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Soft tissue and bone penetration abilities of daptomycin in diabetic patients with bacterial foot infections

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Objectives: In the attempt to overcome increasing glycopeptide- and methicillin-resistant soft tissue infections, daptomycin is presently considered as an attractive alternative to the class of glycopeptides. However, daptomycin dosing and its ability to penetrate into inflamed target tissues are still a matter of controversy. Thus, in the present investigation, we set out to evaluate daptomycin's ability to penetrate into inflamed subcutaneous adipose tissue and bone in diabetic patients presenting with severe bacterial foot infection.

Patients and methods: The microdialysis technique was utilized to collect interstitial space fluid from inflamed subcutaneous adipose tissue and metatarsal bone. Plasma and unaffected subcutaneous adipose tissue served as reference compartments. Serial sampling of specimens at steady-state was performed from 0 to 8 h post-dose in five patients (Group A) and from 8 to 16 h after study drug administration in another group of four patients (Group B). In all subjects, daptomycin was administered intravenously once daily at a dosage of 6 mg/kg body weight for 4 consecutive days at minimum.

Results: Equilibrium between free tissue and plasma concentrations was achieved ~2 h post-infusion. Under steady-state conditions, the degree of tissue penetration was assessed by the calculation of the ratio of free (*f*) AUC of daptomycin in plasma to the *f*AUC in tissues. The mean ratios of the $fAUC_{0-16\ tissue}$ to the $fAUC_{0-16\ plasma}$ were 1.44, 0.98 and 1.08 for healthy tissue, inflamed subcutaneous adipose tissue and bone, respectively. The corresponding ratios of the *f*AUCs from 0 to 24 h were 1.54, 1.06 and 1.17, respectively.

Conclusions: With the reservation that pharmacokinetic–pharmacodynamic targets for daptomycin in tissues are currently not established, we conclude that daptomycin given at intravenous doses of 6 mg/kg body weight once daily may be considered an effective treatment regimen in diabetic patients suffering from bacterial foot infection and osteomyelitis.

Keywords: multiple-dose pharmacokinetics, methicillin-resistant staphylococci, osteomyelitis

Introduction

Current strategies in the therapy of diabetic patients suffering from complicated bacterial foot infections aim at eradicating the causative pathogen and, in severe cases, involve surgical debridement of lacerated or devitalized tissue.[1] *Staphylococcus aureus* and β-haemolytic streptococci are frequently isolated at early stages of diabetic foot infection (DFI).[1,2] After long-term antibiotic therapy, Gram-negative bacilli and anaerobic bacteria may be isolated from wounds in these patients.[1,2] Thus, the Infectious Diseases Society of America recommends starting an empirical antimicrobial therapeutic regimen directed against aerobic Gram-positive bacteria.[1]

In patients at increased risk of wound infection with resistant organisms, such as methicillin-resistant *S. aureus* (MRSA) or vancomycin-resistant enterococci, the lipopeptide daptomycin

is presently considered an alternative antibiotic agent in adults, although cross-resistance between daptomycin and vancomycin, particularly after vancomycin pre-treatment, has been occasionally reported in the literature.[3,4] At present, daptomycin is approved for the therapy of complicated skin and soft tissue infections at an intravenous dose of 4 mg/kg total body weight (TBW) once daily. However, results of recent investigations in critically ill patients and subjects suffering from bone and joint infections suggest doses of 6 mg/kg TBW in an attempt to achieve concentrations of daptomycin sufficiently high to eradicate bacteria in plasma and in deep compartments.[5,6] Other studies in morbidly obese subjects recommend that appropriate dosing of daptomycin should be based on ideal body weight (IBW) and creatinine clearance calculated by the four-variable 'modification of diet in renal disease' (MDRD) formula,[7] rather than on TBW assessment.[8] The extent of tissue penetration is expressed as the ratio of the area under the concentration–time curve from 0 to 24 h (AUC_{0-24}) for tissue to the AUC_{0-24} for plasma. This is independent of dose, as long as a drug displays linear pharmacokinetics.

By utilizing the microdialysis technique, a well-established method for the determination of free antibiotic concentrations in the interstitial space fluid of tissues including bone,[9,10] Kim *et al.*[11] found good penetration of daptomycin into the interstitium of healthy subcutaneous adipose tissue in diabetic patients. No significant difference in soft tissue penetration ratios of free daptomycin was detected between diabetic patients and healthy controls. Based on these investigations, we performed the present clinical microdialysis study, aiming at describing daptomycin's concentration profile in bone and soft tissue after the administration of high doses to diabetic patients presenting with bacterial foot infection and osteomyelitis. Unaffected, healthy subcutaneous adipose tissue of the same lower limb served as the reference tissue, in order to eliminate bias from a reduction in blood flow due to peripheral vascular disease.[12]

Patients and methods

The study was approved by the Ethics Committee of the Medical University of Graz, Austria and was performed according to the seventh revision of the Declaration of Helsinki (dated October 2008) at the Division of Plastic Surgery, Department of Surgery, Landeskrankenhaus Universitätsklinikum Graz (State Hospital University Clinic of Graz), Graz, Austria. Analytical work was performed at the laboratory of J&P Medical Research Ltd, Vienna, Austria.

Study subjects

Ten patients with type II diabetes [3 females and 7 males; aged 44–76 years; body mass index (BMI) 27.2–46.9 kg/m^2] presenting with deep-seated bacterial foot infections were included into the study after written informed consent was obtained. The patients required surgical debridement with partial metatarsal bone resection and adjuvant systemic antimicrobial therapy. Exclusion criteria were known allergy to daptomycin, pre-treatment with daptomycin within 1 week before the screening visit, pregnancy, renal dysfunction indicated by a creatinine clearance of <40 mL/min, as estimated by the Cockcroft–Gault equation, and knowledge of pre-existing myopathy. All patients had received non-invasive, conservative treatment for DFI prior to enrolment in the study. Five of the 10 patients had a history of percutaneous transluminal angioplasty. During the conduct of the present study, the co-administration of antimicrobial agents or medications other than the study drug was permitted, if medically indicated. The co-administrated drugs were insulin, ciprofloxacin, amoxicillin/clavulanic acid, low-molecular-weight heparin, angiotensin-converting-enzyme inhibitors, antacids, statins, metformin, oral antidiabetic drugs, β-blockers, acetylsalicylic acid, antidepressants and non-steroidal anti-inflammatory drugs, none of which is known to interfere pharmacokinetically with daptomycin. The level of angiopathy was not determined consistently in our subjects prior to the start of the study. Patients received four to five intravenous infusions of daptomycin (Cubicin™, Chiron Corp., Uxbridge, UK) at a dose of ~6 mg/kg TBW over 30 min once daily prior to the start of the microdialysis procedure. To keep discomfort from the microdialysis procedure low, the patients were allocated into two groups: Group A comprised subjects of sampling periods from 0 to 8 h post-dose; and Group B included subjects of sampling periods from 8 to 16 h post-dose.

Microdialysis and sampling procedures

The principles of microdialysis are described in detail elsewhere.[9] In brief, during surgery, a microdialysis probe (molecular cut-off 20000 Da; CMA Microdialysis AB, Stockholm, Sweden) was implanted into metatarsal bone close to the resection margin. For this purpose, a channel of ~2 mm in diameter and 2 cm in length was drilled into the bone. Under continuous visual control, the probe was inserted into cancellous healthy bone using an adapted plastic cannula (Venflon™, Becton Dickinson, Heidelberg, Germany). The plastic cannula served as a guidance tool to ensure that the membrane at the tip of the probe was properly and safely placed into the target tissue. Suturing and other procedures related to the management of the surgical wound were not hampered or delayed by probe implantation. The reference probe was inserted into an unaffected region of subcutaneous adipose tissue of the same lower limb. The probes were connected via tubing to a precision pump (SP101i syringe pump, WPI Inc., Sarasota, FL, USA) and constantly perfused with 0.9% sterile saline solution at a flow rate of 1.5 μL/min. Subsequently, microdialysate samples were collected at 30 min intervals from 0 to 4 h post-dose and at 60 min intervals from 4 to 8 h in Group A, and at 60 min intervals from 8 to 16 h post-dose in Group B. Samples of venous blood were drawn at predefined timepoints from an indwelling intravenous cannula contralateral to the site of dosing. *In vivo* calibration of the individual probes was performed by using the 'reverse dialysis method', as previously described.[9] Microdialysates and plasma aliquots collected from venous blood after centrifugation at 1600 **g** for 10 min were immediately frozen at −20°C and stored at −75°C until analysis.

Determination of plasma protein binding

The individual values of plasma protein binding were determined by the ultrafiltration method, as previously described.[13] In brief, plasma aliquots from two defined sampling timepoints were ultrafiltered using centrifugal filter devices (molecular cut-off 10000 Da; Ultrafree-MC, Millipore, Bedford, MA, USA) and the filtrate was analysed for free daptomycin. The mean of the two results was corrected for the non-specific binding of daptomycin to the filter membrane. The plasma protein binding of daptomycin was calculated using the formula: protein binding (%) = $100-(100\times C_{filtrate}/C_{plasma})$.

Chemical analysis

Daptomycin concentrations in plasma, plasma ultrafiltrate and microdialysates were measured by HPLC with ultraviolet detection, according to the method of Dvorchik *et al.*[14] with modification. The samples were diluted as necessary with blank human plasma and 0.9% saline, respectively. The lower limit of quantification was 0.3 mg/L for plasma and 0.1 mg/L for microdialysates. The coefficients of inaccuracy (relative

error) and imprecision (relative standard deviation) for this method were 1.2%–14.8% and 0.6%–5.4% for plasma, and 4.0%–8.8% and 0.6%–7.3% for microdialysates, respectively.

Pharmacokinetic and statistical analysis

The IBW was calculated by using the formula: IBW = 48 kg (females: 45.5 kg) + 2.7 kg (females: 2.2 kg) per inch over 5 feet height.[15] The creatinine clearance was calculated using the Cockcroft–Gault formula and the MDRD formula.[7] Non-compartmental pharmacokinetic analysis was carried out by use of commercially available computer software (Kinetica, version 3.0, Innaphase, PA, USA). For each matrix, a mean pharmacokinetic profile from 0 to 16 h was obtained from the combined data of Groups A and B. For the mean concentrations at 24 h, the baseline steady-state concentrations were utilized. The AUCs were calculated by use of the linear trapezoidal rule. The Wilcoxon rank-sum test was performed to compare individual tissue and plasma concentrations. $P < 0.05$ was considered significant.

Results

Ten diabetes patients with DFI requiring surgical debridement were included in the present study to determine the pharmacokinetic profiles of the anti-MRSA drug daptomycin in plasma, soft tissues and metatarsal bone by microdialysis. However, one patient withdrew his consent to participate in the study because of concern related to the microdialysis probe insertion. Thus, the data of nine patients (five in Group A and four in Group B) were available for pharmacokinetic analysis. The patients' demographic characteristics and physiological markers are summarized in Table 1. No adverse events related to the study drug or to the microdialysis procedure were observed or reported.

Concentration-versus-time profiles of daptomycin from Groups A and B in plasma, healthy and inflamed subcutaneous adipose tissue and bone are depicted in Figure 1. The main pharmacokinetic parameters for the mean profile of the combined data of Groups A and B are summarized in Table 2. The individual values of plasma protein binding ranged from 89.4% to 93.5% (mean 91.4%) in our study group and were found to be independent of daptomycin's concentration.

As shown in Figure 1, daptomycin's free concentrations in subcutis and bone closely mimic those seen in plasma over 8 h, indicating the occurrence of rapid equilibration of free drug between plasma and the interstitial space fluid of tissues. Daptomycin's tissue penetration, as expressed by the mean $f\text{AUC}_{0-16\ \text{tissue}}/f\text{AUC}_{0-16\ \text{plasma}}$ was 1.44, 0.98 and 1.08 for unaffected subcutis, inflamed subcutis and bone, respectively. The corresponding ratios of the $f\text{AUC}_{0-24\ \text{tissue}}/f\text{AUC}_{0-24\ \text{plasma}}$ were 1.54, 1.06 and 1.17 (Table 2). As free daptomycin concentrations were not significantly different in inflamed subcutaneous adipose tissue and bone ($P = 0.163$), we pooled these datasets in Figure 2. Figure 2 depicts the ratio of the concentration of daptomycin in tissue to the concentration in plasma versus time. Two morbidly obese patients (BMI of >40 kg/m^2) in Group A showed a descriptively higher mean plasma AUC_{0-8} than the other subjects in this group (349.0 ± 82.1 versus 231.1 ± 4.7 mg·h/L, respectively).

Table 1. Demographic characteristics and physiological markers of study patients (mean ± SD)

Variable	Subjects: morbidly obese (BMI >40 kg/m^2; $n=2$)	Subjects: normal (BMI <40 kg/m^2; $n=7$)
Gender	2 male	3 female, 4 male
Age (years)	53.0 ± 2.0	61.7 ± 9.8
Height (m)	1.92 ± 0.09	1.75 ± 0.06
TBW (kg)	166.0 ± 9.0	92.1 ± 14.5
IBW (kg)	89.6 ± 9.0	69.1 ± 8.8
BMI (kg/m^2)	45.3 ± 1.6	30.1 ± 3.3
Serum creatinine (mg/dL)	1.1 ± 0.2	1.1 ± 0.2
CL_{CR} CG_{TBW} (mL/min)	182.1 ± 24.4	91.8 ± 29.5
CL_{CR} CG_{IBW} (mL/min)	97.4 ± 8.5	68.2 ± 18.4
CL_{CR} MDRD (mL/min/1.73 m^2)	75.2 ± 14.8	68.7 ± 15.2
PPB of daptomycin (%)	92.1 ± 0.0	91.2 ± 1.3

TBW, total body weight; IBW, ideal body weight; BMI, body mass index; CL_{CR}, creatinine clearance; CG_{TBW}, Cockcroft–Gault equation using total body weight; CG_{IBW}, Cockcroft–Gault equation using ideal body weight; MDRD, 'modification of diet in renal disease' formula; PPB, plasma protein binding.

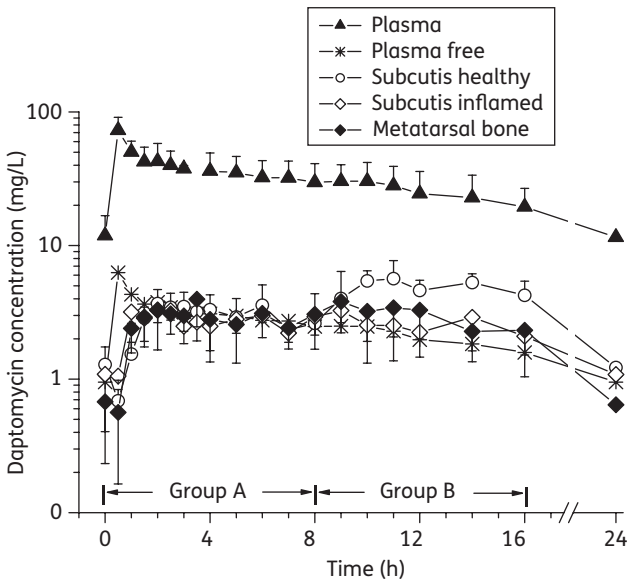

Figure 1. Steady-state pharmacokinetic profiles of free daptomycin in plasma, healthy and inflamed subcutaneous adipose tissue, and metatarsal bone after intravenous administration of 6 mg/kg TBW to diabetic patients presenting with bacterial foot infections (mean ± SD). Combined data from two different patient groups are shown [Group A ($n=5$), data from 0 to 8 h; Group B ($n=4$), data from 8 to 16 h]. The mean steady-state concentrations at baseline were utilized for the concentrations at 24 h.

Discussion

Daptomycin has been on the market in the USA since 2003 and has triggered extensive investigation of potential clinical applications apart from those for which it is currently approved, namely severe soft tissue infection and right-sided bacterial endocarditis. Approved doses of daptomycin are 4 and 6 mg/kg TBW for foot

Table 2. Non-compartmental pharmacokinetic parameters of daptomycin at steady-state (on day 4 or 5) in a total of nine subjects with diabetic foot infections following intravenous administration of ~6 mg/kg TBW once daily; values represent the combined mean profile from Group A (0–8 h) and Group B (8–16 h)

Compartment	C_{max} (mg/L)	T_{max} (h)	$t_{1/2}$ β (h)	V_{ss} (L)	AUC_{0-16} (mg·h/L)	AUC_{0-24}[b] (mg·h/L)	$fAUC_{0-24\ tissue}/fAUC_{0-24\ plasma}$
Plasma	72.9	0.50	10.05	11.04	510.27	619.30	—
Plasma free[a]	6.3	0.50	10.05	—	42.49	51.43	—
Subcutis healthy	4.1	2.33	9.46	—	61.30	80.18	1.54
Subcutis inflamed	4.0	2.38	10.98	—	41.77	54.47	1.06
Metatarsal bone[c]	4.7	4.50	10.72	—	45.84	60.24	1.17

C_{max}, peak concentration; T_{max}, time to peak concentration; $t_{1/2}$ β, half-life at terminal elimination phase; V_{ss}, volume of distribution at steady-state; *f*AUC, AUC of free drug.
[a]Plasma protein binding was determined in duplicate for each subject.
[b]Steady-state concentrations at baseline were used for concentrations at 24 h.
[c]Data from eight patients.

infections and endocarditis, respectively [Summary of Product Characteristics: Cubicin™ powder for concentrate for solution for injection or infusion; Novartis Pharmaceuticals UK Ltd, 2009 (http://emc.medicines.org.uk)]. However, clinicians are increasingly using higher doses based on several reports documenting acceptable side-effect profiles, even at repetitive doses of up to 12 mg/kg TBW in healthy subjects[16] and patients with normal renal function.[17] Thus, daptomycin dosing is still a matter of debate, particularly in light of its high plasma protein binding of ~92% and its relatively high molecular weight of 1620 Da.[18] These characteristics are currently believed to limit the plasma-to-tissue drug exchange rate, resulting in either delayed or incomplete drug equilibrium between compartments. In line with this, distinct studies have shown that inflammation may significantly impair unbound antibiotic concentrations in target tissues.[19] Against this background, we investigated the ability of daptomycin to penetrate inflamed tissues under steady-state conditions following the administration of daptomycin at a dose of 6 mg/kg TBW in diabetic patients presenting with osteomyelitis of the foot.

Our key finding was that free daptomycin in plasma equilibrates completely with soft tissues and bone within 3 h after the start of a 30 min infusion (Figure 1). Inflammation did not affect daptomycin's concentrations at the target site (Table 2 and Figure 1). The mean ratios of the $fAUC_{0-16\ tissue}$ to the $fAUC_{0-16\ plasma}$ were 1.44, 0.98 and 1.08 for unaffected soft tissue, inflamed subcutis and bone, respectively. At steady-state, the free concentrations of daptomycin in all compartments studied were found to exceed the MIC_{90}s for relevant Gram-positive bacteria, such as methicillin-susceptible *S. aureus* and MRSA (MIC_{90} 0.25–0.5 mg/L).[20]

In the past, several research groups compared the pharmacokinetics of antimicrobials in diabetic subjects and healthy controls. In the vast majority of these studies, no statistically significant differences between these study groups were found, as exemplified for fosfomycin, moxifloxacin and daptomycin.[11,19,21] An impaired penetration of vancomycin into target tissues was reported by Skhirtladze *et al.*[22] in a statistically underpowered small subgroup of diabetics among patients undergoing cardiac surgery compared with non-diabetic controls. Similar findings are available in the literature for linezolid in diabetic patients with documented impairment of the arterial

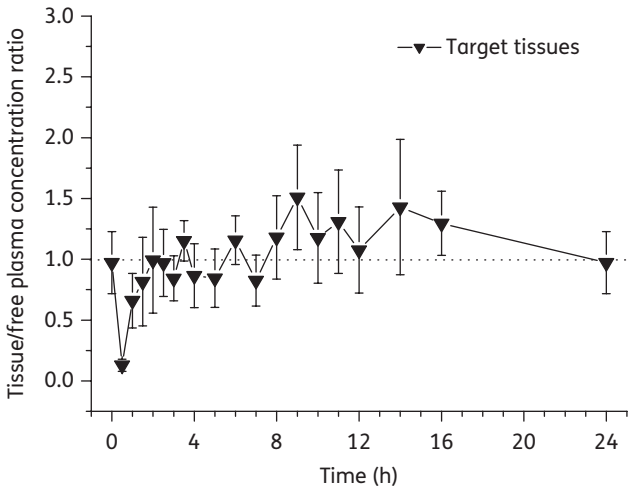

Figure 2. Free target tissue to plasma concentration ratios versus time (mean ± SD). Data from inflamed subcutaneous adipose tissue and bone were pooled.

blood flow of the affected lower limb.[23] Importantly, the microdialysis study on daptomycin by Kim *et al.*[11] looked at the pharmacokinetic profiles of daptomycin in healthy subcutaneous adipose tissue of the thigh in diabetic and non-diabetic subjects following administration of 4 mg/kg TBW. Considering the different doses given and the linear pharmacokinetics of daptomycin,[14,16] our results for healthy subcutaneous tissue are in excellent agreement with those from Kim *et al.*[11] Moreover, we could demonstrate, first, that daptomycin's ability to penetrate tissues is not significantly affected by inflammatory conditions and, second, that plasma AUCs of daptomycin are higher in two morbidly obese patients as compared with subjects of the same group with a BMI of $<40\ kg/m^2$ (349.0 ± 82.1 versus 231.1 ± 4.7 mg·h/L, respectively). The higher plasma AUCs observed in these two morbidly obese patients are related to the higher total dose administered. Thus, instead of using TBW and the Cockcroft–Gault equation for daptomycin's dose adjustment calculations, the assessment and use of MDRD and IBW may help to reduce inter-patient variability in total daptomycin

exposure. It is noteworthy, however, that tissue pharmacokinetic profiles were comparable among obese and non-obese patients, probably due to the small sample size.

Previous experimental data on daptomycin concentrations in infected bone were discouraging. For instance, in a rabbit model of MRSA osteomyelitis, daptomycin's total concentrations were reported to be as low as 0.5 mg/L at 60 min after a single subcutaneous dose of 4 mg/kg. In this model, daptomycin was detectable in infected bone only.[24] These findings do not fit into the overall concept of drug penetration properties into non-specialized tissues, such as subcutaneous adipose tissue and bone, and are in contrast to the results of the present study. It should, however, be mentioned that there is a high variability in the reported methods on bone penetration of antibiotics in pharmacokinetic studies.[25] An attractive approach to gain insight into factors contributing to the inter-subject variability of plasma and tissue pharmacokinetic profiles would be the integration of population pharmacokinetic methods.

For daptomycin, the pharmacokinetic–pharmacodynamic parameter that best correlates with bacterial killing is the ratio of the plasma free AUC_{0-24} to the bacteria's MIC.[26,27] In a neutropenic murine thigh model of infection, a plasma $fAUC_{0-24}$/MIC ratio of ∼100 was calculated for the effective killing of various strains of *S. aureus*.[27] However, pharmacokinetic–pharmacodynamic targets in tissues are currently not available in the scientific literature. In the present study, the $fAUC_{0-24}$/MIC target of 100 was met for staphylococci, including MRSA and coagulase-negative strains (MIC_{90} 0.25–0.5 mg/L), and for β-haemolytic streptococci (MIC 0.25 mg/L) in all compartments tested.[20]

On the other hand, there are anecdotal reports on the progressive loss of *S. aureus* susceptibility after long-term daptomycin use or after vancomycin pre-treatment.[3,28,29] However, respective studies indicated that this effect may be overcome by the currently unapproved dose of daptomycin of >6 mg/kg TBW and/or by combination treatment regimens.[3,28,30]

In chronic DFI, especially after multiple courses of local or systemic antibiotic treatment, Gram-negative enteric bacteria and anaerobic cocci may be isolated against which daptomycin is inactive.[2] There is broad consensus that such cases require broad-spectrum antimicrobial therapy.[1] In these cases, daptomycin may be considered to be administered in combination with the class of penicillins, fosfomycin and metronidazole,[31–34] as *in vitro* experiments reported synergistic or additive antimicrobial effects.[31,33,34]

One limitation of the present investigation is that the effect of surgical procedures, such as debridement and drilling a hole into the bone for microdialysis probe insertion, is not yet clarified. It may be possible that this damage could have had some impact on the measured tissue concentrations.

In conclusion, our microdialysis study showed that free concentrations of daptomycin achieved in subcutaneous adipose tissue and bone after administration of multiple doses of 6 mg/kg TBW were sufficient to cover MRSA and other Gram-positive bacteria commonly found in diabetic patients with foot infections, including osteomyelitis.

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Transparency declarations

F. T., O. M. and M. P. are employees/consultants of J&P Medical Research Ltd, which is an international and independent research institute basically operating according to the Public–Private-Partnership concept. C. J. is managing director of J&P Medical Research Ltd, and owns 100% options. C. J. and K. H. K. are independent consultants for pharmaceutical companies. All other authors: none to declare.

The sponsor was given the opportunity to read and comment on the manuscript before publication.

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