, the relative clearance of Tac is affected by various clinical parameters, such as age, sex, body size measures, race, time on Tac, serum albumin, hematocrit, and the presence of hepatitis B or C infection or other liver diseases.[7]

Patients with a systemic Tac exposure below the target range may be at an increased risk of developing acute or chronic rejection because of insufficient immunosuppression, whereas high systemic Tac exposure increases the risk of adverse effects, such as nephrotoxicity, neurotoxicity, and posttransplant diabetes mellitus.[5]

The Kidney Disease: Improving Global Outcomes clinical practice guideline for the care of kidney transplant recipients recommends pharmacokinetic monitoring of Tac to avoid under- or overexposure. Tac dose adjustments are usually made based on blood Tac trough concentrations (C_0) because it is the easiest approach, and Tac C_0 is generally believed to correlate well with overall systemic exposure, as measured by the area-under-the-concentration-over-time curve (AUC).[8-11] The AUC measures the overall drug exposure and is considered the pharmacokinetic exposure parameter best associated with clinical outcomes.[12-13] However, there are conflicting reports on the correlation between C_0 levels and the AUC.[7,8,14] Some studies reported a reasonable squared correlation coefficient between trough levels and Tac AUC (0–12 h) ($r^2 = 0.60$–0.85),[15-17] but others found poor correlations ($r^2 < 0.50$).[18-19] A retrospective study in >500 patients on TacQD specifically found an r^2 of 0.63, 0.66, and 0.75 in the first 3 mo, months 3 to 12, and beyond 1-y posttransplant, respectively.[20]

Our daily experience confirms that the Tac dose required to meet a specific target C_0 varies highly among patients, with some individuals requiring up to 10-fold-higher doses than others.

The reports showing that trough levels may not be the ideal reflection of systemic exposure raised our concerns particularly regarding those requiring a high Tac dose to attain a therapeutic C_0 level. Our hypothesis is that these patients may show an unexpectedly higher AUC at a therapeutic C_0 level and may therefore be unintentionally overdosed.

The aim of the present study is to compare, retrospectively based on available data, the association between C_0 and AUC in relation to Tac dose, with the hypothesis that patients requiring a high Tac dose for a certain C_0 will also show higher AUC levels.

MATERIALS AND METHODS

Study Design/Participants

This is a single-center, retrospective study of adult kidney transplant recipients treated at the University Hospital of Antwerp (Belgium), for which a Tac AUC time curve from 0 to 24 h (Tac AUC_{24}) was estimated between January 2019 and December 2021.

In some cases, Tac AUC_{24}-monitoring was performed because of concerns about the relatively high Tac doses necessary to obtain an adequate C_0. In other cases, the Tac AUC_{24} was measured in "random" patients who underwent mycophenolic acid AUC monitoring, which provided an opportunity to additionally analyze Tac levels, to obtain maximum insight into the patient's immunosuppressant's status. In the case that a patient underwent >1 Tac AUC_{24} analysis, only the earliest one was included in this study.

All patients were taking the TacQD formula (Advagraf). During 1 wk before the test, there were no modifications in administered Tac doses, nor in concomitant immunosuppressive therapy, to assure steady state. No patient received, during a week before the test, any strong interacting drug.[21]

In general, the Tac dose was targeted to a C_0 level of 8 to 10 ng/mL during the first month posttransplantation, to 6 to 8 ng/mL during the second and third months, and to 5 to 7 ng/mL thereafter. Concomitant immunosuppression generally comprised mycophenolate mofetil and corticosteroids, or occasionally a mammalian target of rapamycine inhibitor.

Pharmacokinetic Analysis

After the first blood sampling for trough concentration (C_0), approximately 24 h after the previous morning dose, the fasting patients took their usual TacQD dose together with their concomitant immunosuppressive medication. Then, blood samples were collected at $20 ± 15$ min (T_1), $60 ± 15$ min (T_2), $120 ± 15$ min (T_3), and $180 ± 30$ min (T_4), and the exact sampling times were recorded.

The C_0/D ratio was calculated by dividing the Tac C_0 by the corresponding daily Tac dose (D).

Whole blood Tac concentrations were analyzed by liquid chromatography with tandem mass spectrometry using isotopically labeled standards on an XEVO TQMS instrument from Waters. Afterward, these data were sent to the laboratory of Pharmacology at the University Hospital of Limoges, where, based on the Immunosuppressant Bayesian Dose Adjustment system, an AUC_{24} estimation was delivered. The Bayesian estimator used to determine Tac AUC_{24} was developed at Limoges University Hospital on the basis of a 1-compartment open model with absorption described as a double gamma distribution, following a classic iterative 2-stage method, and applied to the 3-point limited sampling strategy predose and 1 and 3 h postdose.[20] It was characterized by a mean bias of $4.2\% ± 6.1\%$ on day 14 and $0.2\% ± 7.9\%$ on day 42 posttransplant; the imprecision coefficients of variation were 7.1% and 7.8%, respectively.

Classification of Patients

Patients were divided into a "low-dose" group with a Tac dose ≤0.15 mg/kg and an exposure "high-dose" group with a Tac dose >0.15 mg/kg. This arbitrary cutoff level was determined before the analysis, based on clinical experience.

Statistical Analysis

Statistical analysis was performed using SPSS 28.0 (SPSS, Inc, Chicago, IL). Data with a normal distribution were presented as mean values with their SD.

Nominal data were presented by the number of patients and the percentage of total patients.

Categorical variables were compared between groups with the chi-square test; differences in continuous variables between groups were tested using the independent sample Student t test (parametric variables). All tests were 2-sided, and a P value below 0.05 was considered significant.

To assess whether the association between C_0 and AUC_{24} changes according to dose level, a multiple linear regression model was used with AUC_{24} as the dependent variable and C_0, Tac dose, and interaction between both as independent variables. Tac dose is considered as both a continuous and a categorical variable (dose </> 0.15 mg/kg). In a second stage, this model was adjusted for other influencing factors (age and race). Results were considered significant if $P < 0.05$.

Pearson correlation coefficients between AUC_{24} and C_0 were calculated for each dose group (</> 0.15 mg/kg) and compared using a t test on the Fisher transformed coefficients.

RESULTS

A total of 53 patients were included in this study.

The baseline characteristics of the patients are listed in Table 1. Thirty-six (68%) patients took a Tac dose ≤0.15 mg/kg, and 17 (32%) patients took a Tac dose >0.15 mg/kg. The

TABLE 1.

Demographic and clinical characteristics of patients (N = 53)

	Low dose ≤ 0.15 mg/kg (n = 36)	High dose > 0.15 mg/kg (n = 17)	P
Age, y[a]	53.1 ± 13.0	40.8 ± 13.5	<0.001
Women, %	16 (44)	10 (59)	0.39
BMI, kg/m^2 ± SD[a]	27.2 ± 4.4	25.1 ± 3.8	0.09
Race			
White (%)	30 (83)	7 (41)	<0.001
Other (%)	6 (17)	10 (59)	
Days after transplantation (Q$_1$; Q$_3$)[b]	106 (61; 835)	83 (54; 2094)	0.41
Diabetes, %	11 (31)	4 (24)	0.59
Concomitant immunosuppression			
Corticosteroids, %	30 (83)	16 (88)	0.23
Mycophenolate mofetil, %	31 (86)	14 (82)	0.72
mTOR, %	1 (2)	1 (6)	0.58

[a]Values are expressed as mean ± SD.
[b]Values are expressed as median and interquartile range (Q$_1$; Q$_3$).
BMI, body mass index; mTOR, mammalian target of rapamycine.

mean ages in the groups with low- and high-dose–treated recipients were 53.1 ± 13.0 y and 40.8 ± 13.5 y (mean ± SD), respectively ($P < 0.001$). In the low-dose Tac group, 83% were of White race compared with 41% of the high-dose group ($P < 0.001$). There was no significant difference in sex, body mass index, period after transplantation, and diabetes between patients in the low versus high Tac dose groups.

The pharmacokinetic parameters of Tac were compared between the 17 high-dose–treated recipients and 36 low-dose–treated recipients (Figure 1, Table 2). In the low-dose subgroup, the mean daily Tac dose was 6.7 mg (±2.1 mg), whereas in the high-dose–treated recipients, the mean daily dose was 17.4 mg (±8.5 mg). Despite the large difference in daily Tac dose, the C_0 level was not significantly different between the low-dose group (7.49 ± 2.47 µg/L) and the high-dose group (7.24 ± 2.53 µg/L; $P = 0.74$).

The low-dose subgroup was linked with a higher C_0/D ratio than high-dose–treated recipients (1.21 ± 0.50 ng/mL/mg versus 0.48 ± 0.23 ng/mL/mg, respectively, $P < 0.001$).

However, the mean AUC_{24} was significantly higher in high-dose–treated recipients (320 ± 96 h·µg/L versus 255 ± 81 h·µg/L, $P < 0.001$). The mean Tac C_{max} was also significantly higher in high-dose–treated recipients (28.28 ± 9.85 µg/L versus 21.36 ± 9.26 µg/L, $P < 0.001$).

The correlation between C_0 concentration and AUC_{24} was higher in the low-dose–treated patients than in the high-dose–treated patients (respectively, $r^2 = 0.91$ versus 0.77). This difference was not statistically significant ($P = 0.14$).

Association between C_0 concentration and AUC_{24} was further examined and compared between low-dose–treated recipients and high-dose–treated recipients in a linear regression model. This confirms that, in high-dose–treated recipients, the AUC_{24} is 72 h·µg/L higher than in patients treated with low-dose Tac ($P < 0.001$; Table 3), a difference that also remained significant after adjusting for age and race. Additional exploration, using a model that incorporated a term for interaction between dose-group and C_0, shows no significant difference in the relationship between C_0 and the AUC_{24} in both groups ($P = 0.9$), meaning that the way in which the AUC_{24} increases with higher C_0 levels is similar in patients taking a low versus high dose of Tac (but the high dose group starts at a higher intercept; Figure 2).

When looking at Tac dose as a continuous variable instead of 2 categories (low/high), the conclusion remains that, for the same C_0, a higher Tac dose results in a higher AUC_{24}: For every 0.01 mg/kg increase in the dose of Tac, the AUC_{24} increases by 3.59 h·µg/L.
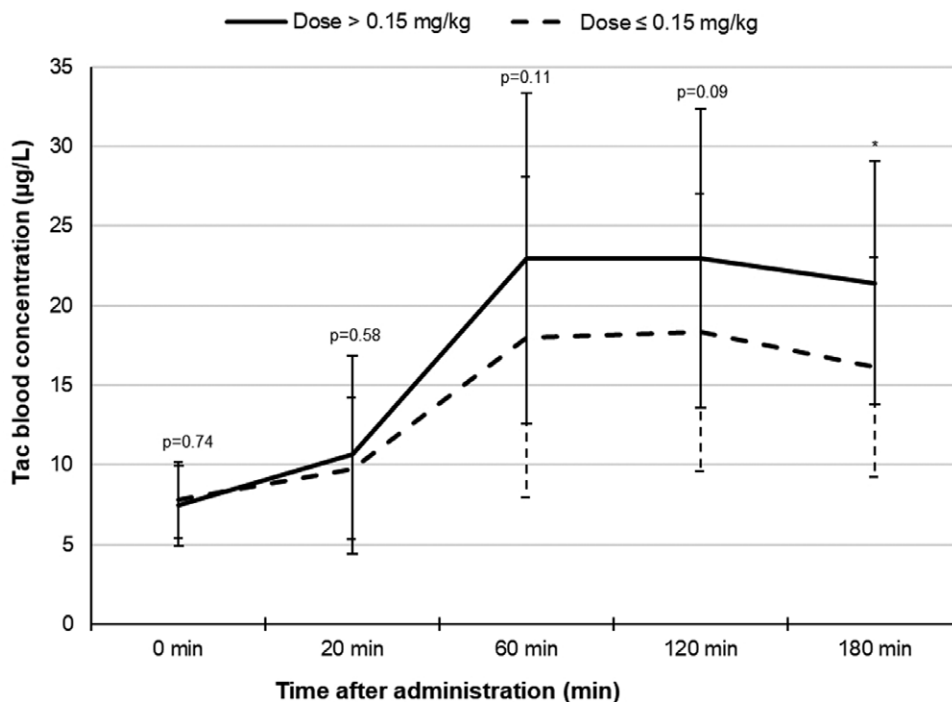
FIGURE 1. A blood-concentration time curve for a Tac dose ≤0.15 mg/kg and a Tac dose >0.15 mg/kg (error bars represent SD of the mean). *Statistically significant. Tac, tacrolimus.

TABLE 2.

Comparison of pharmacokinetic parameters between a Tac dose ≤0.15 mg/kg and a Tac dose >0.15 mg/kg

	Low dose ≤0.15 mg/kg (n = 36)	High dose >0.15 mg/kg (n = 17)	P
Dose, mg	6.7 ± 2.1	17.4 ± 8.5	<0.001
Dose/body weight, mg/kg	0.09 ± 0.03	0.26 ± 0.13	<0.001
C_0, µg/L	7.49 ± 2.47	7.24 ± 2.53	0.74
C_{max}, µg/L	21.36 ± 9.26	28.28 ± 9.85	<0.001
C_0 (ng/mL)/dose, mg)	1.21 ± 0.50	0.48 ± 0.23	<0.001
AUC_{24} (h·µg/L)	255 ± 81	320 ± 96	<0.001
Concentration, µg/L			
C_1	9.76 ± 4.43	10.64 ± 6.23	0.58
C_2	18.02 ± 10.08	22.95 ± 10.38	0.11
C_3	18.31 ± 8.68	22.95 ± 9.40	0.09
C_4	16.13 ± 6.91	21.42 ± 7.63	<0.001

All values are expressed as mean ± SD. Concentration at ±20 min (C_1), ±60 min (C_2), ±120 min (C_3), and ±180 min (C_4).
AUC_{24}, area-under-the-concentration-over-time curve; C_{max}, maximal concentration; Tac, tacrolimus.

DISCUSSION

Our study confirms the hypothesis that patients requiring a high Tac dose (>0.15 mg/kg) to achieve a therapeutic target Tac C_0 level show a significantly higher AUC_{24} than patients requiring a low Tac dose. In other words, we risk to overdose those patients if we only target their Tac dose to the C_0 level.

Despite similar C_0 levels, we found that the AUC_{24} was about 25% (72 h·µg/L) higher in patients taking a high Tac dose than in patients on a low Tac dose. An increase in a Tac dose of 0.01 mg/kg results in an AUC_{24} increase by 3.59 h·µg/L.

The AUC is the most accurate measure of overall drug exposure and is considered the pharmacokinetic exposure parameter best associated with clinical outcomes.[12-13] However, most centers still rely on C_0-level monitoring, despite conflicting data about the correlation with systemic exposure.[7,8,14] We report here a better correlation coefficient between C_0 and AUC_{24} in the low-dose–treated patients than in the high-dose–treated patients (respectively, $r^2 = 0.91$ versus 0.77), which indicates that C_0 level is a less reliable tool to estimate systemic exposure in patients requiring a high Tac dose.

However, only a few studies have investigated the relationship between Tac systemic exposure and the risk of toxicity.[22-23]

The conclusion from these studies was that Tac monitoring may be useful for minimizing the risks of both toxicity and rejection in kidney transplant recipients.[22] The increased risk for Tac-treated patients to develop nephrotoxicity, neurotoxicity, and posttransplant diabetes mellitus may theoretically be reduced by AUC monitoring, although, until now, no prospective trials[19,24] have been published that evaluated the potential benefits on clinical outcomes of AUC monitoring compared with C_0-guided therapy on these side effects.[5]

Recently, the trough concentration/dose (C_0/D) ratio of Tac has been proposed as a prognostic marker for poor outcome after kidney transplantation.[25-26] Patients with a low C_0/D ratio (<1.05 ng/mL/mg, also referred to as fast metabolizers) seem to have more Tac-related nephrotoxicity, more BK viremia, and a shorter graft survival. We confirmed that high-dose–treated recipients showed a C_0/D ratio <1.05 ng/mL/mg (0.48 ng/mL/mg), thus identifying a group of patients with potentially increased risk of poor outcome.

Our data suggest that a Tac AUC estimation might have an added value in patients requiring a high Tac dose for an adequate C_0. Although it is more laborious and costly, it might be worth the effort in this subgroup. However, it may not be feasible to repeat AUC testing at every standard follow-up visit. More research is needed to define the frequency or specific circumstances in which to repeat this. Of note, implementation of home-based dried blood spot Tac sampling could promote regular AUC monitoring. Alternatively, it might be interesting to continue the follow-up by using C_0 levels but to adapt the target C_0 according to the AUC (eg, if you targeted a Tac C_0 of 7 µg/mL but the AUC was too high, then to target a lower C_0). Reassuring, in this respect, is that a large, retrospective study showed no significant changes in the AUC/C_0 ratio over posttransplantation time (before versus after 6 mo), suggesting that, indeed, it could be used to infer an individual C_0 target to achieve a target AUC.[20] Ideally, future prospective studies should compare AUC-guided dosing versus standard C_0 monitoring to determine the impact on the prevention of acute rejection episodes, nephrotoxicity, and other known adverse reactions.

To our knowledge, our study is the first to specifically investigate the impact of a high Tac dose on the relationship between C_0 and AUC. Despite the modest sample size, we were able to demonstrate a statistically significant and clinically relevant increase of the total Tac exposure (AUC_{24}) despite similar C_0 values in such patients. However, AUC_{24} estimates were obtained using an LSS in combination with Bayesian estimation based on a pharmacokinetic model, which despite

TABLE 3.

Factors that influence the relationship between the C_0 and AUC in a linear regression model with AUC as outcome

	Not adjusted		Adjusted[a]	
Parameter	B coefficient (SE)	P	B coefficient (SE)	P
Constant	105.96 (21.27)	<0.001	91.39 (27.64)	0.002
C_0 (µg/L)	29.51 (2.52)	<0.001	29.09 (2.51)	<0.001
Tac dose > 0.15 mg/kg[b]	72 (13)	<0.001	66 (14)	<0.001
Age, y			0.84 (0.49)	0.37
Race: non-White[c]			38.51 (15.34)	0.10

[a]After adjusting for age and race.
[b]Reference category Tac dose ≤ 0.15 mg/kg.
[c]Reference category White.
AUC, area-under-the-concentration-over-time curve; Tac, tacrolimus
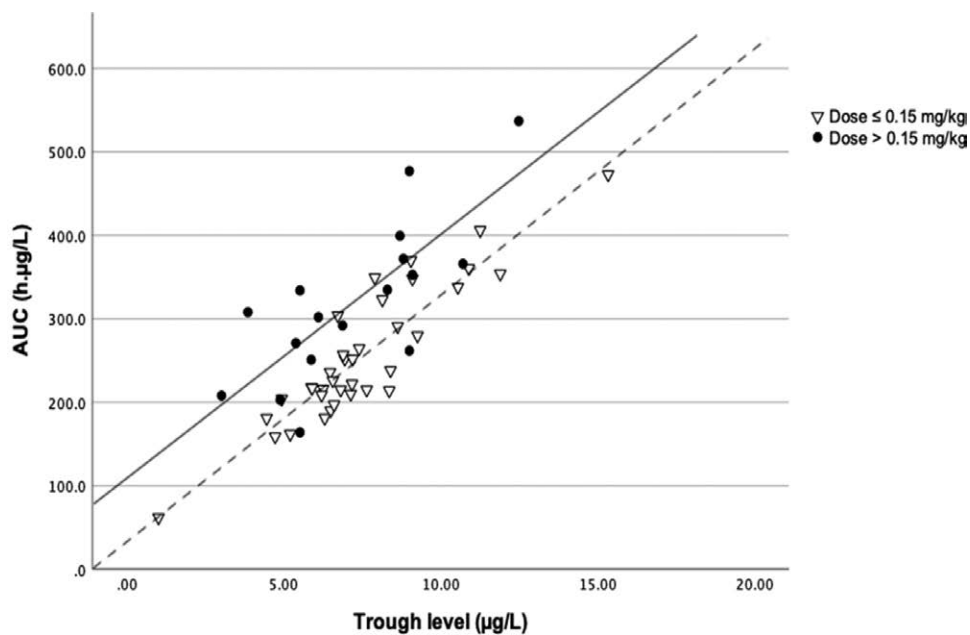
FIGURE 2. Scatterplot graph showing the association between the C_0 and AUC_{24} in 36 patients with a low dose of Tac ($\triangledown$) and in 17 patients with a high dose of Tac ($\bullet$). The dotted line indicates the regression equation. AUC, area-under-the-concentration-over-time curve; Tac, tacrolimus.

low inaccuracy (<5%) and imprecision (<8%) following the authors[27] can be affected by different sources of errors, such as in the sampling times and measured plasma levels, similar to C_0 in this respect. On the other hand, Immunosuppressant Bayesian Dose Adjustment is an expert system routinely used by 140 transplantation centers around the world for the dose adjustment of immunosuppressive drugs in transplant patients.[28] Another strength of this study is that it combined detailed data on Tac pharmacokinetics with clinical patient information so that we could avoid that our findings were confounded by age, by the concomitant use of strongly interacting drugs, and, to some extent, by race. Regarding the latter, it is indeed known that CYP3A4 and CYP3A5 gene polymorphisms can explain some of the interpatient variability in Tac pharmacokinetics and that these genotypes vary by race (eg, more rapid metabolizers in African American and Asian populations).[29-30] Given the highly heterogeneous racial background of our small non-White subgroup and the lack of genotyping, we were unfortunately unable to perform an in-depth analysis of the role of genetic variations on the relationship between Tac dose, C_0, and AUC. Another limitation is the lack of information about the clinical outcome of the recipients. It would be interesting to evaluate potential benefits on clinical outcomes of AUC monitoring compared with C_0-level therapy.

CONCLUSION

The findings of this study challenge the general belief that C_0 levels are sufficiently reliable to estimate the total Tac exposure. We demonstrated that patients requiring a relatively high Tac dose (≥0.15 mg/dL) to achieve therapeutic C_0 levels have a higher total drug exposure and could therefore potentially be overdosed. This study shows that performing a Tac AUC measurement may be worth the effort in such patients, but this does not exclude that it could be valuable for low-dose patients, too, particularly to control low exposure more precisely.

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