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\textbf{T}_2 \textit{Relaxation Study to Evaluate the Crystalline State of Indomethacin Containing Solid Dispersions Using Time-Domain NMR}

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The aim of this study was to demonstrate the usefulness of \textit{T}_2 measurements conducted with a time-domain NMR (TD-NMR) for the characterization of active pharmaceutical ingredients (APIs) containing solid dosage forms. A solid dispersion (SD) and a physical mixture (PM) consisting of indomethacin (IMC) and polyvinylpyrrolidone (PVP) were prepared at different weight ratios as test samples, and then their \textit{T}_2 relaxation curves were measured by TD-NMR. The \textit{T}_2 relaxation curve of IMC was quite different from that of PVP by nature. \textit{T}_2 values of the SD and PM samples became gradually shortened with increasing IMC content. No difference in \textit{T}_2 relaxation curves was observed between SD and PM. By analyzing the \textit{T}_2 relaxation curves in detail, we succeeded in precisely quantifying the IMC contents incorporated in the samples. Next, this study evaluated the \textit{T}_2 relaxation curves of amorphous and crystalline states of powdered IMC. \textit{T}_2 relaxation rate of crystalline IMC was slightly but significantly higher than that of amorphous IMC, proving that the \textit{T}_2 measurement was sensitive enough to detect these differences. Finally, a thermal stress was imposed on SD and PM samples at 60°C for 7 d, and then an amorphous-to-crystalline transformation occurred in IMC in the PM sample and was successfully monitored by \textit{T}_2 measurement. We believe that \textit{T}_2 measurement by TD-NMR is a promising analysis for the characterization of APIs in solid dosage forms, including SD-based pharmaceuticals.

Key words time-domain NMR; \textit{T}_2 relaxation time; solid dispersion; crystal transformation; amorphous; solid state

Introduction

Various technical approaches have been applied in the pharmaceutical industry to improve the solubility of active pharmaceutical ingredients (APIs).\textsuperscript{1–5} Amorphization using the solid dispersion (SD) technique is regarded as a promising pharmaceutical technology.\textsuperscript{5} We recently used the time-domain NMR (TD-NMR) method to evaluate the crystalline state of APIs incorporated into solid oral dosage forms including amorphous SDs.\textsuperscript{6,7} TD-NMR is a benchtop instrument used for measuring the \textit{T}_1-NMR relaxation.\textsuperscript{8} Instead of the \textit{T}_1 measurement for use in the wide range of aspects of molecular structure analysis performed with solid-state NMR and polyvinylpyrrolidone (PVP) were prepared at different weight ratios as test samples, and then their \textit{T}_2 relaxation curves were measured by TD-NMR. The \textit{T}_2 relaxation curve of IMC was quite different from that of PVP by nature. \textit{T}_2 values of the SD and PM samples became gradually shortened with increasing IMC content. No difference in \textit{T}_2 relaxation curves was observed between SD and PM. By analyzing the \textit{T}_2 relaxation curves in detail, we succeeded in precisely quantifying the IMC contents incorporated in the samples. Next, this study evaluated the \textit{T}_2 relaxation curves of amorphous and crystalline states of powdered IMC. \textit{T}_2 relaxation rate of crystalline IMC was slightly but significantly higher than that of amorphous IMC, proving that the \textit{T}_2 measurement was sensitive enough to detect these differences. Finally, a thermal stress was imposed on SD and PM samples at 60°C for 7 d, and then an amorphous-to-crystalline transformation occurred in IMC in the PM sample and was successfully monitored by \textit{T}_2 measurement. We believe that \textit{T}_2 measurement by TD-NMR is a promising analysis for the characterization of APIs in solid dosage forms, including SD-based pharmaceuticals.

In a previous study, we demonstrated that \textit{T}_1 was an effective parameter for distinguishing between amorphous and crystalline APIs (e.g., indomethacin (IMC) and carbamazepine). The crystalline state of APIs can be evaluated even if the APIs are incorporated into solid dosage forms such as SD. We further analyzed the \textit{T}_1 relaxation curves of a physical mixture (PM) consisting of APIs and a carrier polymer for SD, and then precisely quantified the API contents in the samples.\textsuperscript{8} In the course of the studies, we have investigated a wide range of aspects of \textit{T}_1 measurement for use in the evaluation of the crystalline state of APIs. In contrast to the abundant knowledge on \textit{T}_1 measurement, the study of \textit{T}_2 measurement remains limited. In a previous study, we measured the \textit{T}_2 of amorphous and crystalline APIs; however, these \textit{T}_2 relaxation curves appeared to be similar to each other. Thus, at present, it remains uncertain whether \textit{T}_2 is applicable for evaluating the crystalline state of APIs. Besides our work, there are several reports suggesting that \textit{T}_2 measurement is effective for this purpose. For example, in polymer research, \textit{T}_1 is frequently used to identify the amorphous and crystalline regions of polymers.\textsuperscript{9} In pharmaceutical research, Yoshioka and colleagues\textsuperscript{10} investigated the crystalline state of APIs using \textit{T}_2 measurements by TD-NMR for the evaluation of the crystalline state of APIs containing F atoms based on the \textit{T}_2 relaxation.\textsuperscript{10} Instead of the molecular structure analysis performed with solid-state NMR spectroscopy,\textsuperscript{11} TD-NMR enables easy and rapid measurement of the \textit{T}_1 and \textit{T}_2 relaxation times (\textit{T}_1 and \textit{T}_2, respectively) of the samples. Moreover, TD-NMR can be used to evaluate both solid and liquid samples.\textsuperscript{12,13}

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contents in samples. The second objective was to determine whether $T_2$ measurement could characterize the crystalline state of APIs, such as the difference between the amorphous and crystalline forms. As a result of this study, we clarified that, like $T_1$, $T_2$ is a powerful tool for evaluating the crystalline state of APIs in solid dosage forms.

**Experimental**

**Materials** IMC (minimum purity 98.0%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). PVP K30 (Kollidon® 30) was kindly supplied at no charge by BASF (Ludwigshafen, Germany). Other chemicals used were of reagent grades.

**Sample Preparation of Amorphous IMC and PMs** Amorphous IMC was prepared by the melt-quenching method. IMC crystals were placed on a heated Teflon-coated glass plate at ca. 190 and 160°C for a few minutes. The melts were then quench-cooled by immersion in liquid nitrogen. The resulting samples were lightly ground in a mortar, and then passed through a 500 µm sieve. For preparation of physical mixture samples, the amorphous APIs were mixed with PVP at weight ratios (w/w) of 20 : 80, 40 : 60, 60 : 40, and 80 : 20.

**Sample Preparation of Amorphous SDs** SDs containing amorphous IMC were prepared by the melt-quenching method following the solvent evaporation method. Designated amounts of IMC and PVP (20 : 80, 40 : 60, 60 : 40, 80 : 20, 85 : 15, 90 : 10, 95 : 5, and 99 : 1 (w/w)), were dissolved in ethanol–dichloromethane, 1 : 1 (v/v), at a total concentration of 100 mg/mL, and the solvent was removed using an evaporator (Rotavapor® R-134; Büchi, Flawil, Switzerland) at 40–50°C. The resulting samples were further treated by the melt-quenching method to ensure that all incorporated IMC would be amorphous. Namely, all the obtained samples were placed on a heated polytetrafluoroethylene sheet at ca. 160°C for a few minutes, and then the melts were quench-cooled by immersion in liquid nitrogen. The resulting samples were lightly ground in a mortar, and then passed through a 500 µm sieve. As a comparative control, PVP and amorphous API were also prepared by the same method as for the SDs.

**Powder X-Ray Diffraction (PXRD) Measurement** PXRD patterns of all samples were obtained using a D8 DISCOVER apparatus (Bruker BioSpin Corp., Billerica, MA, U.S.A.) with Cu-Kα radiation (λ = 0.154 nm). The count scanning rate was 10°/min and the scanning angle was from 2θ = 5° to 50°.

**TD-NMR Measurement** The $^1$H $T_2$ relaxation curves of the samples were measured by TD-NMR using a Bruker minispec mq20 instrument (Bruker BioSpin Corp.) at a $^1$H frequency of 20 MHz at 25°C. The solid echo sequence was used for the measurement. The following parameters were applied: scans = 16, recycle delay = 10 s, gain = 60 dB, and dummy shots = 4. The $^1$H $T_2$ relaxation time ($T_2$) was calculated from the free induction decay using TD-NMR Analyze software (Bruker BioSpin Corp.). After removing the offset value from intact $T_2$ relaxation curves, the $T_2$ relaxation data were calculated according to Gaussian curve fitting (Eq. 1)\(^{13,14,16}\):

$$M(t) = M_0 \exp \left(-\frac{1}{2} \left(\frac{t}{T_2}\right)^2\right)$$

$$M(t) = M_0 \left[(1-P_{long}) \exp \left(-\frac{1}{2} \left(\frac{t}{T_{2(short)}}\right)^2\right) + P_{long} \exp \left(-\frac{1}{2} \left(\frac{t}{T_{2(long)}}\right)^2\right)\right]$$

where $M(t)$ and $M_0$ are the transverse magnetization at times $t$ and 0 with Gaussian decay, $t$ is the acquisition time, and $T_2$ is the $T_2$ relaxation time. The terms $T_{2(short)}$ and $T_{2(long)}$ are $T_2$s that have fast or slow relaxation speeds, respectively, and $P_{long}$ is the fraction of protons with $T_{2(long)}$.

**Results and Discussion**

**Quantification of IMC Contents in SDs and PMs by Analysis of $T_2$ Relaxation Curves** Figure 1 shows the $T_2$ relaxation curves of the samples consisting of amorphous IMC and PVP at the designated weight ratio. These curves
have clearly changed with the change in the weight ratio of IMC and PVP; the decay of the NMR signal became slower with an increase in IMC content. The \( T_1 \) values were calculated from these curves. For this calculation, Gaussian curve fitting (Eq. 1) was used because of the better approximation. \( T_2 \) values increased with increasing IMC content: 8.4 ± 0.0 and 8.4 ± 0.0 \( \mu s \) for SD and PM containing 20% IMC and 11.8 ± 0.1 and 11.5 ± 0.0 \( \mu s \) for those containing 80% IMC, respectively (Table 1). The \( T_2 \) relaxation curves between SDs and PMs were completely consistent: almost the same values were observed from SD and PM for the same weight ratios of IMC and PVP. In addition to the single-Gaussian curve fitting, the present study analyzed the \( T_2 \) relaxation curves using bi-Gaussian curve fitting (Eq. 1) and \( T_2 \) values were calculated from the bi-Gaussian curve fitting between SD and PM samples containing 20% IMC, the estimated IMC content was estimated from the fraction of protons with the slow relaxation curve of the latter was close to that of PVP, while the slow relaxation curve of the former was close to that of IMC. Furthermore, for each type of PM, the weight ratios of IMC and PVP calculated by the binary analysis were highly consistent with the actual ratios. We thought that both IMC and PVP particles in PMs were large enough to be recognized by the \( T_1 \) measurement, resulting in the biphasic relaxation curves.

It is our opinion that the \( T_2 \) measurement SD and PM was similar to the \( T_1 \) measurement of SD rather than that of PM. There was no obvious difference between the \( T_2 \) relaxation curves of SD and PM. This indicates that the \( T_2 \) measurement could not recognize IMC and PVP particles in PMs as the individual component because these particles were too small for \( T_2 \) measurement. Moreover, good fitting of the \( T_2 \) relaxation curves of SD and PM was achieved with the single-Gaussian approximation: the monophasic relaxation curve also indicated that the \( T_2 \) measurement recognized the both samples as having a uniform structure. Furthermore, the area monitored by the \( T_2 \) measurement was supposed to be much larger than that monitored by the \( T_1 \) measurement, because the proton resonance frequency of \( T_2 \) is lower than that of \( T_1 \). The binary analysis can be working under the condition that the sample consisted of two distinct domains, and they could be separately detected by the measurement. From these issues, we do not think that binary analysis is suitable for the \( T_2 \) measurement to evaluate the IMC contents in the test samples.

To estimate the IMC contents precisely, we next analyzed the \( T_2 \) values calculated from the single-Gaussian fitting in detail (Eq. 1). In NMR studies of a relative liquid, two distinct protons that are rapidly exchanging are frequently expressed as follows:

\[
\frac{1}{T_2} = \frac{1 - P_{long}}{T_{2(short)}} + \frac{P_{long}}{T_{2(long)}}
\]

(3)

where \( T_2 \) is the observed overall \( T_2 \) relaxation time, \( T_{2(short)} \) and \( T_{2(long)} \) are the \( T_2 \) relaxation times of the distinct protons, and \( P_{long} \) is the fraction of proton having \( T_{2(long)} \). The reciprocal of \( T_2 \) has been defined as the \( T_2 \) relaxation rate. Thus, the \( T_2 \) relaxation rate is supposed to proportionally related to \( P_{long} \).

Cooper et al.\(^{18,19}\) used Eq. 3 to evaluate the molecular mobility of water molecules in a silica nanosuspension.

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### Table 1. Gaussian Curve Fitting Analysis of \( T_2 \) Relaxation Curves for SDs and PMs

| Samples                  | Designated IMC content | Single-Gaussian fitting analysis \(a\) | Bi-Gaussian fitting analysis \(b\) |
|--------------------------|------------------------|----------------------------------------|-----------------------------------|
|                          | \( T_1 (\mu s) \) | \( P_{long} \) | Estimated IMC content (%) | \( T_{2(short)} (\mu s) \) | \( T_{2(long)} (\mu s) \) | \( P_{long} \) | Estimated IMC content (%) |
| Solid dispersions (SDs) | 20%              | 8.4 ± 0.0 | 0.14 ± 0.02 | 22.5 ± 2.3 | 8.3 ± 0.0 | 99.8 ± 9.1 | 0.03 ± 0.00 | 4.5 ± 0.3    |
|                          | 40%              | 9.1 ± 0.0 | 0.30 ± 0.00 | 44.3 ± 0.2 | 8.9 ± 0.0 | 53.9 ± 7.8 | 0.03 ± 0.00 | 5.1 ± 0.3    |
|                          | 60%              | 10.2 ± 0.0 | 0.53 ± 0.01 | 66.8 ± 0.9 | 9.5 ± 0.1 | 24.7 ± 2.4 | 0.08 ± 0.02 | 13.6 ± 2.5   |
|                          | 80%              | 11.8 ± 0.1 | 0.78 ± 0.01 | 86.2 ± 0.6 | 9.0 ± 0.5 | 16.1 ± 0.9 | 0.42 ± 0.09 | 56.1 ± 8.4   |
| Physical mixtures (PMs) | 20%              | 8.4 ± 0.0 | 0.15 ± 0.01 | 24.7 ± 0.9 | 8.3 ± 0.0 | 131.4 ± 4.3 | 0.06 ± 0.00 | 10.6 ± 0.1   |
|                          | 40%              | 9.2 ± 0.0 | 0.34 ± 0.02 | 48.4 ± 1.7 | 8.9 ± 0.0 | 365.5 ± 3.8 | 0.04 ± 0.00 | 7.6 ± 0.8    |
|                          | 60%              | 10.1 ± 0.0 | 0.54 ± 0.02 | 67.7 ± 1.4 | 9.2 ± 0.2 | 22.6 ± 3.5 | 0.11 ± 0.03 | 18.8 ± 4.4   |
|                          | 80%              | 11.5 ± 0.0 | 0.76 ± 0.01 | 85.0 ± 1.0 | 8.9 ± 0.2 | 16.5 ± 0.5 | 0.37 ± 0.02 | 51.6 ± 2.4   |
| Amorphous IMC            | 100%             | 13.8 ± 0.0 | — | — | — | — | — | — |
| PVP                      | 0%               | 7.9 ± 0.0 | — | — | — | — | — | — |

\( a \) Calculated according to Eq. 1 and 3. \( b \) Calculated according to Eq. 2. Each value represents the mean ± standard deviation (S.D.) (n = 5).
They assumed that water molecules, solvent molecules of the test nanosuspension, were divided into two molecular states of free and bound water molecules interacting with the silica nanoparticles. As these water molecules were rapidly exchanged, the $T_2$ relaxation curve showed a monophasic relaxation behavior. From these experiments, the authors successfully identified the distinct water molecules in the suspension by using Eq. 3. As far as the present study is concerned, it is unlikely that protons of IMC and PVP were rapidly exchanged because of the solid sample; however, the observed $T_2$ relaxation curves appeared to have a monophasic behavior. Thus, we thought that there was a good possibility that this equation was applicable. Namely, $T_2$ relaxation rate was supposed to change proportionally as a function of the weight ratio of IMC and PVP.

The changes in $T_2$ relaxation rate as a function of IMC contents are shown in Fig. 2. As expected, the observed values of the SDs and PMs became proportionally shorter with increasing IMC content in a dose-dependent manner (Figs. 2a, b); their determination coefficients were very high ($r^2 = 0.991$ and 0.990). Next, the IMC content in the samples was estimated using Eq. 3. Namely, $T_2$ values of amorphous IMC and PVP were plugged in for $T_2^{(long)}$ and $T_2^{(short)}$, and then the $P_{long}$ values, the fractions of the IMC proton, were calculated. Next, experimental IMC contents were estimated by taking the following parameters into account: $P_{long}$, proton mass, molecular weight, and the weight ratio of components in PMs (the parameters of PVP were calculated from its monomer unit, vinylpyrrolidone). As a result of the analysis, precise estimation of IMC contents in the sample was successfully achieved (Table 1). The estimated IMC content calculated from the results of PMs containing 20, 40, 60, and 80% IMC were 24.7, 48.4, 67.7, and 85.0%, respectively; the estimation was much higher than that obtained from the binary analysis (Table 1). We further stress that the same $T_2$ relaxation rates can be obtained regardless of SD or PM: e.g., $8.4 \pm 0.0$ and $8.4 \pm 0.0$ ms$^{-1}$ for SD and PM containing 20% IMC, compared with $11.8 \pm 0.1$ and $11.5 \pm 0.0$ ms$^{-1}$ for SD and PM containing 80% IMC, respectively. From these findings, we concluded that this analysis has achieved precise estimation of IMC contents in the test samples. As mentioned in the Introduction, TD-NMR enables the measurement of $T_2$ rapidly and easily. Furthermore, the measurement is carried out nondestructively. Thus, this technique could be powerful for component analysis of solid dosage forms, including SD-based pharmaceuticals.

### Evaluation of the Crystalline State of IMC Based on $T_2$ Relaxation Curves

In the next stage of this study, the crystalline state of IMC was evaluated. The $T_2$ relaxation curves of crystalline and amorphous IMC were measured, and the $T_2$ relaxation rates calculated by the single-Gaussian curve fitting (Eq. 1) were compared. Although the $T_2$ relaxation curves appeared to be almost the same (Fig. 3a), the calculated values

![Fig. 2. $T_2$ Relaxation Rate ($1/T_2$) of (a) SDs and (b) PMs as a Fraction of IMC Protons](image)

Each value represents the mean ± S.D. ($n = 3$).
of crystalline IMC were significantly higher than those for amorphous IMC: 76.2 ± 0.2 and 71.0 ± 0.3 ms⁻¹ for the crystalline and amorphous forms, respectively. We note that the same result was observed from carbamazepine and ursodeoxycholic acid (see Supplementary Materials, Fig. S1). It is well known that the relationship between relaxation time and rotational time reflects the molecular mobility of a compound. In general, the lower the molecular mobility, the shorter the $T_2$. Thus, the findings in this study are quite reasonable. As mentioned in Introduction, we have previously verified that the $T_1$ value measured by TD-NMR was an effective parameter for evaluating the crystalline state of API powders: the $T_1$ of crystalline APIs was clearly longer than that for amorphous forms. By contrast, at that time, from the $T_2$ measurement, we could not notice any difference between them. However, in the present study, we confirmed that there were slight but significant differences in $T_2$ relaxation rate between amorphous and crystalline APIs (Fig. 3b and Fig. S1). Our findings led us to suggest that the $T_2$ measurement is also useful for the evaluation of the crystalline state of API powders.

**Continuous Monitoring of the Crystalline State of IMC in PMs and SDs during the Thermal Stress Test** In the final phase of this study, we monitored the IMC amorphous-to-crystalline transformation accompanied by the change in $T_2$ relaxation rate behavior. A thermal stress test was conducted at 60°C for 7 d to enhance the crystalline transformation. SDs and PMs containing 20% of amorphous IMC were used for this experiment as a test sample. In a preliminary study, we conducted a thermal stress test at 60°C for several weeks on the same SD and PM samples. The SD was quite stable, and no crystalline transformation was observed throughout the experimental period. As for the PM sample, a clear crystalline amorphous-to-crystalline IMC transformation started to be detected after only 3 d of storage. Based on these findings, the present study considered the SD and PM as being stable and unstable samples, respectively. At the designated intervals, the $T_2$ relaxation rates and the PXRD spectra of the sample were continuously monitored.

From the continuous monitoring of PM, a clear prolongation of the $T_2$ relaxation rates could be observed during the thermal stress test; the values increased from 117.0 ± 0.4 to 120.9 ± 0.2 ms⁻¹ after storage for 7 d. By contrast, the $T_2$ relaxation rates of the SDs were almost constant throughout the experimental period; the values at the initial point and after 7 d of storage were 119.5 ± 0.4 and 119.7 ± 0.3 ms⁻¹, respectively (Fig. 4a). The amorphous IMC and PVP were also monitored as reference samples. Similar to PM, no change in $T_2$ relaxation rate was observed from the amorphous IMC powder; the values expanded from 74.3 ± 0.4 to 76.2 ± 0.5 ms⁻¹ until the end of the 7-d experimental period (Fig. 4b). As for PVP, no change in $T_2$ relaxation rate was observed throughout the experimental period, similar to the SD sample: 127.1 ± 0.6 and 126.6 ± 0.4 ms⁻¹ for the samples at the initial point and after 7 d of storage, respectively (Fig. 4c).

The PXRD patterns during the thermal stress test are shown in Fig. 5. The PM sample began to show clear crystalline diffraction peaks after 3 d of storage (Fig. 5b), and then the peak intensity clearly increased after 7 d of storage, indicating that
a crystalline transformation steadily developed with prolonging storage period. By contrast, the SD showed a halo pattern during the test (Fig. 5a). In addition, a reasonable change can be observed from comparative controls, amorphous IMC and PVP. The amorphous IMC showed clear crystalline diffraction peaks after 3 d (Fig. 5c), whereas PVP showed a halo pattern during the test (Fig. 5d). From these findings, we concluded that the amorphous-to-crystalline transformation occurring in PM and SD samples of IMC can be evaluated by $T_2$ measurement.

In general, monitoring the crystalline state of APIs in SD by TD-NMR is very difficult with $T_1$ measurements because the spin diffusion of protons on average equals the $T_1$ relaxation of neighboring protons. However, $T_2$ is not affected by the spin diffusion of protons. This property of $T_2$ relaxation enables quantification of the API in both SD and PM (see Fig. 2). The APIs could be quantified regardless of the miscibility of the two components. For example, if a small amorphous domain appeared in the crystalline API powder because of some external factor, the $T_1$ relaxation would be averaged and could not be quantified. Additionally, even with conventional PXRD, the halo pattern would be too small to quantify the amorphous API. However, because the quantification of the $T_2$ relaxation does not depend on miscibility, the amorphous part can be quantified from the API powder by $T_2$ measurement. Moreover, compared with $T_1$ measurements, more detailed and precise data analysis can be achieved by $T_2$ measurements because they allow the acquisition of a massive amount of data points compared with the $T_1$ relaxation curves. In this study, we confirmed that the differences in $T_2$ between crystalline and amorphous APIs were slight but significant; thus, we believe that $T_2$ measurement could be a promising technique to evaluate the crystalline state of APIs incorporated into solid dosage forms as well as $T_1$ measurements.

**Conclusion**

In the first part of this study, we focused on the estimation of IMC contents in SD and PM samples. As a result of the detailed analysis of the $T_2$ relaxation curves measured by TD-NMR, a precise estimation of the IMC content in the sample was successfully achieved. In the latter part of this study, we confirmed that $T_2$ measurement could detect the difference between the amorphous and crystalline IMC; furthermore, it was effective in monitoring the transformation from amorphous to crystalline during a thermal stress test. In conclusion, the $T_2$ relaxation behaviors measured by TD-NMR could offer profound insights into the characterization of APIs incorporated in solid dosage forms.

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**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

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