Pharmacokinetics of Betamethasone After Single-Dose Intramuscular Administration of Betamethasone Phosphate and Betamethasone Acetate to Healthy Subjects

Isam I. Salem, PhD; and Naji M. Najib, PhD
International Pharmaceutical Research Center (IPRC), Amman, Jordan

ABSTRACT

Background: Betamethasone is used for its antiinflammatory and immunosuppressive effects in disorders of many organ systems. However, the pharmacokinetic properties of betamethasone in plasma after intramuscular injection of betamethasone sodium phosphate and betamethasone acetate dual-acting suspension need further investigation.

Objectives: The main aim of this study was to determine the pharmacokinetic parameters of betamethasone, betamethasone acetate, and betamethasone phosphate after the administration of a single intramuscular dose of the dual-acting suspension to healthy human volunteers.

Methods: Two different studies were conducted in healthy males. Volunteers were judged healthy based on their medical history, physical examination, and laboratory test results. Before confinement, all volunteers were tested for freedom from alcohol and drugs of abuse. Following a 10-hour overnight fasting, a single dose of 1 mL of the dual-acting suspension containing 3 mg of betamethasone phosphate and 3 mg of betamethasone acetate was administered by intramuscular injection. Blood sampling covered 48 hours. The plasma samples obtained in the second study were stabilized to enable pharmacokinetic profiling of betamethasone esters.

Results: Twenty-four healthy males with mean (SD) age of 27 (6.62) years participated in each study. No incidences of serious adverse events were recorded during the studies. Six mild adverse events were reported in 2 subjects in the second study. One subject suffered from pain at the injection site and insomnia, and another subject complained of heartburn and drowsiness. Betamethasone phosphate appeared to be readily absorbed with a mean AUC₀–t of 96.01 ng/h/mL and an AUC₀–H₁₁₀₀₉ of 97.96 (23.38) ng/h/mL. Betamethasone peak plasma concentration reached a mean t₁/₂ of 12.92 hours. Betamethasone acetate was not detected in the volunteers’ plasma in either study (total of 2208 plasma samples).

Conclusion: The observed pharmacokinetic parameters suggested that the acetate ester, and not the phosphate ester, of betamethasone acts as a prodrug or reservoir for betamethasone, conferring on it sustained- and extended-release characteristics. (Clin Ther. 2012;34:214–220) © 2012 Elsevier HS Journals, Inc. All rights reserved.

Key words: acetate, betamethasone, pharmacokinetics, phosphate.

INTRODUCTION

Betamethasone (BET), one of many synthetic analogs of the naturally occurring glucocorticoids, is used for its antiinflammatory and immunosuppressive effects in disorders of many organ systems. It reduces the incidence and severity of respiratory distress syndrome, one of the most common causes of morbidity and mortality in premature newborns. However, the use of this drug may carry some risk of fetal death in pregnancies complicated by severe hypertension-edema-proteinuria syndromes.¹

Approved products are formulated based on a fast-releasing betamethasone phosphate (BP) ester prodrug or a dual-acting suspension formulation containing BP and betamethasone acetate (BA) esters. Both esters are expected to be hydrolyzed in vivo to the active glucocorticoid BET.² Whereas BP solution is intended for a fast release (either via intravenous or intramuscular administration), the solution/suspension formulation is designed to provide a combination of fast- and sustained-release properties, respectively.

Following the discovery that glucocorticoid hormones are capable of crossing the placenta and triggering the maturational process, Liggins and Howie¹ selected a mix-
ture containing equal quantities of BP and BA to maximize the effect on the fetus while minimizing maternal exposure. The investigators aimed to maximize the drug’s efficacy by administering only 1 injection per day. Nevertheless, other investigators still have doubts about the efficacy or safety of this formulation.\textsuperscript{3–6}

In 1975, Ballard et al\textsuperscript{7} determined serum levels of BET in 20 mothers who received intramuscular administration of a mixture of 6 mg BP and 6 mg BA for the prevention of respiratory distress syndrome. The authors utilized indirect radioreceptor assay for the determination of glucocorticoids. The technique measures steroids with glucocorticoid activity in proportion to both their concentration and their affinity for the cytoplasmic receptor.\textsuperscript{7} Ballard et al reported that the BET level was maximal at 1 hour (755 \(\mu\)g cortisol equivalents per 100 mL) and then declined with a mean \(t_{1/2}\) of 6 hours.

In their 1983 paper, Petersen et al reported the pharmacokinetic (PK) properties of BET and BP ester in 8 healthy adults after intravenous bolus injection of 10.6 mg of BP.\textsuperscript{8} In 1984,\textsuperscript{9} Petersen et al reported the BET PK findings in 9 patients treated with the dual-acting suspension formulation. The authors reported that after intramuscular administration of a dual-acting suspension formulation, BP plasma concentrations did not reach a defined peak and were found to be erratic. The bioavailability of the dual-acting suspension formulation was reported to be poor and less reliable than the solution in terms of the achieved BET plasma concentrations.\textsuperscript{9}

With more emphasis on clinical outcome than the determination of PK parameters, Gyamfi et al\textsuperscript{10} investigated maternal delivery and cord serum BET concentrations, comparing singletons with twins and obese with non-obese women. The authors indicated that maternal and umbilical cord blood serum BET concentrations were not different in twin gestations or obese women.

In this context, Della Torre et al\textsuperscript{11} evaluated the effect of maternal body size and multiple gestation on the PK parameters and their observed variability. The authors reported that the relationship between the PK parameters and lean body weight remained linear over a wide range of maternal body sizes.

The PK properties of BET, BA, and BP in plasma after intramuscular injection of the combination BP and BA suspension need further investigation. No detailed PK data analysis has been performed on the dual-acting formulation of acceptable inferences about the PK properties of BET, BA, and BP. This may be related to inherent release properties of BET esters (BP and BA). Other reasons related to a lack of population PK studies; a lack of strict, controlled conditions during the execution of trials; or even an inadequate amount of sampling time. The discrepancy in results also may be related to lack of specific and highly sensitive methods of analysis.

Establishment of the PK properties of BET, BA, and BP, and therefore reassessment of the use of this dual-acting formulation, is needed to evaluate the risk of exposing patients to steroid levels that are of no therapeutic benefit. Hence, the main aim of our work was to determine the detailed PK properties of BET, BA, and BP by simultaneous calculation of the data obtained in 2 PK studies, each with 24 healthy volunteers. In addition, and since the PK results obtained in the first 1 of the 2 studies did not reveal whether the acetate or the phosphate ester of BET acted as a reservoir or as a prodrug slowly releasing BET within the blood, we decided to determine the differences in PK parameters when plasma samples were stabilized as described in our second study.

**SUBJECTS AND METHODS**

**Drugs**

For the 2 studies, the dual-acting formulation suspension 3 mg BP/3 mg BA per 1 mL was used.\textsuperscript{*}

**Subjects**

Volunteers were included in the study if they were judged healthy based on their medical history; physical examination; and hepatic, renal, respiratory, cardiac, gastrointestinal, complete blood count, and serum chemistry results. In addition, subjects were required to be medication free, including over-the-counter drugs, for 7 days before beginning the study. Subjects were excluded if they tested positive for HIV or hepatitis B and C viruses. Persons who participated in any clinical study or donated blood or were hospitalized within the last 3 months of the initiation of the studies were excluded.

**Experimental and Assay Procedure**

Both studies were approved by the Institutional Review Board at the International Pharmaceutical Research Center (IPRC) and by Jordan’s Food and Drug Adminis-
tation. The studies were performed in 2007 in accordance with the declaration of Helsinki (revised, Tokyo 2004) and current Good Clinical Practice guidelines.

Sixty males gave written, informed consent to participate in the studies before screening.

Consumption of alcohol-containing beverages and foods was prohibited before dosing and until donating the last sample in each study. The consumption of grapefruit-containing beverages and foods was prohibited before dosing and throughout the entire study. Before confinement, all volunteers were tested for the presence of alcohol and drugs of abuse.

Following an overnight fasting of at least 10 hours, subjects participating in each study were administered a single dose of 1.0 mL of a sterile BA/BP suspension accurately drawn into a sterile syringe. The drug was delivered by intramuscular injection into the upper outer quadrant of the buttocks. The site was examined to ensure that the injection was administered to the muscle. Subjects were administered the dose by qualified medical staff. Aseptic technique was maintained when preparing and administering the injection. Meals were provided at approximately 4 and 12 hours after drug administration. In addition, snacks were served 8 hours after drug administration. Subjects remained ambulatory or seated upright for the first 4 hours following drug administration and did not engage in strenuous activity at any time during the housing period. Subjects were housed after dosing until the 24-hour blood draw. Subjects returned to donate the remaining samples.

Seven milliliters of blood samples were withdrawn via an indwelling cannula placed in the forearm antecubital vein. The cannula was flushed with 1 mL of heparinized saline solution (2 IU/mL) after each sample collection. A small volume (0.2 mL) of blood was discarded from the cannula before each sample withdrawal.

In the first study, blood samples were collected in light-protected, heparinized plastic tubes before dosing (0.00 hours) and at the following times after the dose: at 2, 4, 6, 8, and 10 minutes and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 10.00, 16.00, 24.00, 36.00, and 48.00 hours. Blood samples were harvested immediately to plasma by centrifugation for 5 minutes at 1789g (at 5°C) and then stored at −70°C until analysis.

In the second study, blood samples were collected in light-protected, already prechilled plastic heparinized tubes containing 10 μL of 2 M (molar) sodium arsenate solution per mL blood. The blood withdrawal schedule followed that in the first study. Blood samples (kept over ice) were harvested to plasma within a maximum of 15 minutes by centrifugation for 5 minutes at 1789g (at 5°C). Plasma was siphoned into prechilled plastic tubes containing 10 μL of 50% (mass/volume [w/v]) potassium fluoride solution per mL plasma. Plasma samples were stored at −70°C.

In both studies, vital signs (blood pressure, respiratory rate, and body temperature) were measured at predose and 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours following the dose. Adverse events were elicited at scheduled physical examinations and throughout the study by the attending physicians.

Follow-up medical tests (eg, vital signs, physical examination, electrocardiograph, biochemistry, hematology and urine analysis) were conducted in both studies on all volunteers at discharge.

Two different liquid chromatography, tandem mass spectrometry (LC-MS/MS) methods were developed and validated previously for BET, BA, and BP determinations in human plasma at IPRC. The first method involved a simple liquid-liquid extraction technique of BET, BA, and the IS (prednisolone). Separation was accomplished by high-performance liquid chromatography using a C8 column and methanol and ammonium formate buffer as mobile phase. Detection was carried out on an Applied Biosystems API 4000 Mass Detector (Applied Biosystems, Streetsville, Ontario, Canada). Chromatograms of BET were extracted at a mass-to-change ratio (m/z) of 393.2 > 373.10; for BA the m/z was 435.20 > 415.10. Internal standard chromatograms were extracted at an m/z of 361.01 > 343.

The second LC-MS/MS method (for BP determination) involved solid phase extraction of BP and IS on MCX (Mixed-mode cation exchange) solid-phase cartridges and subsequent separation by high-performance liquid chromatography equipped with a C18 column. Detection was carried out on an Applied Biosystems API 4000 Mass Detector. Chromatograms of BP were extracted at an m/z of 473.00 > 435.10. Internal standard chromatograms were extracted at an m/z of 441.12 > 423.00. To prevent the in vitro hydrolysis of BA or BP esters to BET during blood collection and the handling and storage stages of plasma samples, and hence to better profile BET esters, the second PK study was designed as indicated earlier, whereby a potassium arsenate solution was used to prevent esters hydrolysis during blood collection, whereas
a potassium fluoride solution was used during plasma collection.\(^{12}\)

Stability of BET, BA, and BP was studied in blood and plasma covering the interval from the time the blood samples were withdrawn from the volunteers until the moment the samples were stored in the freezer after centrifugation to plasma.\(^{12}\)

### Data Analysis

Establishing the PK parameters for all individuals was accomplished by using Kinetica version 4.1 (Thermo Electron Corporation, Waltham, Massachusetts). Plasma profiles were best characterized by a noncompartmental model.\(^{13}\) The elimination rate constant (\(\lambda_e\)) was obtained as the slope of the linear regression of the log transformed concentration values versus time data in the terminal phase. The \(t_{1/2}\) was calculated as 0.693/\(\lambda_e\). The AUC\(_{0-t}\) was calculated by the linear trapezoidal rule. The AUC\(_{0-\infty}\) was calculated as AUC\(_{0-\infty}\) = C\(_e\)/\(\lambda_e\), where C\(_e\) is the last measurable concentration. The mean residence time (MRT) was calculated as AUMC\(_{0-\infty}\)/AUC\(_{0-\infty}\) by standard methods. AUMC\(_e\), area under the first moment curve, was calculated as \(0.693/\lambda_e\). The volume of distribution (\(V_{ss}/F\)) was calculated as CI \(\times\) MRT/F.

### RESULTS

Forty-eight subjects were found eligible to participate. Twenty-four volunteers participated in each study. The mean (SD) age of the participants was 27 (6.62) years. The mean (SD) weight of the subjects was 68.04 (7.72) kg, and the mean (SD) height was 163.37 (5.92) cm.

The dual-acting suspension formulation was well tolerated by all volunteers. None of the subjects experienced clinically relevant changes in baseline conditions. No incidences of serious adverse events were recorded during the studies. Six mild adverse events were reported in 2 subjects in the second study. One subject suffered from pain at the injection site and insomnia, and another subject indicated heartburn and drowsiness. All were resolved without medical intervention. No unexpected incidents that could have influenced the outcome of the studies occurred. There were no dropouts during the studies, and all the volunteers who started the studies continued to the end and were discharged in good health.

The BET and BA LC-MS/MS method was linear for BET determination over the range 0.50 to 50.00 ng/mL (\(r^2 > 0.999\)). The lower limit of quantitation (LLOQ) was 0.50 ng/mL with 95.33\% accuracy and a %CV of 4.53.\(^{12}\) Plasma samples were stable for 6 hours at room temperature (96.51\%, %CV 3.01). The betamethasone and BA LC-MS/MS method was linear for BA over the range 1.0 to 20.0 ng/mL (\(r^2 > 0.999\)). The LLOQ was 1.00 ng/mL with 95\% accuracy and %CV of 5.77.\(^{12}\) Plasma samples were stable for 6 hours at room temperature (103.04\%, %CV 5.62).

The BP LC-MS/MS method was linear over the range 2.0 to 200.0 ng/mL (\(r^2 > 0.999\)). The LLOQ was 2.00 ng/mL with 100.83\% accuracy and %CV of 8.54. Plasma samples were stable for 5 hours at room temperature (100.72\%, %CV 2.48).\(^{12}\)

The results of stability testing in blood indicated that the arsenate stabilizer prevented the in vitro hydrolysis of BA to BET, as the latter was undetectable (<0.2 ng/mL). BP hydrolysis in blood was also prevented by the addition of arsenate solution to blood.\(^{12}\)

The PK parameters obtained from the PK analysis of the individual plasma concentration-time data of both studies are depicted in Table I. The profiles of the mean

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without Stabilizers</th>
<th>With Stabilizers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>AUC(_{0-t}) (ng/h/mL)</td>
<td>466.51 (115.11)</td>
<td>486.20 (84.51)</td>
</tr>
<tr>
<td>AUC(_{0-\infty}) (ng/h/mL)</td>
<td>506.95 (125.03)</td>
<td>542.17 (90.08)</td>
</tr>
<tr>
<td>C(_{max}) (ng/mL)</td>
<td>33.21 (8.71)</td>
<td>25.86 (4.11)</td>
</tr>
<tr>
<td>(t_{max}) (h)</td>
<td>1.56 (1.32)</td>
<td>2.52 (0.98)</td>
</tr>
<tr>
<td>(k_e) (1/h)</td>
<td>0.0570 (0.01)</td>
<td>0.0565 (0.01)</td>
</tr>
<tr>
<td>(T_{1/2}) (h)</td>
<td>12.47 (1.91)</td>
<td>12.92 (3.18)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>18.07 (2.67)</td>
<td>19.37 (4.57)</td>
</tr>
<tr>
<td>CI/F (l/h)</td>
<td>12.62 (3.45)</td>
<td>11.49 (1.96)</td>
</tr>
<tr>
<td>(V_{ss}/F) (l)</td>
<td>226.00 (61.64)</td>
<td>221.00 (57.33)</td>
</tr>
</tbody>
</table>

BA = betamethasone acetate; BP = betamethasone phosphate; CI/F = plasma clearance; MRT = mean residence time; \(V_{ss}/F\) = volume of distribution.

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values of BET plasma concentration-time data of the volunteers who participated in both studies are represented in Figure 1.

The mean C\textsubscript{max} value for BET in the first study (non-stabilized samples) was 33.21 ng/mL (range 16.10–50.00 ng/mL), whereas in the stabilized study it was 25.86 ng/mL (range 16.36–32.60 ng/mL). BET was measurable in the first study at the 2-minute time point, with peak plasma concentrations at T\textsubscript{max} 1.56 (1.32) hours, whereas in the stabilized study BET was detected after 4 minutes with a T\textsubscript{max} of 2.52 (0.98) hours.

The AUC\textsubscript{0–t} relative to AUC\textsubscript{0–∞} values in both studies indicated adequate blood sampling, since the individual % AUC\textsubscript{extrapolated} was below 20% (mean 7.92% and 9.07%, respectively) and had a small contribution to the total AUC (AUC\textsubscript{0–∞}).

At 48 hours postdosing, the plasma concentrations of BET were measurable in all volunteers in both studies. The t\textsubscript{½} in the first study (without stabilizers) was 12.47 (1.91) hours, whereas in the stabilized study the t\textsubscript{½} was 12.92 (3.18) hours.

The PK results obtained in both studies were submitted to statistical comparisons using the t test. The test depicted significant differences in C\textsubscript{max} and T\textsubscript{max} parameters (P < 0.001). Moreover, the Wilcoxon rank sum test showed significant difference in T\textsubscript{max} (P < 0.05). However, no significant difference (P = 0.375) was observed for the AUC values. C\textsubscript{max} and AUC comparisons were performed after logarithmic transformation to ensure normality. It was also observed that the variability of PK parameters of the first study (nonstabilized plasma samples) was 1.4- to 2.2-fold higher than that of the second study (stabilized samples).

BP appeared to be readily absorbed and was measurable at the first sampling point (2 minutes) in all volunteers. Peak plasma concentrations (C\textsubscript{max} 128.2 [44.32] ng/mL) were achieved at a mean T\textsubscript{max} of 0.24 (0.09) hours. BP fell below LLOQ after 3 hours in all volunteers. The t\textsubscript{½} was determined to be 0.43 (0.16) hour. The AUC\textsubscript{0–t} relative to AUC\textsubscript{0–∞} values indicated adequate blood sampling, since the individual % AUC\textsubscript{extrapolated} was < 20% with negligible contribution to the total AUC (AUC\textsubscript{0–∞}).

The PK parameters of BP are depicted in Table II, and Figure 2 illustrates the profile of the mean values of the BP plasma concentration-time data of the 24 volunteers who participated in the second study.

BA was not detected at any sampling point in either of the 2 studies.

![Figure 1. Mean plasma concentration-time profiles of betamethasone (BET) in 2 different studies after single-dose intramuscular administration of a dual-acting suspension formulation containing 3 mg betamethasone acetate and 3 mg betamethasone phosphate.](image)

Table II. Pharmacokinetic parameters of betamethasone phosphate after single-dose intramuscular administration of a dual-acting suspension formulation containing 3 mg BA and 3 mg BP (mean [SD]).

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>24</td>
</tr>
<tr>
<td>AUC\textsubscript{0–t} (ng/h/mL)</td>
<td>96.01 (23.44)</td>
</tr>
<tr>
<td>AUC\textsubscript{0–∞} (ng/h/mL)</td>
<td>97.96 (23.38)</td>
</tr>
<tr>
<td>C\textsubscript{max} (ng/mL)</td>
<td>128.2 (44.32)</td>
</tr>
<tr>
<td>T\textsubscript{max} (h)</td>
<td>0.24 (0.09)</td>
</tr>
<tr>
<td>k\textsubscript{e} (1/h)</td>
<td>1.7998 (0.61)</td>
</tr>
<tr>
<td>T\textsubscript{½} (h)</td>
<td>0.43 (0.16)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.69 (0.20)</td>
</tr>
<tr>
<td>Cl/F (l/h)</td>
<td>32.40 (8.00)</td>
</tr>
<tr>
<td>V\textsubscript{ss}/F (l)</td>
<td>22.00 (9.78)</td>
</tr>
</tbody>
</table>

BA = betamethasone acetate; BP = betamethasone phosphate; Cl/F = plasma clearance; MRT = mean residence time; V\textsubscript{ss}/F = volume of distribution.
DISCUSSION

In this report we described detailed studies that reveal the PK properties of BET, BA, and BP after the intramuscular administration of a dual-acting suspension formulation of BA/BP to healthy volunteers.

Petersen et al\(^9\) reported that BP levels after intramuscular administration of a dual-acting suspension formulation did not reach defined peak plasma concentrations and were found to be erratic. Our findings revealed that PB appeared to be readily absorbed and was bioavailable with a mean (SD) of AUC\(_{0–t}\) 96.01 (23.44) ng/h/mL and a mean (SD) of AUC\(_{0–H1100}\) 97.96 (23.38) ng/h/mL. In their study, Petersen et al\(^9\) could not determine the T\(_{\text{max}}\) or t\(_{1/2}\) of BP, whereas we report here that PB peak plasma concentrations were determined at a mean (SD) T\(_{\text{max}}\) of 0.24 (0.09) hour and declined with a t\(_{1/2}\) of 0.43 (0.16) hour. The very frequent blood sampling and the use of 2 stabilizers and the sensitive LC-MS/MS method\(^1\) allowed the determination of BP in all subjects at a very early stage, nearly immediately after dosing (2 minutes). In addition, this method allowed the follow-up of plasma levels of BP for a period of 3 hours. No BP was detected after this sampling time. Our reported T\(_{\text{max}}\) and t\(_{1/2}\) for BP are similar to those reported by Petersen et al\(^9\) after intramuscular dosing with a BP solution, in which BP peak plasma concentrations were observed within 18 to 30 minutes, declining with a mean (SD) t\(_{1/2}\) of 23.3 (7.6) minutes.

In our study without the stabilizers we detected BET in all subjects at 2 minutes, whereas in the plasma-stabilized study, BET was determined in all subject samples at 4 minutes, demonstrating the immediate hydrolysis of BP to BET. BET was bioavailable, as reflected in Table I. In addition, we have been able to determine BET plasma levels until the last sampling time at 48 hours in all subjects in both studies (mean 2.27 ng/mL). This finding allowed the accurate estimation of the t\(_{1/2}\) of BET in this dual-acting suspension formulation, which we report to be 12.92 (3.18) hours. This value is different from that reported by Petersen et al (mean [SD] t\(_{1/2}\), 317 [74] minutes)\(^9\) and that reported by Ballard et al (6 hours).\(^7\)

BA was not detected in any plasma samples donated by the participants (total of 2208 samples). The addition of stabilizers to the blood and plasma did not make any difference, as BET reflected a comparable extent of release in both studies (Table I).

Based on the previous report, the t\(_{1/2}\) was longer than that reported for BET (6 hours) after either intravenous or intramuscular administration of solution.\(^8,9\) This observation suggests that the t\(_{1/2}\) was reflective of the absorption process, whereas the observed PK results were due to the slow release of BET from the BA ester within the muscle. This finding results, of course, because the acetate, unlike the BP ester of BET, is poorly soluble and is retained within the muscle longer before it reaches the blood. The reported long t\(_{1/2}\) for BET would be helpful in the future when determining possible negative effects of prolonged-acting glucocorticoids.

CONCLUSIONS

The reported PK parameters for BET, BA, and BP suggested that the BA ester, and not the BP ester, of BET acts as a prodrug or reservoir for BET, conferring on it the sustained- and extended-release characteristics.

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Both authors contributed equally to the conduct of the study and creation of the manuscript.
CONFLICTS OF INTEREST
The authors have indicated that they have no conflicts of interest regarding the content of this article.

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Address correspondence to: Isam I. Salem, PhD, International Pharmaceutical Research Center (IPRC), Sports City Circle, PO Box 963166, Amman 11196, JORDAN. E-mail: dr.salem@iprc.com.jo