OBSTETRICS

A pharmacokinetic assessment of optimal dosing, preparation, and chronotherapy of aspirin in pregnancy

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BACKGROUND: The benefit of aspirin in preventing preeclampsia is well established; however, studies over the years have demonstrated variability in outcomes with its use. Potential contributing factors to this variation in efficacy include dosing, time of dosing, and preparation of aspirin.

OBJECTIVE: We aimed to compare the difference in pharmacokinetics of aspirin, through its major active metabolite, salicylic acid, in pregnant women and nonpregnant women, and to examine the effect of dose (100 mg vs 150 mg), preparation (enteric coated vs non−enteric-coated), and chronotherapy of aspirin (morning vs evening) between the 2 groups.

MATERIALS AND METHODS: Twelve high-risk pregnant women and 3 nonpregnant women were enrolled in this study. Pregnant women were in 1 of 4 groups (100 mg enteric coated, 100 mg non−enteric-coated, 150 mg non−enteric-coated morning dosing, and 150 mg non−enteric-coated evening dosing), whereas nonpregnant women undertook each of the 4 dosing schedules with at least a 30-day washout period. Blood samples were collected at baseline (before ingestion) and at 1, 2, 4, 6, 12, and 24 hours after ingestion of aspirin. Plasma obtained was analyzed for salicylic acid levels by means of liquid chromatography−mass spectrometry. Pharmacokinetic values of area under the curve from time point 0 to 24 hours point of maximum concentration, time of maximum concentration, volume of distribution, clearance, and elimination half-life were analyzed for statistical significance with SPSS v25 software.

RESULTS: Pregnant women had a 40% ± 4% reduction in area under the curve from time point 0 to 24 hours ($P < .01$) and 29% ± 3% reduction in point of maximum concentration ($P < .01$) with a 44% ± 8% increase in clearance ($P < .01$) in comparison to that in nonpregnant women when 100 mg aspirin was administered. The reduction in the area under the curve from time point 0 to 24 hours, however, was minimized with the use of 150 mg aspirin in pregnant women, with which the area under the curve from time point 0 to 24 hours was closer to that achieved with the use of 100 mg aspirin in nonpregnant women. There was a 4-hour delay ($P < .01$) in the time of maximum concentration, a 47% ± 3% reduction in point of maximum concentration ($P < .01$) and a 48% ± 1% increase in volume of distribution ($P < .01$) with the use of 100 mg enteric-coated aspirin compared to non−enteric-coated aspirin, with no difference in the overall area under the curve. There was no difference in the pharmacokinetics of aspirin between morning and evening dosing.

CONCLUSION: There is a reduction in the total drug metabolite concentration of aspirin in pregnancy, and therefore a dose adjustment is potentially required in pregnant women. This is likely due to the altered pharmacokinetics of aspirin in pregnancy, with an increase in clearance. There was no difference in the total drug metabolite concentration of aspirin between enteric-coated and non−enteric-coated aspirin and between morning and evening dosing of aspirin. Further pharmacodynamic and clinical studies are required to examine the clinical relevance of these pharmacokinetic findings.

Key words: aspirin, dose, pharmacokinetics, preeclampsia, pregnancy

The pharmacokinetics of medications in pregnancy is influenced by the maternal physiological changes that occurs through all three trimesters of pregnancy. These changes lead to an alteration in the absorption, distribution, and elimination of commonly used medications in pregnancy.[1,2] However, most pharmacokinetic studies of medications commonly used in pregnancy, such as aspirin, have been conducted in healthy males.[3,4]

The prophylactic use of aspirin to prevent preeclampsia has been studied over the last 40 years, but results are contradictory because of unanswered questions relating to its optimal application. The varying risk reduction of between 10% and 60% observed in previous studies has been largely attributed to the heterogeneity of studies for dosing, timing of ingestion, gestation at initiation of therapy, and type of aspirin preparation.[5−7]

Initial studies that demonstrated a prophylactic benefit of aspirin prescribed a daily dose of 150−300 mg.[8] Subsequent studies, however, argued for the use of low-dose therapy ranging from 75 to 150 mg daily,[5,9] whereas more recent studies suggest better clinical outcomes with the use of a 150-mg dose.[10,11] Current guidelines do not specify a recommended dose of aspirin but suggest a range of 75−150 mg, with 100 mg being the most commonly suggested dose.[12,13] The use of 150 mg in recent studies is likely to have an impact on clinical guidelines in the future[14]; however, there remains a significant paucity in clinical and pharmacokinetic data that directly compares the use of 100 mg to 150 mg aspirin in pregnant women to support such a change in clinical practice. It therefore remains unclear whether the use of 150 mg daily results in better bioavailability of aspirin and consequently better clinical outcomes.

Pharmacology studies comparing varying preparations of aspirin in

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AJOG at a Glance

Why was this study conducted?

To address the lack of data and understanding on the pharmacokinetics of aspirin in pregnancy and its impact on the variable outcomes observed with its prophylactic role in preventing preeclampsia.

Key findings

The total drug exposure of aspirin is reduced in pregnancy compared to that in nonpregnant women and suggests the need for dose adjustment in pregnancy.

What does this add to what is known?

The physiological changes in pregnancy alters the pharmacokinetic of medication in pregnancy. Aspirin in now commonly used in high-risk pregnant women, and this finding will provide more insight into the potential need for dose adjustment in pregnancy.

healthy male and female volunteers have demonstrated better platelet inhibition activity with non−enteric-coated aspirin (non-EC) compared to enteric-coated aspirin (EC), which is often used for gastrointestinal protection.[15] Bhatt et al demonstrated a lack of platelet inhibition activity with EC aspirin in patients with diabetes, whereas others have demonstrated a lack of difference in platelet inhibition activity between the 2 preparations in healthy volunteers.[16,17] Once again, the pharmacokinetics of the various preparations of aspirin in pregnant women have not been examined, and the influence on obstetric clinical outcomes remains unknown.

Another area of growing interest is the chronotherapy of daily aspirin. Recent studies demonstrated that ingestion of aspirin at bedtime results in better ambulatory blood pressure control and reduced incidence of hypertensive disorders of pregnancy among high-risk women.[18] However, the mechanism of this effect is not understood, and the current recommendation on this remains unclear.

Based on the current gaps in the literature and clinical practice, we aimed to compare the pharmacokinetics of aspirin in pregnant vs nonpregnant women, and to examine the effect of dose (100 mg vs 150 mg), preparation (EC vs non-EC), and chronotherapy of aspirin (morning vs evening dosing) between the 2 groups.

Materials and Methods

Sample collection

Twelve pregnant women from high-risk pregnancy clinics within the South Western Sydney Local Health District (SWSLHD), NSW, Australia, gave written informed consent to participate in this study. Women were in 1 of 4 groups (100 mg EC, 100 mg non-EC, 150 mg non-EC morning dosing, and 150 mg non-EC evening dosing). Three nonpregnant women undertook each of the 4 dosing schedules with at least a 30-day washout period (Figure 1). Baseline clinical characteristics of the participants included age, ethnicity, body mass index (BMI), weight, gestation at time of study, and smoking status (Table 1). The type of aspirin consumed by healthy nonpregnant women was standardized. The aspirin consumed by pregnant participants was unaltered from their prescribed aspirin. At the time of publication, 150 mg EC aspirin was not commercially available for clinical use in Australia. Patients who were prescribed 150 mg aspirin by their clinicians were therefore advised to use half a tablet of 300 mg non-EC aspirin or one-and-a-half tablets of 100 mg non-EC aspirin. The pregnant and nonpregnant women in our study used half a tablet of 300 mg non-EC aspirin. Morning dosing of aspirin was set at 8 am ± 0.5 hour, and evening dosing was set at 2000 ± 1 hour. Ingestion and time of ingestion of aspirin were witnessed and verified by the attending investigator. Participants in this study took their aspirin right after consuming food. Women with underlying renal and liver dysfunction were excluded from this study.

Blood samples (4 mL) were collected via a 23G BD Vacutainer Push Button needle (Becton Dickinson, Franklin Lakes, NJ) at baseline (before ingestion) and 1, 2, 4, 6, 12, and 24 hours after ingestion of aspirin. Blood samples were collected into VACUETTE® K2EDTA tubes (Greiner Bio-One International) and were centrifuged immediately at 3000 rpm for 10 minutes. Plasma was then aliquoted and stored at −80°C until analysis.

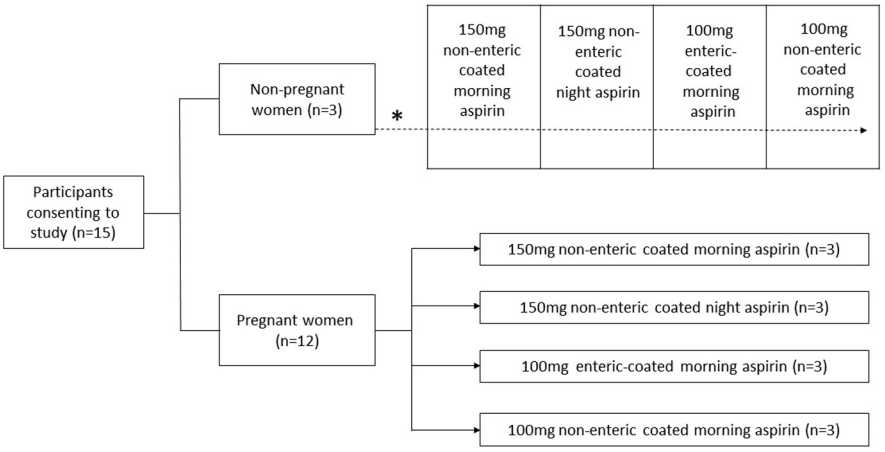
Ethics approval for this study was obtained from the SWSLDH ethics committee (HE 16/184).

Sample preparation for liquid chromatography−mass spectrometry analysis

Standards were prepared using 100 μL blank human plasma with known concentrations of salicylic acid (SA) (Sigma Aldrich, Castle Hill, NSW, Australia). Salicylic acid was dissolved and diluted with 100% methanol before spiking blank plasma with known concentrations of 0 ng/mL, 10 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL, and 500 ng/mL. The standards were then spiked with a fixed concentration of 125 ng deuterated salicylic acid (D4-SA) (Santa Cruz Biotechnology, Santa Cruz, CA) as an internal control and vortexed for 1 minute. Similarly, 100 μL of plasma at each time point was transferred into Eppendorf tubes and spiked with a fixed concentration of 125 ng D4-SA as internal control.

To precipitate protein, 400 μL of 100% of acetonitrile (ACN) (Lichrosolv; Merck Milipore, Baywater, VIC, Australia) was added to standards and samples and vortexed for 30 seconds. Samples and standards were then centrifuged at 16,168 *g* for 10 minutes (Eppendorf Microcentrifuge Model 5415R, Hamburg Germany), after which the supernatant was transferred into glass culture tubes (12 × 75-mm disposable culture tubes) and evaporated at 45°C using an Eppendorf Concentrator (Model 22331) for

FIGURE 1
Patient distribution. A total of 12 pregnant volunteers were subdivided into 4 groups as above



*Three nonpregnant women were used for all 4 subgroups in a crossover pattern with a washout period of at least 30 days between varying aspirin exposures.

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approximately 90 minutes. Samples and standards were then reconstituted and acidified with 1 mL of 0.1% formic acid (FA) (Lichrosolv; Merck Milipore, Baywater, VIC, Australia).

Samples and standards underwent solid phase extraction using Discovery DSC-18 1 mL solid phase extraction cartridges (Supelco, Bellefonte, PA) with a vacuum manifold. Cartridges were preconditioned with 1 mL of 100% methanol, then washed with 2 mL of 0.1% trifluoroacetic acid (TFA) (Lichrosolv; Merck Milipore, Baywater VIC, Australia). The sample and standards were applied to the column and then washed with 1 mL of water, after which they were eluted with 1 mL of 100% methanol. The methanol eluate was then evaporated at 45°C for 90 minutes. Samples were then reconstituted with 100μL of 0.1% FA and spun at 1.4×10^4 RPM for 5 minutes before transfer into high-performance liquid chromatography (HPLC) vial inserts.

Liquid chromatography—mass spectrometry methodology

Analysis was performed on an Agilent 1290 series UHPLC system coupled with 6460A triple quadrupole mass spectrometers (Agilent Technologies, Santa Clara, CA). The separation of SA in plasma was achieved by using an Agilent Zorbax Eclipse XDB-C18 (4.6 × 50 mm, 1.8 μm) column fitted with a UHPLC Zorbax Eclipse XDB-C18 (4.6 × 5 mm, 1.8 μm) guard column, with mobile phase A containing 0.1% formic acid in water and mobile phase B consisting of 0.1% formic acid in 90% acetonitrile in water. The injection volume was 5 μL with a total run of 7 minutes at flow rate of 0.5 mL/min. The gradient was started at 30% of B and increased to 90% at 2 minutes and was then maintained at 90% for another 2 minutes. It then increased to 100% in the next 0.5 minute, maintained at 100% for another minute, decreased to 30% by 6 minutes, and re-equilibrated for another minute at 30% before the next injection. Tandem mass spectrometry was performed using electrospray ionization equipped with jet stream technology in the negative mode using the following parameters: capillary spray voltage was held at 3500 V, drying gas flow of 10 L/min with temperature set at 325°C and nebulizer pressure at 45 psi. The optimal fragmentor voltage (90 V) and collision energy voltage (15 V) was obtained by flow injection analysis in MS2-product ion scan mode. The following MRM ion transitions were monitored: 136.9 ->93.10 (SA) and 141.00 -> 97.00 (D4-SA). Peak areas for SA relative to the internal standard D4-SA was used to interpolate a standard curve and then to calculate the SA present in standard and sample.

Data analysis

Data acquisition was performed using MassHunter B.07.01, and data analysis was conducted using MassHunter qualitative and quantitative software (version B.07.00; Agilent Technologies). The pharmacokinetic parameters of maximum concentration (C_{max}), time point of maximum concentration (T_{max}), area under the curve from time point 0 to 24 hours [$AUC_{(t\text{-}24)}$], volume of distribution (Vd), clearance (CL), and elimination half-life ($t_{½}$) were determined through a 2-compartmental analysis using PKSolver.[19] One-way analysis of variance (post hoc testing with Tukey test), 4-way analysis of variance (post hoc testing with Tukey test), and *t* tests were used for analysis of mean values with SPSS v25 software.

Results

The characteristics of the participants are described in Table 1. There was no statistically significant difference in the clinically relevant characteristic between the participants. This included age, weight, BMI, and gestation in pregnant women. None of the participants were smokers, and none were on a proton pump inhibitor or histamine H2 receptor antagonist.

Effect of varying dose of aspirin

The 150-mg non-EC group had a 40% ± 6% higher $AUC_{(t\text{-}24)}$ ($P = .01$) and 31% ± 2% higher C_{max} ($P = .02$) compared to 100-mg non-EC group for both pregnant and nonpregnant women (Figure 2 and Table 2). There was no difference in the $t_{½}$ and T_{max} in pregnant and nonpregnant women regardless of dosage. However, the mean $AUC_{(t\text{-}24)}$ was 41% ± 2% and 34% ± 4% lower in pregnant women in both the 100-mg non-EC and 150-mg non-EC aspirin groups, respectively ($P < .01$), with a 43% ± 8% increase in CL, in comparison to that in nonpregnant women. Similarly, the C_{max} was 25% ± 2% and

TABLE 1
Participant characteristics

	100 mg enteric-coated aspirin		100 mg non–enteric-coated aspirin		150 mg non–enteric-coated aspirin, morning dosing		150 mg non–enteric-coated aspirin, evening dosing	
	Pregnant women (n = 3)	Nonpregnant women (n = 3)[a]	Pregnant women (n = 3)	Nonpregnant women (n = 3)[a]	Pregnant women (n = 3)	Nonpregnant women (n = 3)[a]	Pregnant women (n = 3)	Nonpregnant women (n = 3)[a]
Age (mean)	32.2 ± 2	37.4 ± 5	31.4 ± 1	37.4 ± 5	31.8 ± 3	37.4 ± 5	33.2 ± 2	37.4 ± 5
Weight (kg) (mean)	71.8 ± 5	75.6 ± 7	72.9 ± 4	75.6 ± 7	70.8 ± 9	75.6 ± 7	73.9 ± 5	75.6 ± 7
BMI (mean)	25.7 ± 2	26.9 ± 2	26.4 ± 1	26.9 ± 2	26.1 ± 2	26.9 ± 2	26.8 ± 2	26.9 ± 2
Gestation (wk) (Mean)	24.6 ± 1	N/A	26.1 ± 1	N/A	25.2 ± 1	N/A	26.3 ± 1	N/A
Pre-existing comorbidities	Hypertension (2) Prior preeclampsia (2) SLE (1)	N/A	Hypertension (3) Type 2 DM (1) Prior preeclampsia (2)	N/A	Hypertension (2) SLE (1) Prior preeclampsia (3)	N/A	Hypertension (2) Prior preeclampsia (2) Type1 DM (1)	N/A
Ethnicity	White (2) South East Asian (1)	Mediterranean (2) South Asian (1)	White (1) South Asian (1) Middle Eastern (1)	Mediterranean (2) South Asian (1)	White (2) South Asian (1)	Mediterranean (2) South Asian (1)	White (1) South East Asian (1) African (1)	Mediterranean (2) South Asian (1)
Smoking status	Nonsmoker	Nonsmoker	Nonsmoker	Nonsmoker	Nonsmoker	Nonsmoker	Nonsmoker	Nonsmoker

Data are mean ± standard deviation. Numbers in parentheses are numbers of women.
BMI, body mass index; *DM*, diabetes mellitus; *N/A*, not applicable; *SLE*, systemic lupus erythematosus.
[a] Nonpregnant female participants were examined in a crossover pattern with a washout period of at least 30 days between aspirin groups.
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31% ± 3% lower in pregnant women in both the 100-mg non-EC and 150-mg non-EC aspirin groups, respectively ($P < .01$).

When comparing 150 mg in pregnancy against a regular dose of aspirin in nonpregnant women (100 mg), the difference in the pharmacokinetics between groups was minimal, with a difference of 5% in the $AUC_{(t\text{-}24)}$ and 2.3% in the C_{max}. This suggests that a higher dose of aspirin (150 mg) minimizes the pregnancy-related reduction in total drug exposure of 100 mg aspirin (Figure 2 and Table 2).

Effect of varying preparation of aspirin

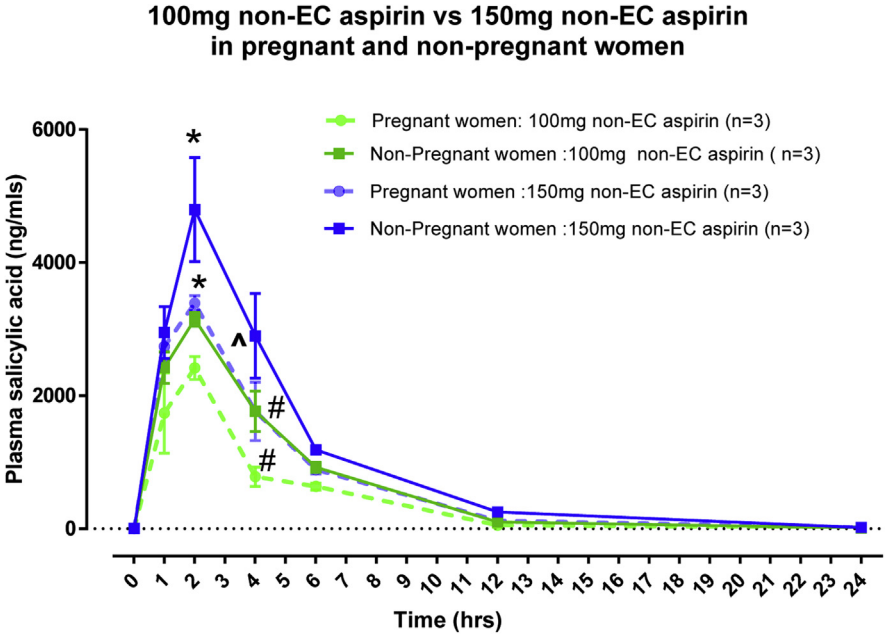
There was no difference in the $AUC_{t\text{-}24}$ and $t_{½}$ between the 100-mg EC and non-EC dosing. There was, however, a 4-hour delay ($P < .01$) in the T_{max} and a 47% ± 2% reduction in C_{max} ($P < .01$) as well as a 48% ± 1% increase in Vd ($P < .01$) with the use of 100 mg EC aspirin compared to non-EC aspirin (Figure 3 and Table 3).

Once again, pregnant women had a 43% ± 2% lower $AUC_{t\text{-}24}$ ($P = .03$) and a 27% ± 1% lower C_{max} ($P = .01$) respectively, with a 44% ± 1% increase in CL ($P < .01$) in both the EC and non-EC preparations of 100 mg aspirin compared to that in nonpregnant women (Figure 3 and Table 3).

Effect of chronotherapy of aspirin

In examining the influence of chronotherapy on the pharmacokinetics of aspirin, we compared morning (8 am ± 0.5 hour) and evening (8 pm ± 1 hour) dosing of 150 mg aspirin in pregnant and nonpregnant women. There was no difference in the $AUC_{(t\text{-}24)}$, C_{max}, T_{max}, and $t_{½}$ half between the 2 dosing regimens, indicating a lack of influence of chronotherapy on the pharmacokinetics of aspirin (Figure 4 and Table 4). We once again found that pregnant women had a 35% ± 4% lower $AUC_{(t\text{-}24)}$ ($P = .01$) and 24% ±1% lower C_{max} ($P = .02$) with a 46% ± 4% increase in CL compared to that in nonpregnant women in both morning and evening dosing of 150 mg non-EC aspirin groups (Figure 4 and Table 4).

FIGURE 2
Pharmacokinetics of 100 mg non−enteric-coated (non-EC) aspirin (green) compared to 150 mg non-EC aspirin (blue) in pregnant (dotted lines) and nonpregnant women (solid lines)



*Area under the curve from time point 0 to 24 hours [$AUC_{(t-24)}$] of 150 mg non-EC aspirin is 42% lower in pregnant women compared to nonpregnant women ($P < .01$). #Area under the curve of 100 mg non-EC aspirin is 38.6% lower in pregnant women compared to nonpregnant women ($P < .01$). ^Use of 150 mg aspirin in pregnancy minimizes the pregnancy-related reduction in the area under the curve (AUC) and the point of maximum concentration (C_{max}) with the use of 100 mg aspirin in pregnancy.

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A 4-way analysis of variance examining for multifactor relationship did not demonstrate a statistically significant 4-way interaction among dose, chronotherapy, pregnancy, and preparation ($F_{3,16} = 0.773$, $P = .5$).

Comment

Our study demonstrated a consistent reduction in total drug metabolite concentration of aspirin (measured through SA) in pregnancy, with an average 40% ± 4% reduction in $AUC_{(t-24)}$ and 29% ± 3% reduction in C_{max} with a 44% ± 8% increase in CL in comparison to that in nonpregnant women when 100 mg aspirin was administered. The reduced AUC with the use of 100 mg, however, was minimized with the use of 150 mg aspirin in pregnant women. Use of the 150-mg dose in pregnant women achieved total drug metabolite concentration similar to that from 100 mg aspirin in nonpregnant women. This therefore suggests that the physiological changes, such as increase in clearance, that occur in pregnancy highlight the potential need for aspirin dose adjustment in pregnancy. The only pharmacokinetic study of aspirin in pregnancy to date was conducted in 1994 by Rymark et al, who demonstrated that 75 mg aspirin in pregnant women in the second and third trimesters had a lower SA plasma peak

TABLE 2
Comparison of mean pharmacokinetic values between pregnant and nonpregnant women with both doses of non−enteric-coated (non-EC) aspirin

	100 mg Non-EC aspirin			150 mg Non-EC aspirin			
	Pregnant women (n=3)	Nonpregnant women (n=3)	*P* value[a]	Pregnant women (n=3)	Nonpregnant women (n=3)	*P* value[a]	*P* value[b]
$AUC_{(t-24)}$ (ng/mL*h)	12187.3 ± 100	21096.6 ± 532	<.01	19993.3 ± 240	29288.3 ± 430	<.01	.01
Tmax (h)	2	2	NS	2	2	NS	NS
C_{max} (ng/mL)	2417.2 ± 71	3214.7 ± 98	<.01	3289.3 ± 117	4797.5 ± 182	<.01	.02
t½ (h)	2.5 ± 0.3	2.7 ± 0.2	NS	2.6 ± 0.2	2.6 ± 0.4	NS	NS
Vd mg/(ng/mL)	0.08 ± 0.005	0.06 ± 0.005	NS	0.09 ± 0.002	0.07 ± 0.003	NS	NS
CL mg/(ng/mL)/h	0.006 ± 0.001	0.003 ± 0.001	<.01	0.009 ± 0.001	0.005 ± 0.001	<.01	NS

Data are mean ± standard deviation.
$AUC_{(t-24)}$, area under the curve from time point 0 to 24 hours; *CL*, clearance; *C_{max}*, point of maximum concentration; *NS*, not significant; *T_{max}*, time of maximum concentration; *t½*, elimination half-life; *Vd*, volume of distribution.

[a] *P* value represents the comparison between pregnant and nonpregnant women in the 100 mg non-EC aspirin group and the 150 mg non-EC aspirin groups, respectively; [b] *P* value represents the comparison between 100 mg non-EC aspirin and 150 mg non-EC aspirin.

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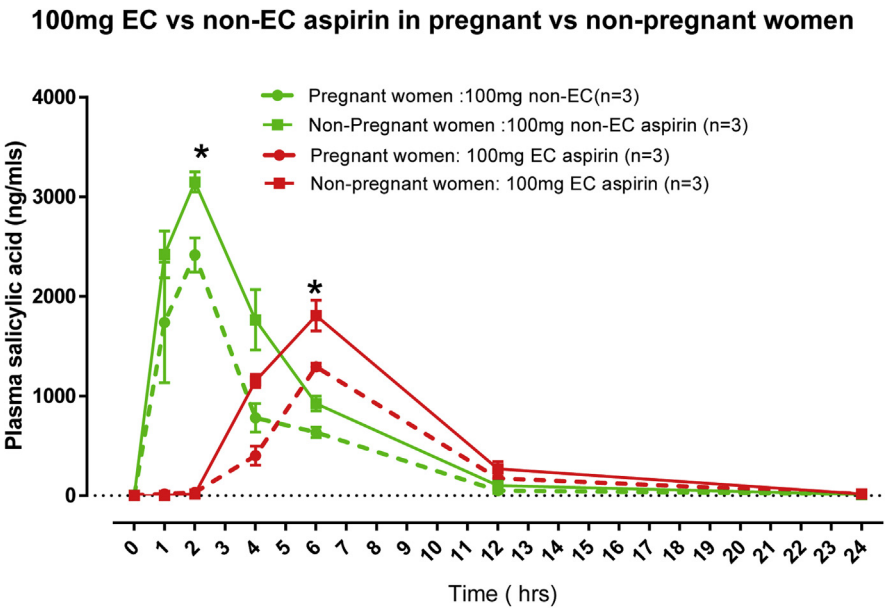
FIGURE 3
Pharmacokinetics of 100 mg enteric-coated (EC) aspirin (red) and 100 mg non—enteric-coated (non-EC) aspirin (green) in both pregnant (dotted lines) and nonpregnant (solid lines) women



*EC aspirin demonstrated a 4-hour delay in time of maximum concentration (T_{max}) ($P < .01$) and a 47% reduction in point of maximum concentration (C_{max}) ($P < .01$) compared to non-EC aspirin, with no difference in area under the curve from time point 0 to 24 hours [$AUC_{(t\text{-}24)}$] ($P =$ not significant).

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compared to that in nonpregnant women, with no intertrimester variation.[1] Despite the widespread use of aspirin in pregnancy, its altered pharmacokinetics in pregnancy and the impact of this on optimal dosing and clinical outcomes have not been adequately examined and remain unclear. This evidence suggests that we should stop extrapolating pharmacokinetic data from nonpregnant women and should undertake the relevant studies in pregnant women.

Aspirin is absorbed rapidly from the stomach and intestine by passive diffusion and is rapidly hydrolyzed, with a short half-life of 15 minutes, into salicylic acid.[3,4] Salicylic acid is responsible for aspirin's anti-inflammatory effects.[20] With repeated dosing, salicylic acid has been found to be the principal substance in plasma, with a half-life of 2 hours.[21] For these reasons, we chose to examine the pharmacokinetics of aspirin through detection of its hydrolyzed product, salicylic acid, as did Rymark et al. About 50—70% of salicylic acid in the blood is bound to albumin, whereas the rest remains in the active, ionized state.[3,20] The protein binding of salicylic acid is concentration dependent, and therefore saturation of binding sites leads to more free salicylic acid and eventually to toxicity.[20] The volume of distribution (Vd) of salicylic acid is low, at 0.1—0.2 L/kg, indicating that the majority of salicylic acid is confined to the plasma.[4] This is consistent with what we demonstrate in this study. As for its metabolism, 80% of salicylic acid is metabolized in the liver through conjugation with glycine to form salicyluric acid and with glucuronic acid to form 2 different glucuronide esters.[20]

TABLE 3
Comparison of mean pharmacokinetic values between pregnant and nonpregnant women in enteric-coated (EC) and non—enteric-coated (non-EC) 100-mg aspirin

	100 mg Non-EC aspirin			100 mg EC aspirin			
	Pregnant women (n = 3)	Nonpregnant women (n = 3)	*P* value[a]	Pregnant women (n = 3)	Nonpregnant women (n = 3)	*P* value[a]	*P* value[b]
$AUC_{t\text{-}24}$ (ng/mL*h)	12187.3 ± 100	21096.6 ± 532	.03	11927.3 ± 104	22007.3 ± 118	.03	NS
Tmax (h)	2	2	NS	6	6	NS	<.01
Cmax (ng/mL)	2417.2 ± 71	3214.7 ± 98	.01	1275 ± 97	1808.3 ± 154	.01	<.01
t½ (h)	2.5 ± 0.3	2.7 ± 0.2	NS	2.6 ± 0.1	2.5 ± 0.3	NS	NS
Vd mg/(ng/mL)	0.08 ± 0.005	0.06 ± 0.005	NS	0.16 ± 0.01	0.13 ± 0.01	NS	<.01
CL mg/(ng/mL)/h	0.006 ± 0.001	0.003 ± 0.001	<.01	0.007 ± 0.001	0.004 ± 0.001	<.01	NS

Data are mean ± standard deviation.
$AUC_{(t\text{-}24)}$, area under the curve from time point 0 to 24 hours; *CL*, clearance; C_{max}, point of maximum concentration; *NS*, not significant; T_{max}, time of maximum concentration; t½, elimination half-life; *Vd*, volume of distribution.

[a] *P* value represents the comparison between pregnant and nonpregnant women in the 100-mg enteric-coated (EC) aspirin group and the non—enteric-coated (non-EC) group, respectively; [b] *P* value represents the comparison between 100 mg EC and non-EC aspirin.

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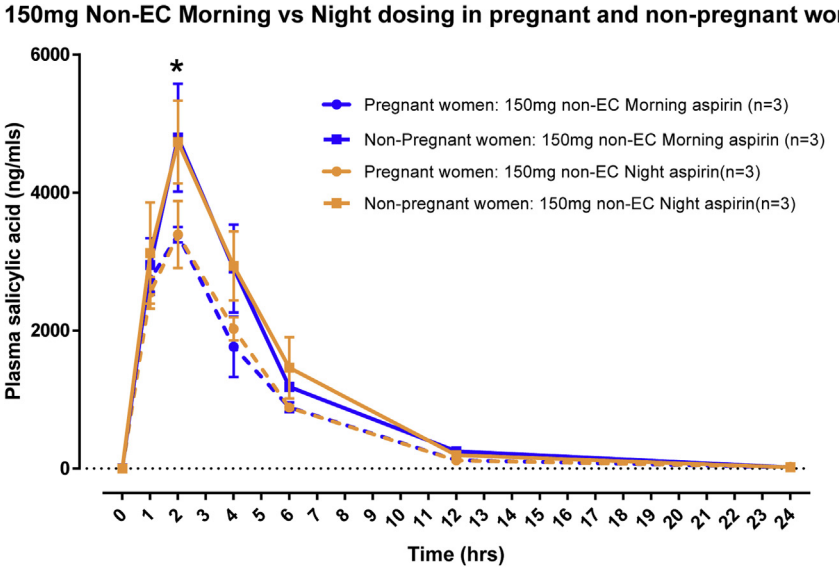
FIGURE 4
Pharmacokinetics of morning (blue) and evening (purple) dosing of 150 mg non—enteric coated (non-EC) aspirin in pregnant (dotted line) and nonpregnant (solid line) women



*There was no difference in the pharmacokinetics of morning and evening dosing of 150 mg aspirin.

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Salicylic acid is excreted mainly by the kidneys as salicyluric acid (75%), free salicylic acid (10%), salicylic phenol (10%), and acyl glucuronides (5%).[3,4]

In pregnancy, there is a progesterone-driven delay in gastrointestinal motility which results in a 30% increase in gastric emptying time and an approximately 40% reduction in gastric acidity.[22,23] These changes can result in ionization of weak acids such as aspirin and could potentially affect its absorption. Similarly, there have been arguments that the use of enteric-coated aspirin can also reduce its absorption.[15] Evidence to validate this assumption, however, has been contradictory. Our assessment on the influence of enteric coating demonstrated an expected delay in T_{max} with reduced C_{max} and an increase in Vd with no overall difference in $AUC_{(t\text{-}24)}$ between EC and non-EC aspirin. However, the influence of EC aspirin on the pharmacodynamic of aspirin in pregnancy remains unknown and is subject to further research.

A stronger influence on the alteration of pharmacokinetics in pregnancy is the variation in drug distribution and elimination. The physiological plasma expansion of 50% in pregnancy often results in altered drug volume of distribution (Vd) and is most prominent toward the end of the first trimester.[24,25] The total mean increase in the maternal body volume is approximately 8 L, and the resulting volume expansion results in a decrease in peak serum concentration (C_{max}), particularly of hydrophilic agents such as salicylate acid. The increased circulating estrogen and progesterone in pregnancy can either increase or decrease hepatic metabolism of drugs based on its stimulatory or inhibitory influence on the cytochrome P450 isoenzymes and uridine 5'-diphosphoglucuronosyltransferase isoenzyme activity.[24,26,27] Glucuronidation activity may be a critical determinant of aspirin efficacy; however, the enzymes responsible for this conjugation have yet to be

TABLE 4
Comparison of mean pharmacokinetic values between pregnant and nonpregnant women in both morning and evening dosing of 150 mg non—enteric-coated (non-EC) aspirin

	150 mg Non-EC aspirin, morning dosing			150 mg Non-EC aspirin, evening dosing			
	Pregnant women (n = 3)	Nonpregnant women (n = 3)	*P* value[a]	Pregnant women (n = 3)	Nonpregnant women (n = 3)	*P* value[a]	*P* value[b]
AUC $_{t\text{-}24}$ (ng/mL*h)	19993.3 ± 240	29288.3 ± 430	.01	21417.7 ± 428	30048.2 ± 296	.01	NS
Tmax (h)	2	2	NS	2	2	NS	NS
Cmax (ng/mL)	3289.3 ± 117	4797.5 ± 182	.02	3439.6 ± 256	4276.5 ± 238	.02	NS
t½ (h)	2.6 ± 0.2	2.6 ± 0.4	NS	2.8 ± 0.5	2.5 ± 0.5	NS	NS
Vd mg/(ng/mL)	0.09 ± 0.002	0.07 ± 0.003	NS	0.08 ± 0.01	0.07 ± 0.01	NS	NS
CL mg/(ng/mL)/h	0.009 ± 0.001	0.005 ± 0.001	<.01	0.008 ± 0.001	0.003 ± 0.001	<.01	NS

Data are mean ± standard deviation. *$AUC_{(t\text{-}24)}$*, area under the curve from time point 0 to 24 hours; *CL*, clearance; *C_{max}*, point of maximum concentration; *NS*, not significant; *T_{max}*, time of maximum concentration; *t½*, elimination half-life; *Vd*, volume of distribution.

[a] *P* value represents the comparison between pregnant and nonpregnant women in the morning and evening dosing groups, respectively; [b] *P* value represents the comparison between morning and evening dosing of aspirin.

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identified and characterized.[28] Therefore, the influence of pregnancy on the hepatic metabolism of salicylic acid remains subject to further research.

The relationship between increased creatinine clearance and drug excretion in pregnancy is well recognized and has been demonstrated through various studies, including ours, which demonstrated a 45% increase in clearance of SA.[24,29,30] Renal drug excretion is dependent on the glomerular filtration rate (GFR), tubular secretion, and resorption. In pregnancy, GFR is increased by 50% from the first trimester and continues to increase until the last week of pregnancy.[24,31] Therefore, the clearance of drugs and metabolites that are excreted renally, such as salicyluric acid and salicylic acid, is thought to parallel the change in GFR in pregnancy, leading to lower C_{max} and $AUC_{(t\text{-}24)}$ as demonstrated in our study.[32] Plasma protein binding of drugs is known to reduce in pregnancy because of reduced albumin concentration and estrogen-induced reduced protein-binding of drugs.[29,33] Decreased protein binding increases the concentration of free drug and consequently increases the clearance of drugs and metabolites such as salicylates via the increased renal clearance in pregnancy. The net effect of reduced protein binding, however, is counterbalanced by the increased renal clearance of drugs and metabolites such as salicylates.

Although our study did demonstrate a reduced total drug metabolite concentration of aspirin in pregnancy with a potential need for dose adjustment in pregnancy, the clinical relevance of this will need to be examined through a randomized clinical study in pregnancy that directly compares the clinical outcomes with the use of 100 mg and 150 mg aspirin. In addition, more data on the safety of 150 mg aspirin in pregnancy is required. Our study did not demonstrate a difference in the pharmacokinetics between EC and non-EC aspirin in the pregnant and nonpregnant states. Similarly, we did not demonstrate a difference in the pharmacokinetics of aspirin in relation to chronotherapy. The current thought on the mechanism of aspirin in the prevention of preeclampsia focuses on 2 pathways that potentially influence placentation. This involves aspirin's influence on platelet aggregation (through the cyclooxygenase [COX]−1 pathway) and its immunomodulatory effect through the COX-2 pathway.[34,35] Given this, in translating our pharmacokinetic findings to clinical studies, there is a need to further examine a dose-dependent response to platelet aggregation and the immune pathway of preeclampsia. In addition to this, there is a need to examine its overall effect on the pertinent end-point—namely, the clinical outcomes of these women and their infants.

Our study has a few limitations. The study excluded pregnant women with renal disease. Given that salicylate acid is renally excreted, women with renal disease can potentially have higher drug exposure. Although this is beyond the scope of our study, the pharmacokinetics of aspirin in women with renal disease and the optimal dose in these women will require further exploration. Another limitation to our study is the small number of participants. Although our post hoc power analysis was close to 100% to address the main aim of this study, the sample size limited our ability to examine for further confounders such as intergestational variation of pharmacokinetics and ingestion of aspirin in relation to intake of food. Although Rymark et al did not demonstrate an intergestational variation in the pharmacokinetics of aspirin in the second and third trimesters,[1] the potential difference in the first trimester remains unknown and will be worth exploring in future pharmacokinetic studies. Similarly, the optimal timing of aspirin ingestion in relation to food intake remains unclear, with a recent systematic review demonstrating a lack of difference in overall bioavailability.[36] This, again, is worth exploring in future pharmacokinetic studies.

In conclusion, the physiological changes that occur in pregnancy alter the pharmacokinetics of aspirin in pregnancy, suggesting the need for aspirin dose adjustment in pregnancy. ■

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