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Systemic detection of doxycycline after local administration

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Abstract

Objectives. Controlled release delivery (CRD) systems are used to extend the half-life of topical antibiotics in gingival crevicular fluid (GCF), while avoiding systemic contamination with antibiotics. When multiple periodontitis sites are treated by subgingival application of a one-component copolymer gel containing 14% doxycycline, it is likely that low levels of the antibiotic can be detected in blood by high performance liquid chromatography (HPLC). ***Methods.*** Twelve patients with severe periodontitis and one single defect per patient were treated with one single subgingival application of a new one-component doxycycline gel (14%) in each defect (the UNISITE group). Furthermore, 12 patients with between 3 and 9 periodontal defects were treated with a single application of the same doxycycline gel in each defect, resulting in 3–9 applications per patient (the MULTISITE group). Doxycycline was separated and quantitatively measured with HPLC using a UV detector. ***Results.*** In saliva, the maximum doxycycline concentration of the MULTISITE patients was nearly 10-fold higher than in the UNISITE group. In GCF specimens, maximum doxycycline concentrations were the same magnitude in both the MULTISITE and UNISITE groups. Only one UNISITE patient showed detectable levels of doxycycline in blood serum (maximum application: 0.18 μg/ml). Six MULTISITE patients exhibited measurable concentrations of doxycycline in their serum samples (maximum values: 0.12–0.76 μg/ml). The mean systemic concentration following application of the doxycycline-containing gel to multiple sites was as high as 160 ng/ml within minutes following application. Within approximately 1 h, this fell to levels below the limit of detection by HPLC (<50 ng/ml). ***Conclusions.*** Systemic contamination with doxycycline after topical administration may occur even after unisite application if no periodontal dressing is used. Locally administered doxycycline can be identified in the systemic circulation at levels far below those expected to have antibacterial effects. Systemic concentration following application to a single site was always below levels capable of detection by HPLC.

Key Words: *Antibiotics, controlled release, local delivery, pharmacokinetics, systemic contamination*

Introduction

For the successful treatment of periodontitis, the microbial levels of the bacteria that are responsible for the periodontal infection within the oral cavity have to be minimized significantly [1,2]. Both local and systemic applications of antibiotics are effective in eliminating bacterial infections [3]. Since periodontal infections are characterized by the accumulation of bacterial pathogens mainly within periodontal pockets, research efforts have focused on local delivery of antibiotic agents into the periodontal pocket as a possible treatment strategy [4–8]. With the help of this local route of administration, the concentration of the antimicrobial agent within the pocket and the gingival crevicular fluid (GCF) can be increased significantly, whereas the systemic concentration of the antibiotic can, at the same time, be kept comparatively low [9]. During the past 10 years, different systems have been developed to deliver a variety of antimicrobial substances to the bacterial pathogens within periodontal pockets [10]. Antibiotic agents that are administered in the form of a liquid or gel have been shown to be cleared ultimately from the pocket by normal crevicular fluid

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flow. The half-life of a fluorescein gel deposited into periodontal pockets was 12.5 min [11]. In order to overcome the problems associated with crevicular fluid flow clearance, 0.5 mm diameter extruded ethylene vinyl acetate fibers were loaded with 25% tetracycline HCI [4]. Following fiber placement into periodontal pockets, GCF levels of tetracycline were shown to be sustained at levels in excess of 1500 μg/ml for 10 days. Once the fiber was removed, the half-life of the residual tetracycline was 4.5 h. Serum tetracycline during such treatment was close to undetectable levels (0.1 μg/ml) for the entire 10-day period that the fibers were in place [4]. The pharmacokinetic properties of a biodegradable controlled-release delivery system containing doxycycline hyclate (DH) (8.5% w/w) (Atridox®) have been described [12]. The components of the system were contained in two separate syringes that have to be mixed immediately before use. One syringe contained DH and the other the polymer of polylactic acid (poly-D-L-lactide) dissolved in a biocompatible carrier, N-methyl-2-pyrrolidone (NMP). The constituted product had a concentration of 8.5% w/w DH. When the DH was injected into the periodontal pocket, the NMP carrier that was highly miscible in an aqueous environment was replaced by water, which caused the polymer to return to its solid state. As the polymer degraded, the doxycycline was released into the pocket environment [12]. The clinical and antimicrobial efficacy of this doxycycline gel in terms of attachment gain, as well as its pharmacokinetics, has already been investigated in previous studies after unisite application conditions [13–17].

So far, no studies are available in the literature describing the pharmacokinetics of the polyethylene glycol-lactid/glycolid copolymer doxycycline gel (Ivoclar, Vivadent AG, Schaan, Liechtenstein) in the human body after multisite local delivery. The aim of this clinical study was to investigate the pharmacokinetic profile of this doxycycline gel after multisite (between 3 and 9 pockets) application. Pharmacokinetics of the doxycycline gel were analyzed in GCF, saliva and serum samples. The hypothesis to be tested with this study was: When multiple periodontitis sites are treated with subgingival application of a one-component copolymer gel containing 14% doxycycline, it is likely that low levels of the antibiotic can be detected in blood by HPLC.

Material and methods

Patients

Test group: multisite application. The test group (MULTISITE) comprised 12 patients (5 F, 7 M) with a mean (SD) age of 49.3 (11.8) years. Inclusion criteria for participation in the study was the existence of at least three teeth per patient with persisting or recurring pockets of similar probing depth (PD) and shape (PD $\geq$5 mm and bleeding on probing or PD $\geq$6 mm) after thorough mechanical treatment (non-surgical or surgical) of chronic generalized periodontitis. Exclusion criterion for participation in the study was the administration of any local or systemic antibiotic during the 4 weeks prior to initial application of the local doxycycline gel. Patients were recruited during regular supportive maintenance. The time elapsed after completion of active periodontal treatment varied from 9 months (minimum) to 6 years (maximum).

Control group: unisite application. The control group (UNISITE) comprised 12 patients (7 F, 5 M) with an age of 55.9$\pm$11.2 years (mean$\pm$SD). An inclusion criterion for participation in the study was one tooth with a persisting or recurring pocket of similar PD and shape (PD $\geq$5 mm and bleeding on probing or PD $\geq$6 mm) after thorough mechanical treatment (non-surgical or surgical) of chronic generalized periodontitis. An exclusion criterion for participation in the study was the administration of any local or systemic antibiotic during the 4 weeks prior to initial application of the local doxycycline gel. Patients were recruited during regular supportive maintenance. Time elapsed after completion of active periodontal treatment varied from 6 months (minimum) to 4 years (maximum).

The patients of both the UNISITE and the MULTISITE groups were informed about the procedures, risks, and benefits of the study, and informed written consent was obtained. The study was approved by the Institutional Review Board for Human Studies of the University of Heidelberg. There were no dropouts during the course of the study.

Pharmacokinetic evaluation

After chewing for 10 min on a paraffin block (Vivacare; Ivoclar/Vivadent AG, Schaan, Liechtenstein), 5 ml samples of saliva were collected and sent to the laboratory for further investigation. GCF samples were obtained at baseline and throughout the course of the study. Prior to collecting GCF, the teeth were isolated, dried, and supragingival plaque was removed. Filter paper strips (Periopaper; Oraflow® Inc. Plainview, N.Y., USA) were then placed subgingivally for a period long enough to collect 0.15–0.70 μl of GCF (minimum application time 5 s; maximum application time 40 s). Because the amount of GCF that was produced within a defined interval differed from collection site to collection site, no fixed time for GCF collection was used. GCF was collected from all the sites where

the gel had been applied (in the MULTISITE group between 3 and 9 sites per patient, in the UNISITE group one site per patient). Upon removal, the quantity of fluid on the strip was recorded. The absolute quantification of GCF on the filter paper strip was performed using an electronic device (Periotron® 8000; Oraflow® Inc., Plainview, N.Y., USA). After GCF sampling, 5 ml blood samples were obtained from a vein in the arm, stored for 60 to 120 min at 6°C until completion of blood clotting and then centrifuged at 5000 rpm for 15 min to separate serum from all cellular and clotted particles. Saliva, GCF and blood samples were collected at 2 h and 5 h, 1, 2, 3, 4, 7, 9, and 11 days after subgingival application of the doxycycline gel. For further analysis of blood samples, serum was used instead of heparinized plasma. All samples were stored at −18°C until HPLC analysis.

Clinical examination and therapy

Immediately before the baseline sampling of GCF, plaque (PlI) and gingival index (GI) were scored and PD and vertical attachment levels (CAL-V) were measured to the nearest 0.5 mm using a periodontal probe (PCPUNC 15; HuFriedy, Chicago, Ill., USA) at 6 sites of the respective test teeth. Thereafter, the test teeth were kept dry using cotton rolls. The tip of the doxycycline cartridges was inserted into the pocket and doxycycline gel was pressed in [13] (14% doxycycline-free amine in a polyethylene glycol-lactid/glycolid copolymer gel; Ivoclar/Vivadent AG, Schaan, Liechtenstein). Using a precision balance, the cartridges were weighed to the nearest 1.0 mg before and after instillation of DOXY gel to assess the instilled amount of gel. The excess amount of material flowing from the pocket after the instillation was collected with the help of a periodontal curette and laid on the balance together with the emptied cartridge (Sartorius Research R300S; Sartorius GmbH, Göttingen, Germany).

Laboratory assays

The GCF collected on the filter strips was analyzed for doxycycline concentration (DOXY) utilizing reverse phase high performance liquid chromatography (HPLC; pump: L-6200 A intelligent; Hitachi High Technologies, San Jose, Calif., USA) and UV detection ($\lambda = 260$ nm; detector: L-7455 (Hitachi High Technologies).

Prior to HPLC analysis, GCF was eluted from the paper strips following a standardized procedure. First, 0.5 ml methanol was added to each paper strip and stored for 60 min at 4°C. Then, 0.5 ml acetone was added and the sample was centrifuged at 5000 rpm for 5 min. After completion of the centrifugation, the internal standard (tetracycline) was added. After replacement of the sample into a brown colored HPLC vial, the sample was directly injected from the HPLC vial. A symmetry shield RP 8 column (5 µm 3.0 × 150 mm; Waters, Milford, Mass., USA) was used for the HPLC analysis. The injected volume was 100 µl, with water:acetronitrile:70% $HClO_4$ (699:298.5:2.5 V/V), Na_2EDTA (0.6 mmol/l) and oxalate (5 mmol/l) as a mobile phase. The flow rate was adjusted at 0.7 ml/min. The results of this assay were expressed in µg of doxycycline per ml of GCF. The concentration of doxycycline in saliva was also determined by HPLC and expressed in ng/ml of saliva. For the expression of doxycycline concentration in blood serum, ng per milliliter of blood serum was used as the unit of measurement. The applied method has been described in detail in a previous study [15].

Statistical methods

The sampled data were collected using a software program for table calculation (Excel®; Microsoft, Redmond, Wash., USA) and later transferred to a scientific statistical software program (SPSS® software, v. 12.0 Chicago, Ill., USA) for statistical analysis. Differences in concentrations of doxycycline that were measured in GCF, saliva, and serum between UNISITE and MULTISITE application were tested with a non-parametric test for independent samples (Mann-Whitney U-test). The amount of applied doxycycline was correlated with the maximum doxycycline concentration that could be found in GCF, blood, and saliva, and the Pearson coefficient for correlation was calculated and subsequently tested for statistical significance.

Results

The clinical parameters of the sites treated subgingivally with the doxycycline gel are given in Table I for the UNISITE and the MULTISITE groups, respectively. The mean amounts of doxycycline administered subgingivally were 12.5 mg (UNISITE) and 20.0 mg (MULTISITE).

Precision, linearity, and recovery

Quantitative analysis of the doxycycline exhibited an inter-assay variability between 2.69% and 3.66% (depending upon concentration). The coefficient of variability ranged between 1.31% and 1.46% for the intra-assay precision. The lower detection threshold was 50 ng/ml. A linear relationship between estimated and real concentration could be found between 50 and 1000 ng/ml ($r = 0.998$). Recovery of doxycycline was 105.88–111.37% (saliva), 93.95–104.56% (GCF) and 105.46–112.54% (serum), respectively.

Table I. Clinical data of patients with UNISITE and MULTISITE application of doxycyline gel (test sites).

	UNISITE ($n=12$)	MULTISITE ($n=12$)
PPD[1] (mm)*		
Mean	7.04	7.33
Median	7.25	7.25
SD	1.48	1.13
PAL-V[2] (mm)*		
Mean	8.58	9.08
Median	8.50	9.00
SD	2.66	0.87
GI*		
Mean	2.00	2.08
Median	2.00	2.00
SD	0.00	0.29
PlI*		
Mean	0.58	0.42
Median	0.00	0.00
SD	0.79	0.67
Amount of applied Doxy-Gel (mg)		
Mean	89.3	147.4
Median	89.5	133.9
SD	20.5	70.2
Doxycycline (mg)		
Mean	12.47	20.6
Median	12.50	18.7
SD	2.86	9.8

[1]PPD = Pocket probing depth.
[2]PAL-V = Probing attachment level vertical.
*Median values only of the treated sites.

Pharmacokinetics

The data for pharmacokinetics of doxycycline in GCF, saliva, and serum are summarized in Tables II–IV. In saliva, the mean (SD) maximum concentration of DOXY (2 h after application) in the MULTISITE group (32083.9 (5306.3) ng/ml) was about 8 times higher compared to that in the UNISITE group (3942.4 (3223.0) ng/ml). After 11 days (2 days), DOXY could not be detected in saliva in either the MULTISITE or UNISITE groups with the HLPC method used in this study. In GCF, the concentration of DOXY was much higher (maximum values: MULTISITE 1798.4 (1560.9) μg/ml, UNISITE 1452.3 (1535.6) μg/ml) than in saliva. Only one patient in the UNISITE group (maximum: 183 ng/ml) and six in the MULTISITE group (maximum values: 117.0–764.0 ng/ml) exhibited detectable concentrations of DOXY in blood serum.

Correlations between the applied amount of doxycycline and maximum detectable doxycycline concentrations in serum, saliva, and GCF were calculated and analyzed for their statistical significance. The Pearson coefficient for correlation was statistically significant for saliva ($r=0.855$; $p<0.001$) and serum ($r=0.443$; $p<0.05$), but not for GCF.

Discussion

Owing to the infective nature of periodontitis, different antimicrobial agents have been used to support mechanical debridement [4,18–21]. Up until now, there have been mainly five antimicrobial agents used in local delivery systems: tetracycline hydrochloride, doxycycline, minocycline, metronidazole, and chlorhexidine [10]. The local delivery system applied in this study contained doxycycline as effective antimicrobial agent. Doxycycline is a synthetic tetracycline compound, its main advantages over tetracycline hydrochloride being increased oral resorption, prolonged serum half-life, and decreased gastrointestinal side effects [22].

After oral administration, absorption of doxycycline is rapid; traces of the antibiotic can be found in the blood within 15 min [23]. After a single oral administration of 100 to 200 mg, the peak serum concentrations range from 1.7to 5.7 μg/ml and occur between 2 and 3.5 h after administration [23–26]. Following absorption, approximately 80% to 90% of doxycycline is bound to serum proteins [24,27]. About one half of an orally administered dose of doxycycline is transformed into inactive compounds. Since more than 60% of the administered dose is eliminated by hepatic and intestinal clearance, no accumulation occurs after repeated doses, even in patients with anuria [28].

Table II. Doxycycline concentration in GCF after subgingival application MULTISITE vs. UNISITE.

		Mean (μg/ml)		SD (μg/ml)		
Evaluation time	n	MULTISITE	UNISITE	MULTISITE	UNISITE	
Baseline	12	0.0	0.0	0.0	0.0	n.s.
2 h	12	1798.4	1452.3	1560.9	1535.6	n.s.
5 h	12	1171.3	981.0	647.8	1123.4	n.s.
1 d	12	570.3	684.7	350.2	507.5	n.s.
2 d	12	103.8	407.1	81.1	536.2	n.s.
3 d	12	59.5	153.5	81.8	138.2	n.s.
4 d	12	58.1	154.2	118.5	194.0	n.s.
7 d	12	0.0	68.9	0.0	84.5	$p=0.039$
9 d	12	0.0	36.3	0.0	86.9	n.s.
11 d	12	0.0	18.8	0.0	65.1	n.s.

Table III. Doxycycline concentration in saliva after subgingival application MULTISITE vs. UNISITE.

		Arithmetic mean (ng/ml)		SD (ng/ml)		
Evaluation time	*n*	MULTISITE	UNISITE	MULTISITE	UNISITE	
Baseline	12	0.0	0.0	0.0	0.0	n.s.
2 h	12	32083.9	3942.4	5306.3	3223.0	$p<0.001$
5 h	12	9096.5	1044.3	9405.4	1097.1	$p<0.001$
1 d	12	717.5	295.17	684.9	539.7	n.s.
2 d	12	138.6	0.0	177.7	0.0	$p<0.001$
3 d	12	107.8	0.0	72.1	0.0	$p<0.001$
4 d	12	178.5	0.0	178.4	0.0	$p=0.003$
7 d	12	25.0	0.0	26.0	0.0	$p=0.006$
9 d	12	104.5	0.0	182.2	0.0	$p=0.006$
11 d	12	0.0	0.0	0.0	0.0	n.s.

The concentration of three different tetracyclines (tetracycline, minocycline, doxycycline) in plasma, GCF, and saliva has been investigated after oral administration [29], with doxycycline (single dose: 100 mg) showing a better absorption than tetracycline and minocycline. The highest concentrations were measured 2 h after administration. The highest concentration of doxycycline could be found in blood plasma (2.35 μg/ml). GCF showed an intermediate doxycycline concentration of 1.65 μg/ml, whereas the concentration of doxycycline in saliva was lowest (0.47 μg/ml). These data confirm that the average concentration of doxycycline in GCF after systemic administration of doxycycline is lower than the doxycycline concentration in blood plasma. The concentration of tetracyclines in GCF was strongly associated with plasma concentration, indicating a primary role of drug absorption in the delivery of these systemically administered antibiotics to the site of action in periodontal therapy. The average GCF concentration in individuals varied widely (between 0 and 8 μg/ml), with approximately 50% of the samples achieving levels of $<$ μg/ml.

Suspected periodontal pathogens have a susceptibility to doxycycline ranging from 0.1 μg/ml to 2.0 μg/ml [30]. In biofilms, the required MICs are about 50 times higher [31–34]. Thus, systemic administration of doxycycline obviously cannot guarantee a sufficient concentration of the antibiotic in GCF. Therefore, local delivery systems for doxycycline have been developed. These so-called controlled release delivery (CRD) systems aim at an increased concentration of doxycycline in GCF, while keeping the systemic concentration of the antibiotic comparatively low [35]. By using a topical antibiotic-like tetracycline gel without CRD in combination with SRP, the treatment results could not be improved compared to SRP alone [36]. Repeated topical application of a tetracycline solution with a microbrush instead of SRP may reduce inflammation parameters in locally persistent periodontitis comparable to SRP alone [37]. On the other hand, for subgingivally applied doxycycline gels with true CRD characteristics, better clinical and microbiological results could be demonstrated when used as an adjunct compared to SRP alone under different clinical circumstances [13,14,38], at least for short-term follow-up. However, in a recent study, Bogren et al. [39] found no additional benefit of repeated adjunctive application of a CRD doxycycline for an observation period of 3 years compared to mechanical debridement alone in periodontal maintenance patients. For the doxycycline gel that was administered in this study, previous studies have already demonstrated that a single dose of the investigated gels provides sufficient antimicrobial activity up to 10 days in most cases and fulfils the conditions for a "controlled release device" [15,16]. The release

Table IV. Doxycycline concentration in serum after subgingival application MULTISITE vs. UNISITE.

		Mean (ng/ml)		SD (ng/ml)		
Evaluation time	*n*	MULTISITE	UNISITE	MULTISITE	UNISITE	
Baseline	12	0.0	0.0	0.0	0.0	n.s.
2 h	12	160.8	15.25	236.9	52.8	n.s.
5 h	12	119.8	13.0	136.1	45.5	n.s.
1 d	12	49.4	0.0	72.7	0.0	$p=0.039$
2 d	12	45	0.0	105.3	0.0	n.s.
3 d	12	40	0.0	94.2	0.0	n.s.
4 d	12	14.9	0.0	51.6	0.0	n.s.
7 d	12	0.0	0.0	0.0	0.0	n.s.
9 d	12	0.0	0.0	0.0	0.0	n.s.
11 d	12	0.0	0.0	0.0	0.0	n.s.

rate is determined by the local drug concentration, but is also influenced by the degradation rate and the barrier properties of the drug carrier. In the local delivery system applied in this study, the initial drug concentrations in the carrier were 14% (DOXY) per weight doxycycline-free amine. Although this available drug concentration in the carrier is lower than that of tetracycline HCl used at 25% loading in tetracyline fibers (Actisite®), the MIC of doxycycline for suspected periodontal pathogens was exceeded for at least 10 days after application of the gel. These conclusions are supported by several clinical studies that could prove the efficacy of locally administered doxycycline in terms of gain of attachment [13,40,41]. In two multicenter clinical trials designed to study the efficacy of a doxycycline containing drug delivery system, a reduction of PD of 1.3 mm and clinical attachment gain of 0.8 mm were obtained at month 9 [40]. Our results confirm the high inter-individual variability of concentration in GCF after local delivery of doxycycline observed by other authors [12].

Calculation of the mean doxycycline concentration in GCF and saliva showed significant inter-individual variability. The release of doxycycline peaked at 2 h, both in GCF and in saliva after UNISITE and MULTISITE administration. In the GCF samples, the concentration of doxycycline was higher than the MIC_{50} for *Aggregatibacter* (formerly: *Actinobacillus*) *actinomycetemcomitans* in biofilms for a period of 10 days after administration of the gel in both the UNISITE and MULTISITE groups [3]. In a previous study, after a single topical subgingival administration of the doxycycline gel, no elevation of the systemic concentration of the antibiotic in blood was observed [15], though a periodontal dressing was not placed.

On the other hand, up to now, whether systemic effects, or at least an increased concentration, of doxycycline in blood can occur after MULTISITE administration of the 14% doxycycline gel in the oral cavity has not been investigated scientifically.

Only one patient from the UNISITE group (maximum: 183 ng/ml) and 6 from the MULTISITE group (maximum values: 117.0–764.0 ng/ml) exhibited detectable concentrations of DOXY in blood serum.

For the local doxycycline ATRIDOX®, multisite administration within the oral cavity has already been investigated [12]. In the cited study, doxycycline concentrations in serum never exceed 0.1 μg/ml; swallowing and mucosal absorption of the gel were inhibited with a periodontal dressing. The doxcycline gel used in our study had a higher concentration of doxycycline (14% vs. 8.5% in ATRIDOX®). The two doxycycline gels use different carrier systems. Furthermore, the doxycycline gel used in this study is a one-component gel that is ready to use; no mixture of the two components prior to application is necessary.

After application of the 14% doxycycline gel, the doxycycline concentrations in blood were up to 7 times higher compared to the data from Stoller et al. [12], who used a periodontal dressing to avoid systemic contamination caused by swallowing. Even after application of the new 14% doxycycline gel into one single pocket, systemic contamination may occur if no periodontal dressing is placed, as could be shown for one patient in the UNISITE group. Doxycycline that has been delivered locally may reach blood circulation by two different routes: either via direct resorption from the periodontal tissues, e.g. within the pocket, or via intestinal resorption after swallowing, which is likely to be the main route for systemic contamination with doxycycline.

For maximum detectable concentration of doxcycline, we could demonstrate statistically significant correlations with the applied amount in saliva and serum, but not in GCF. The lack of correlation in GCF can be explained by the fact that the amount of doxycycline gel administered per pocket depended only on the size of the pocket. Thus, in patients with multisite application, the average maximum concentration calculated from the GCF samples taken from 3–9 sites did not correlate with the total amount of doxycycline applied in all pockets.

Within the limitations of this study, the following conclusions may be drawn for the new 14% doxycycline gel.

Systemic contamination with doxycycline after topical administration may occur even after unisite application if no periodontal dressing is used. Maximum concentrations of doxycycline in serum and saliva show a significant correlation with the total amount of doxycycline that has been applied. Even the patients with a maximum number of defects (9), and thus a maximum amount of applied doxycycline gel, showed doxycycline peaks in blood serum that were about one-third of the maximum doxycycline concentration compared to the systemic mode of administration (100–200 mg p.o.). Locally administered doxycycline can be identified in the systemic circulation at levels far below those expected to have antibacterial effects.

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Conflict of interest

We declare that there are no conflicts of interest.

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