ANESTHESIOLOGY

Influence of St. John's Wort on Intravenous Fentanyl Pharmacokinetics, Pharmacodynamics, and Clinical Effects

A Randomized Clinical Trial

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- The popular herbal medicine, St. John's wort, is a potent inducer of several cytochrome P450 enzymes, including CYP3A4, which plays a role in the metabolism of fentanyl. St. John's wort may also influence the expression of P-glycoprotein, which can alter the movement of drugs across the blood–brain barrier.

What This Article Tells Us That Is New

- The pharmacokinetics and pharmacodynamics (pupillary diameter over time) were examined in volunteers before and after treatment with St. John's wort. No differences were seen, suggesting that this herbal medication does not influence the clinical behavior of fentanyl.

ABSTRACT

Background: Patients often use complementary and alternative herbal medicines, hence, potential exists for adverse herb–drug interactions. Fentanyl is metabolized by hepatic CYP3A4 and considered transported by blood–brain barrier P-glycoprotein. Both disposition processes could be upregulated by the herbal St. John's wort. This investigation evaluated effects of St. John's wort on fixed-dose and apparent steady-state IV fentanyl pharmacokinetics, pharmacodynamics, and clinical effects.

Methods: Healthy volunteers received a fentanyl fixed-dose infusion and an individually tailored target controlled infusion on separate days, before and after 30-day St. John's wort (300 mg thrice daily; n = 8) or placebo control (n = 8) in a randomized parallel-group design. Fentanyl plasma concentrations, pupil diameter, analgesic response to experimental pain (cold pressor), subjective side effects, and cognitive effects were measured. Plasma fentanyl concentrations and changes in pupil diameter were subjected to pharmacokinetic–pharmacodynamic modeling.

Results: St. John's wort did not alter fentanyl pharmacokinetics. Clearance (l/min) before and after St. John's wort (1.13 ± 0.29 and 1.24 ± 0.26, respectively) or placebo (0.96 ± 0.28 and 1.12 ± 0.27, respectively) were not different. St. John's wort also did not affect fentanyl pharmacodynamics as measured by pupil constriction after fixed-dose and tailored fentanyl infusions. EC_{50} (ng/ml) was 1.1 ± 0.7 and 1.4 ± 0.9 before and after St. John's wort *versus* 1.2 ± 0.8 and 1.4 ± 1.7 before and after placebo. Effect site equilibration time, $T_{1/2,ke0}$ (min), was 12.8 ± 5.3 and 11.3 ± 6.4 before and after St. John's wort *versus* 11.4 ± 6.4 and 11.1 ± 5.6 before and after placebo. St. John's wort had no influence on analgesia, cognitive performance, or somatic cognitive–affective effects of fentanyl.

Conclusions: St. John's wort did not alter fentanyl pharmacokinetics, pharmacodynamics or clinical effects, suggesting no effect on hepatic clearance or blood-brain barrier efflux. Patients taking St. John's wort will likely not respond differently to IV fentanyl for anesthesia or analgesia.

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Surveys have found that 22 to 32% of surgical patients report using herbal supplements.[1,2] In addition, patients are often reluctant to disclose the use of herbal medications to their healthcare providers.[2,3] One study found that as many as 70% of patients taking herbal supplements were not forthcoming about their use during preoperative evaluation.[2] Presurgical use of herbal therapy, together with multiple drug use during anesthesia, presents a high potential for adverse herb–anesthetic interaction. Currently, there is little data regarding the many potential herb–anesthetic interactions.

St. John's wort has gained popularity as an antidepressant whose clinical effectiveness is supported by randomized, controlled trials in patients with mild to moderate depression.[4] However, serious adverse interactions have been observed between St. John's wort and a number of prescription drugs.[5] These interactions involve the upregulation of two major drug disposition mechanisms: cytochrome P450 enzymes and the active efflux transporter P-glycoprotein, which could lead to a lower oral bioavailability, accelerated clearance, and/or altered organ distribution of the affected drugs, and in turn, can result in a potential loss of therapeutic efficacy.

This article is featured in "This Month in Anesthesiology," page 1A.

Hyperforin, a major bioactive constituent of St. John's wort, is the most potent known agonist of the pregnane X receptor (PXR, NR1I2), which activates the transcription of CYP3A4 and P-glycoprotein.[6] Studies have demonstrated induction of CYP3A4 in the human intestine and liver after St. John's wort treatment.[7] St. John's wort has also been shown to induce the intestinal expression of the active efflux transporter P-glycoprotein.[7,8] P-glycoprotein is constitutively expressed in human tissues other than intestinal mucosa, notably the blood–brain barrier.[9] P-glycoprotein at the blood–brain barrier functions to restrict drug entry into the brain and has been shown to govern the delivery of a variety of neuropharmacologic agents, including opioids.[10,11] The role of P-glycoprotein in the distribution kinetics of opioids to the brain depends on the opioid and the competing processes of entry and efflux across the blood–brain barrier.[12,13] There is evidence from animal studies that brain P-glycoprotein is inducible.[14,15] Fentanyl, one of the most commonly used intraoperative opioids, is a known substrate of CYP3A *in vitro* and *in vivo* and has also been shown to be a P-glycoprotein substrate *in vitro*.[10,16,17] Thus, potential drug interactions may occur between fentanyl and St. John's wort *via* multiple mechanisms involving hepatic CPY3A4 and blood–brain barrier P-glycoprotein activity.[16,18,19]

The overall objective of this study was to test the hypothesis that pretreatment with St. John's wort, using a formulation and dose that is known to induce hepatic and intestinal CYP3A activity (*i.e.*, increased midazolam clearance after oral and IV administration) and increase intestinal P-glycoprotein function, will alter the pharmacokinetics and pharmacodynamics of IV fentanyl with resultant changes in clinical effects as objectively measured and subjectively reported.[20] The study was designed to evaluate two potential mechanisms of St. John's wort–fentanyl interaction: (1) St. John's wort may increase the systemic clearance of fentanyl through induction of hepatic CYP3A4; and (2) St. John's wort may attenuate brain uptake of fentanyl *via* upregulation of P-glycoprotein expression at the blood–brain barrier. Two experimental paradigms were used: first, pharmacokinetic–pharmacodynamic modeling of the time course of pupil constriction (miosis) and arterial plasma fentanyl concentration during and after short (30 min) IV fixed-dose fentanyl infusion, before and after St. John's wort; and second, the influence of St. John's wort on analgesia, miosis, and side effects during a pharmacokinetically tailored infusion targeted at a predefined steady-state plasma concentration of fentanyl.

Materials and Methods

Subjects and Study Protocol

The protocol was approved by the University of Washington Institutional Review Board and all subjects provided written informed consent. Sixteen healthy subjects, eight males and eight females, 21 to 41 yr, 72 ± 9 kg (mean ± SD; range, 61

to 91 kg) were enrolled in the study which was conducted before the requirement for clinical trials registration. No subject withdrew from the study, all completed the protocol, and results from all subjects are presented. Sample size was estimated based upon miosis and response to pain stimuli observed before and after placebo treatment in an earlier study from our laboratory that deployed a similar repeated-measures, within-subject, across-days design.[21] For sample size calculations, a 50% decrement in opioid effect from St. John's wort treatment was sought, and α and β were set at 5% and 80%, respectively. A sample of at least five to six subjects per treatment group was deemed to provide adequate power.

The study followed a randomized, double-blinded, parallel-arm design. The sequence of fentanyl administration and St. John's wort or control treatment is detailed in figure 1. Subjects were randomized to either the St. John's wort or placebo treatment arm. Each subject underwent two separate days of IV fentanyl testing 1 to 2 weeks apart: 30-min fixed-dose infusion followed by pharmacokinetically tailored 180-min infusion. Subjects then received either St. John's wort (one 300-mg Kira tablet; Lichtwer Pharma, Berlin, Germany) or placebo tablet three times a day from day 1 through day 21. Kira St. John's wort is the same formulation as the LI-160 product marketed in Germany by Lichtwer Pharma. At the same dose used in this study, LI-160 induced hepatic and intestinal CYP3A and intestinal P-glycoprotein.[20] To verify the bioactive content, we assayed each batch of purchased Kira tablets for hypericin and hyperforin.[22] During St. John's wort/placebo treatment, subjects received another round of IV fentanyl testing: 30-min fixed-dose fentanyl infusion on a day between day 14 and 18, and a pharmacokinetically tailored 180-min infusion on day 21.

Fixed-dose IV Fentanyl Infusion. For pharmacokinetic–pharmacodynamic modeling of pupil response, subjects received a 30-min constant rate fixed-dose IV infusion of fentanyl (2.5 µg/kg). Resting, dark-adapted pupil diameter was measured before; at 5, 10, 15, and 30 min during infusion; and at 5, 10, 15, 20, 30, 40, 60, 75, 90, 120, 150, and 180 min and 4, 5, 6, and 12 h after infusion was discontinued. Blood samples (5 ml each) were collected from an arterial catheter immediately after each pupil measurement. Plasma fentanyl concentration–time data were fitted to a three-compartment model with IV infusion input (WinNonlin 5.01; Pharsight Corp, USA).

Pharmacokinetically Tailored IV Fentanyl Infusion. Effects of St. John's wort or placebo on fentanyl analgesia, miosis, and side effects were assessed during a pharmacokinetically tailored IV fentanyl infusion. The three-compartment model parameters estimated from the preceding fixed-dose IV fentanyl infusion study were deployed in programming a computer-controlled IV infusion regimen based on the bolus/elimination/transfer algorithm to achieve a steady-state plasma fentanyl concentration of 1.0 ng/ml for 3 h.[23]
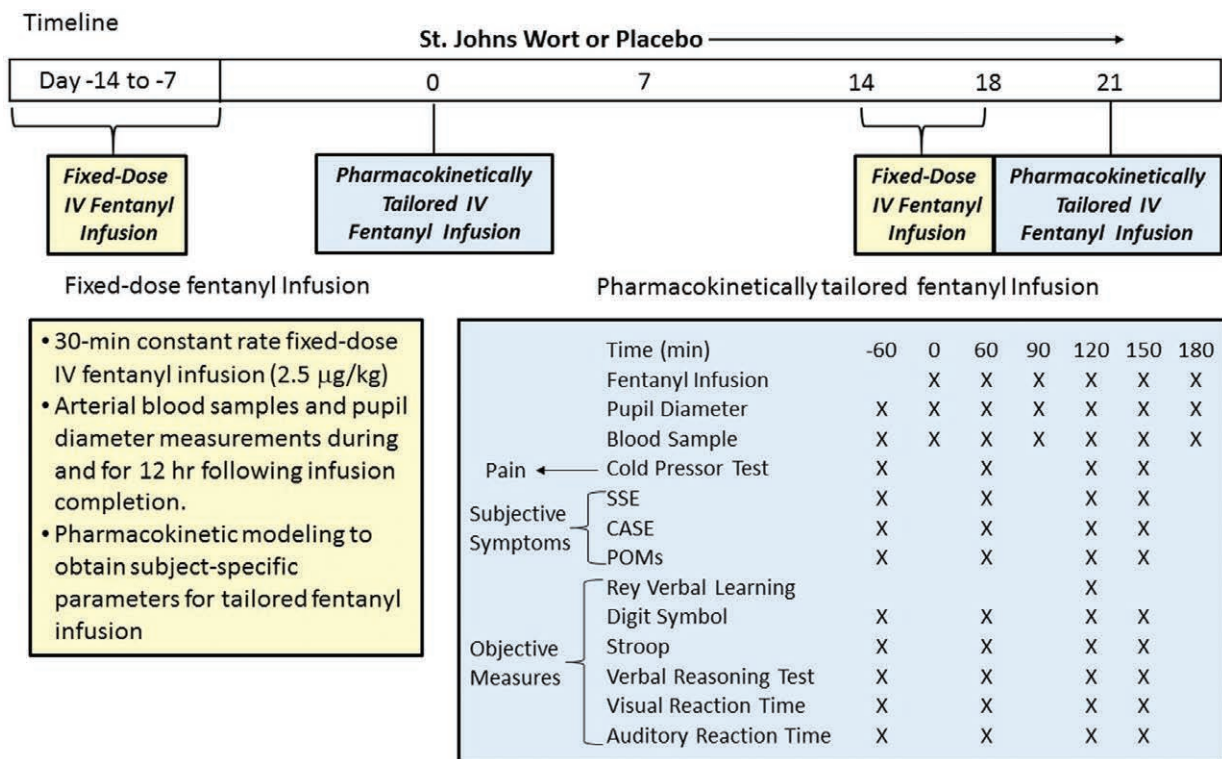
Fig. 1. Study protocol. CASE, Cognitive–Affective Side Effects scale; IV, intravenous; POMS, Profile of Mood States; SSE, Somatic Side Effects scale.

This target plasma fentanyl concentration is above the reported minimum analgesic concentration of 0.50 to 0.75 ng/ml.[24,25] Blood samples were collected at baseline and at 60, 90, 120, 150, and 180 min during tailored infusion. Dark-adapted pupil diameter was measured immediately after blood sampling.

During a 60-min baseline period before the start of the fentanyl infusion, pain response to cold-pressor test, subjective symptoms (Somatic Side Effects, Cognitive–Affective Side Effects, profile of mood states) and standardized objective measures of cognitive function (Rey Verbal Learning Test, Digit Symbol Substitution Test, Stroop Color and Word Test, Verbal Reasoning Test, Visual Reaction Time, and Auditory Reaction Time) were evaluated. The test battery was repeated three times, beginning at 60, 120, and 150 min after initiation of the tailored fentanyl infusion. Because the Rey Verbal Learning Test cannot be given more than once during each study day, it was only administered once during the second period of testing (120 min); hence, data from the training day were used as preopioid baseline in the statistical analysis.

Assessments of Opioid Effects

Dark-adapted pupil diameter was measured using a Pupilscan II Model 12A infrared pupillometer (Keeler Instruments, Inc., USA). The room was kept dark for 5 min before readings. The left eye was assessed, while the right eye was covered. The average of three successive readings was recorded.

The Cold Pressor Test, a well-accepted experimental pain paradigm known to be responsive to opioids,[26] was performed before and during the tailored infusion. The subjects first placed their dominant hand in a bath of warm water (35 ± 2°C) for 2 min, and then submerged the hand in a container of recirculating cold water set at 3.5 ± 0.5°C. The subjects were instructed to verbally indicate when they first felt painful sensations (*i.e.*, "pain threshold"). The subjects continued to hold their hand in the water until they could no longer bear the pain (*i.e.*, "pain tolerance") or a maximum duration of 300 sec was reached. Times to pain threshold and tolerance were recorded.

Subjective side effects were assessed on the tailored infusion day. Subjects rated side effects that could be related to opioids on a 37–item Somatic Side Effects scale and a 44–item Cognitive–Affective Side Effects scale with each item rated from 0 ("not at all") to 4 ("extremely").[27] Internal consistency reliability was strong for both somatic side effects ($\alpha = 0.91$) and Cognitive–Affective Side Effects ($\alpha = 0.86$). Subjects also completed the 30-item Profile of Mood States Short Form, a widely used, valid, and reliable measure of mood disturbance to measure general dysphoric affect.[28]

Digit Symbol Substitution Test is a well–established tool adapted from the Wechsler Adult Intelligence Scale–Revised and has been used in a number of drug studies, including opioids.[29,30] Subjects match abstract symbols with numbers according to a key provided above a long series of numbers. This timed test assesses visual–motor coordination, learning, visual scanning, response speed, and sustained attention.

The Rey Verbal Learning Test was used to assess immediate recall and delayed memory.[31] Subjects were presented with 15 words and asked to repeat the words immediately. Twice more, subjects were read the list and asked to repeat the words, for a total of three trials. Next, as an "interference" short delay recall test, a different list of 15 words was read and subjects were asked to recall the second list, followed by a request to recite the first list again. Twenty minutes later subjects were asked to recall the first list of words one last time.

The Verbal Reasoning Test was adapted from the Baddeley Three-minute Reasoning Test.[32] Subjects read through each statement and circled "true" or "false" depending on whether the letter pair after each statement accurately described the preceding statement; an example of items would be "A does not precede B: BA." This test demonstrates adequate reliability and validity, and is a sensitive measure of verbal reasoning.

The Stroop Color and Word Test is a widely used test of cognitive flexibility and specifically the ability to inhibit cognitive interference.[33] In each of three conditions, total reading time and errors were recorded, with reading time reported here. This test has been shown to be reliable and valid in detecting attentional deficits after numerous types of brain insult, and is sensitive to cognitive changes.[34]

Psychomotor function was tested with two measures, simple Auditory and Visual Reaction Time. The tests are part of a computerized psychomotor battery developed for use in evaluating effects of psychoactive drugs.[32] The battery has been used to assess reaction time and attention in numerous opioid and nonopioid laboratory trials.[32,35] The simple reaction time tests require a subject to hit a computer key as quickly as possible after seeing or hearing a cue on the computer.

Pharmacokinetic and Pharmacodynamic Analysis

Compartmental Analysis. A semilogarithmic plot of the fixed–dose plasma fentanyl concentration–time data revealed a tri-exponential washout profile. Two- and three-compartment models featuring elimination from central compartment were fit to plasma concentration data during and after the fixed-dose infusion from each subject using the WinNonlin program (Model 19, WinNonlin 5.01; Pharsight Corp, USA). The three-compartment model provided a statistically superior fit based on residual analysis and Akaike Information Criterion.

Pharmacokinetic–Pharmacodynamic Modeling. A plot of the decrease in pupil diameter from prefentanyl baseline *versus* the corresponding plasma fentanyl concentration at successive sampling times demonstrated counterclockwise hysteresis. This observation is consistent with either an equilibration delay between plasma and the effect site, or contribution of active metabolite(s) to the pupil response. The latter scenario is unlikely, since the main metabolite of fentanyl— norfentanyl—is devoid of pharmacologic activity.[36] An initial estimate of the effect site equilibration constant k_{e0} for each subject was obtained by analyzing the hysteresis data using the k_{e0} program (Department of Anesthesiology, Stanford University, Stanford, California).

An inhibitory effect Emax model (Model 103, WinNonlin 5.01, Pharsight Corp.) was used to relate the effect site concentration to pupil response as presented in equation 1.

$$E = E_0 \cdot \left(1 - \frac{C_e}{C_e + EC_{e,50}} \right) \qquad (1)$$

E_0 is the baseline pupil diameter. It should be noted that Emax is assumed equal to E_0, *i.e.*, there is complete constriction of the pupil at very high effect-site concentration. $EC_{e,50}$ is the concentration resulting in pupil constriction to 50% of the maximum. C_e is the effect site concentration at a given time. In the final output from WinNonlin, an estimate of the plasma concentration corresponding to $EC_{e,50}$ at steady-state (*i.e.*, EC_{50}) is provided and reported here.

Analytical Methods

Plasma samples were analyzed for the concentration of fentanyl using previously published methods. Sample extraction was performed according to the procedure described by Szeitz *et al.*,[37] using fentanyl-d_5 (Cerrillant, USA) as the internal standard. Analysis of extracts by liquid chromatography-mass spectrometry followed a modified method of Koch *et al.*[38] Limit of detection and limit of quantification were 0.05 ng/ml and 0.0625 ng/ml, respectively. Calibration curves were linear up to a concentration of 10 ng/ml. Inter-day coefficient of variation for low (0.2 ng/ml,) and high (1.5 ng/ml,) quality controls were 6.2% and 3.8%, respectively.

Statistical Analysis

Generalized Estimating Equations (GENMOD SAS software version 9.0; SAS Institute Inc., USA) were used to assess the effect of treatment (St. John's wort or placebo), day (pre- or posttreatment), and time (testing at baseline or during fentanyl infusion).[39] Interactions between treatment, day, time, and sex (all two–factor, three–factor, and four–factor interactions) were included in the model.

Analysis was primarily focused on the treatment-by-day interactions; that is, was there a difference between pre– and

posttreatment day that was dependent on whether the subject received St. John's wort or placebo treatment? Furthermore, for each of the pharmacodynamic measures, it was also important to assess whether time was a significant variable. Specifically, was there a difference between measures taken at baseline and during fentanyl infusion, *i.e.*, was an opioid effect evident? If time was not a significant factor, any significant day-by-treatment interactions would not be pertinent as they would not be related to a measurable opioid effect.

In several cases where Generalized Estimating Equations would not specifically address changes during tailored fentanyl infusions, within-day comparisons were evaluated by repeated-measure ANOVA.

Results

Fixed-dose IV Fentanyl Infusion

Systemic Pharmacokinetics. Figure 2 shows the time course of mean arterial plasma fentanyl concentration on the pre- and posttreatment days. The washout of fentanyl followed a triexponential pattern, with average initial distribution half-lives of 2.4 min and 21 min, and a terminal half-life of approximately 200 min. The plasma concentration-time profiles from the four study days are nearly superimposable; St. John's wort did not appear to have altered the systemic disposition of fentanyl. Table 1 shows pharmacokinetic parameters before and after St. John's wort and placebo. The parameters agree with previously published data for sampling durations of at least 8 h.[40] Analysis by General Estimating Equations showed that none of the pharmacokinetic parameters had a significant treatment-by-day interaction (*i.e.*, St. John's wort treatment had no effect on fentanyl systemic pharmacokinetics).
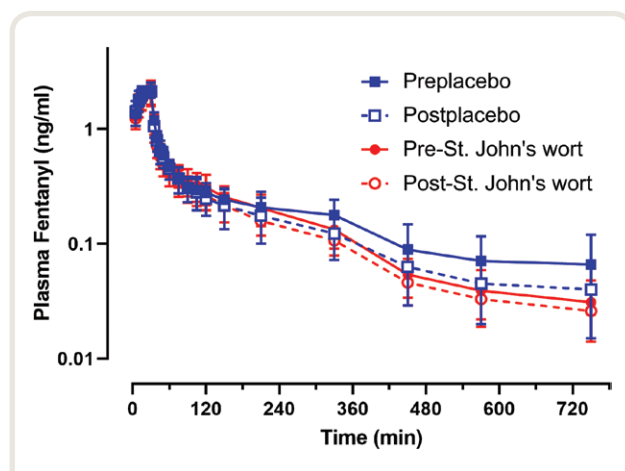

Fig. 2. Plasma concentration time course of fentanyl in healthy human subjects after a 2.5 µg/kg intravenous infusion of fentanyl on pre- (*closed symbol*) and post- (*open symbol*) treatment days in the placebo (*blue squares*) or St. John's wort (*red circles*) groups (n = 8 in each). Results are the mean ± SD.

Pharmacokinetic–Pharmacodynamic Modeling. Figure 3A shows pupil diameter *versus* time during and after the brief fixed-dose IV fentanyl infusion in the two treatment groups. A robust miotic response was observed with an average reduction of 58% in pupil diameter at the peak; peak pupil constriction was observed at 2.5 min after the cessation of IV infusion. St. John's wort pretreatment did not alter the magnitude or time course of miosis after IV fentanyl infusion. A replot of the change in pupil response (miosis) *versus* arterial plasma fentanyl concentration (fig. 3, B and C) revealed a counter-clockwise hysteresis, which is best explained by a delay in the equilibration of fentanyl between plasma and the opioid receptor site mediating the pupillary response. Table 2 presents the mean estimates of the pharmacodynamic model parameters. The effect compartment equilibration half-life of fentanyl averaged 12 min. The predicted steady-state EC50 was 1.3 ng/ml, which agrees with previous steady-state plasma concentration-response data.[25] None of the pharmacodynamic parameters differed significantly between the placebo and St. John's wort groups (Generalized Estimating Equation analysis revealed no treatment-by-day interaction).

Individually Tailored Target-concentration IV Fentanyl Infusion

The pharmacokinetically tailored IV fentanyl infusion day was intended to provide a confirmation of a change in steady-state plasma fentanyl concentration-effect relationship, if St. John's wort treatment was found to have altered fentanyl pharmacokinetics. We also anticipated that in the event of no pharmacokinetic effect, it would still be necessary to investigate any change in the fentanyl concentration-effect relationship since a pharmacodynamic interaction between St. John's wort and fentanyl at the blood–brain barrier, opioid receptor, and neural circuitry level is still possible.

By tailoring or customizing the bolus-elimination-transfer-based IV infusion regimen using each subject's fentanyl pharmacokinetic parameters obtained from the preceding fixed-dose IV infusion study, we could achieve the same target plasma fentanyl concentration in each subject, thereby reducing inter-subject variation. The tailoring approach further allowed us to normalize any effect of St. John's wort on systemic fentanyl kinetics.

Plasma Fentanyl. In most subjects, plasma fentanyl concentration reached an apparent steady-state by the first blood sampling time of 60 min after initiation of the tailored infusion; in a few subjects, steady-state was achieved between 60 and 90 min (fig. 4). It is possible that using arterial plasma data to develop the tailored IV infusion led to lower than expected venous plasma concentrations at the first sampled time point due to longer than expected arteriovenous equilibration. Repeated-measure ANOVA on

Table 1. Pharmacokinetic Parameter Estimates of Fentanyl for a Three-compartment Model Derived from the Fixed-dose IV Infusion Pre- and Postplacebo or St. John's Wort

Parameter	Placebo		St. John's Wort	
	Pre	Post	Pre	Post
V_1 (l)	12.4 ± 4.4	12.4 ± 4.0	14.0 ± 4.5	14.9 ± 5.4
V_2 (l)	16.9 ± 3.6	23.7 ± 12.4	28.2 ± 24.8	25.6 ± 15.3
V_3 (l)	186 ± 30	153 ± 55	136 ± 47	151 ± 44
Vss (l)	215 ± 28	189 ± 45	178 ± 46	191 ± 50
CL_{10} (l/min)	0.96 ± 0.28	1.12 ± 0.27	1.13 ± 0.29	1.23 ± 0.26
CL_{12} (l/min)	1.40 ± 0.96	1.19 ± 0.31	1.27 ± 0.60	1.16± 0.55
CL_{13} (l/min)	1.48 ± 0.47	1.16 ± 0.51	1.31 ± 0.45	1.35 ± 0.31
k_{10} (min^{-1})	0.083 ± 0.032	0.101 ± 0.051	0.087 ± 0.028	0.092 ± 0.042
k_{12} (min^{-1})	0.144 ± 0.134	0.103 ± 0.039	0.101 ± 0.059	0.086 ± 0.048
k_{21} (min^{-1})	0.085 ± 0.048	0.057 ± 0.019	0.063 ± 0.030	0.058 ± 0.030
k_{13} (min^{-1})	0.138 ± 0.073	0.104 ± 0.064	0.106 ± 0.050	0.099 ± 0.039
k_{31} (min^{-1})	0.0080 ± 0.0023	0.0078 ± 0.0021	0.0098 ± 0.0032	0.0092 ± 0.0017
$T_{1/2,\alpha}$ (min)	2.3 ± 1.3	2.3 ± 0.9	2.5 ± 1.1	2.5 ± 0.7
$T_{1/2,\beta}$ (min)	16.5 ± 6.9	21.2 ± 8.6	24.3 ± 20	21.5 ± 9.5
$T_{1/2,\gamma}$ (min)	258 ± 72	212 ± 65	181 ± 52	178 ± 32
MRT (min)	250 ± 103	183 ± 70	165 ± 52	158 ± 37

Generalized Estimating Equation analysis did not reveal a statistically significant treatment-by-day interaction for any of the parameter estimates. n = 8 in each group.

CL_{10}, clearance out of central compartment; CL_{12}, clearance from compartment 1 to compartment 2; CL_{13}, clearance from compartment 1 to compartment 3; k_{10}, elimination rate constant from compartment 1; k_{12}, transfer rate constant from compartment 1 to compartment 2; k_{21}, transfer rate constant from compartment 2 to compartment 1; k_{13}, transfer rate constant from compartment 1 to compartment 3; k_{31}, transfer rate constant from compartment 3 to compartment 1; MRT, mean residence time; $T_{1/2,\alpha}$, rapid distribution half-life; $T_{1/2,\beta}$, slow distribution half-life; $T_{1/2,\gamma}$, terminal half-life; V_1, volume of compartment 1 or central compartment; V_2, volume of compartment 2 or shallow peripheral compartment; V_3, volume of compartment 3 or deep peripheral compartment; Vss, steady-state volume.

pooled data for each treatment day combination indicated no statistically significant difference in the observed plasma fentanyl concentration across the duration of infusion. The overall average plasma fentanyl achieved during tailored infusion was 0.90 ± 0.18 ng/ml (n = 32 subject days) as compared to the intended target of 1 ng/ml. The mean prediction error (bias) was −0.10 ng/ml, and the root mean squared prediction error (precision) was 0.20 ng/ml; hence, our target-controlled infusion was fairly accurate and reproducible across subjects.[41] Comparable mean steady-state plasma concentrations of fentanyl, ranging between 0.8 and 1.0 ng/ml, were achieved in both the placebo and St. John's wort groups (Generalized Estimating Equation analysis showed no treatment-by-day interaction).

Fentanyl Effects. Pupil constriction quickly reached a steady-state by 60 min after initiation of the tailored IV fentanyl infusion, as would be expected given the rather short equilibration half-life observed in the fixed-dose infusion study. A remarkable miotic response was observed during tailored IV fentanyl infusion (fig. 4); on average, pupil diameter decreased to about 60% from baseline, which was statistically significant (Generalized Estimating Equation time variable; $P = 0.003$). Observed miotic response was compared to response predicted by applying the baseline pupil diameter and average plasma fentanyl level achieved on each tailored infusion day to the pharmacodynamic model using the EC_{50} estimated on the preceding fixed

dose infusion day. The mean prediction error (bias) was −0.41 mm across the 32 subject days, or the observed miosis was, on average, 7.5% lower than predicted. The root mean squared prediction error (precision) was 1.05 mm, which reflects the variability in prediction error across subjects and between days. Pupillary response to pharmacokinetically tailored fentanyl infusion was not statistically different between the St. John's wort and placebo treatments.

Pain tolerance to Cold Pressor Test increased significantly during tailored IV infusion of fentanyl (Generalized Estimating Equation time variable; $P = 0.022$) (fig. 5), indicating significant analgesia at the apparent steady-state plasma fentanyl concentration of 0.9 ng/ml. Mean pain tolerance did not vary across the three testing periods for all four treatment day combinations (repeated-measure ANOVA); analgesic response appeared to equilibrate as rapidly as pupil response. There was no significant difference in response to Cold Pressor Test before and after treatment with either placebo or St. John's wort (Generalized Estimating Equation showed no treatment-by-day interaction). Steady-state analgesic effect of fentanyl was not altered by concomitant St. John's wort therapy.

Subjective side effects are shown in figure 6 for the three side effect scales: Somatic Side Effects scale for physical symptoms, Cognitive–Affective Side Effects scale for mental effects, and Profile of Mood States–Short Form for negative mood state. Subjects reported significant opioid-related effects at the first test period. For all three scales, the mean scores remain relatively stable across the
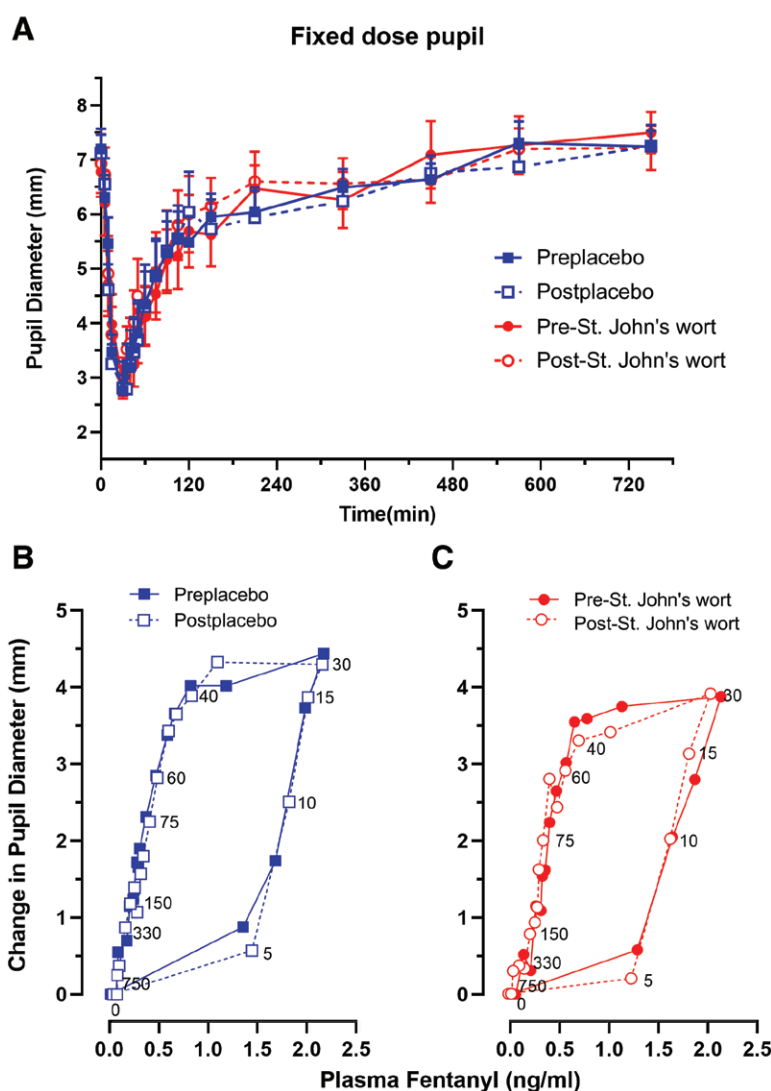
Fig. 3. Time course of pupil diameter over 12 h (*A*) in 16 healthy human subjects afer a 2.5 µg/kg intravenous infusion of fentanyl and hysteresis plots (*B, C*) of mean change in pupil diameter *versus* mean plasma fentanyl concentration in the order of time on the pre- (*closed symbol*) and post- (*open symbol*) treatment days in the placebo (*blue squares*) and St. John's wort (*red circles*) groups.

Table 2. Pharmacodynamic Parameter Estimates for Fentanyl-derived from Effect Site Modeling of Pupil Diameter during and after a Fixed-dose IV Infusion Pre- and Posttreatment in the Placebo and St. John's Wort Groups

| Parameter | Placebo | | St. John's Wort | |
	Pre	Post	Pre	Post
Emax (mm)	7.64 ± 0.86	7.49 ± 0.88	7.53 ± 1.63	7.50 ± 1.29
EC_{50} (ng/ml)	1.22 ± 0.83	1.16 ± 1.56	1.13 ± 0.74	1.38 ± 0.89
k_{e0} (min^{-1})	0.080 ± 0.044	0.070 ± 0.059	0.062 ± 0.023	0.090 ± 0.060
$T_{1/2,ke0}$ (min)	12.9 ± 5.5	10.9 ± 6.3	11.9 ± 5.8	13.0 ± 9.3

Results are mean ± SD. n = 8 in each group. Generalized Estimating Equation analysis did not reveal a statistically significant treatment-by-day interaction for any of the parameter estimates.

EC_{50}, predicted steady-state plasma concentration that results in pupil constriction to 50% of maximum; Emax, maximum constriction in pupil diameter; k_{e0}, effect site equilibration constant; $T_{1/2,ke0}$, effect site equilibration half-life.
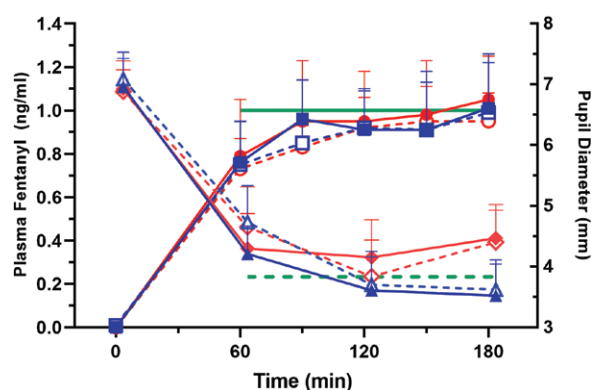
Fig. 4. Plasma fentanyl concentrations and pupil diameter during the pharmacokinetically tailored intravenous infusion in the placebo (*blue squares* for concentration, *blue triangles* for pupil diameter) and St. John's wort (*red circles* for concentration, *red diamonds* for pupil diameter) groups. *Solid symbols* represent St. John's wort/placebo pretreatment values and *open symbols* posttreatment values. *Solid green line* denotes the target plasma fentanyl at 1 ng/ml, and *dashed green line* represents the overall predicted pupil diameter based upon the baseline pupil diameter, achieved plasma fentanyl, and EC_{50} derived from the fixed-dose fentanyl study day for all 32 subject days. Results are mean ± SD.

three testing periods. As expected, all three side effect scales showed a significant opioid effect (Generalized Estimating Equation time variable; *P* values ranged from 0.003 to 0.01). The mean scores for the three testing periods did not differ between pre- and posttreatment in both the placebo and St. John's wort group (Generalized Estimating Equation showed no treatment-by-day interaction).

Of the six cognitive tests in our battery (fig. 7), only Digit Symbol Substitution Test, Stroop, and Auditory Reaction Time showed a significant change in response to fentanyl with longer response time or fewer items completed on fentanyl (Generalized Estimating Equation time variable; *P* values ranged from 0.0007 to 0.004). Visual Reaction Time, Rey Verbal Learning Test, and Verbal Reasoning Test were not responsive to opioid treatment. The test scores for the three fentanyl-responsive cognitive tests were stable across the three testing periods (repeated-measures ANOVA). Moreover, St. John's wort treatment did not alter the opioid-related side effects of fentanyl at the apparent steady-state plasma concentration of 0.9 ng/ml for any cognitive tests (Generalized Estimating Equation showed no treatment-by-day interaction).

Discussion

The first aim of this study was to determine if St. John's wort would induce CYP3A-mediated fentanyl metabolism,
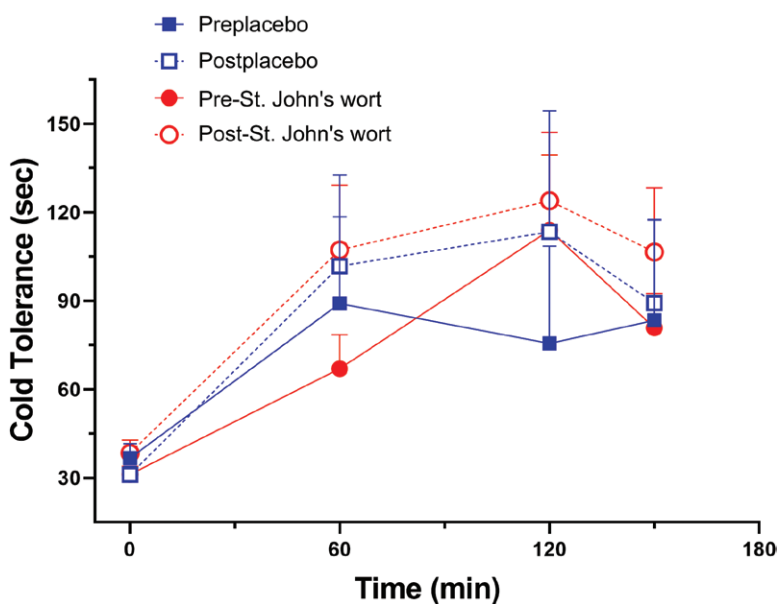

Fig. 5. Pain tolerance in the Cold Pressor Test at baseline and the three testing periods during pharmacokinetically tailored intravenous infusion of fentanyl on pre- (*closed symbol*) and post- (*open symbol*) treatment days in the placebo (*blue squares*) and St. John's wort (*red circles*) groups (n = 8 in each). Results are mean ± SD.
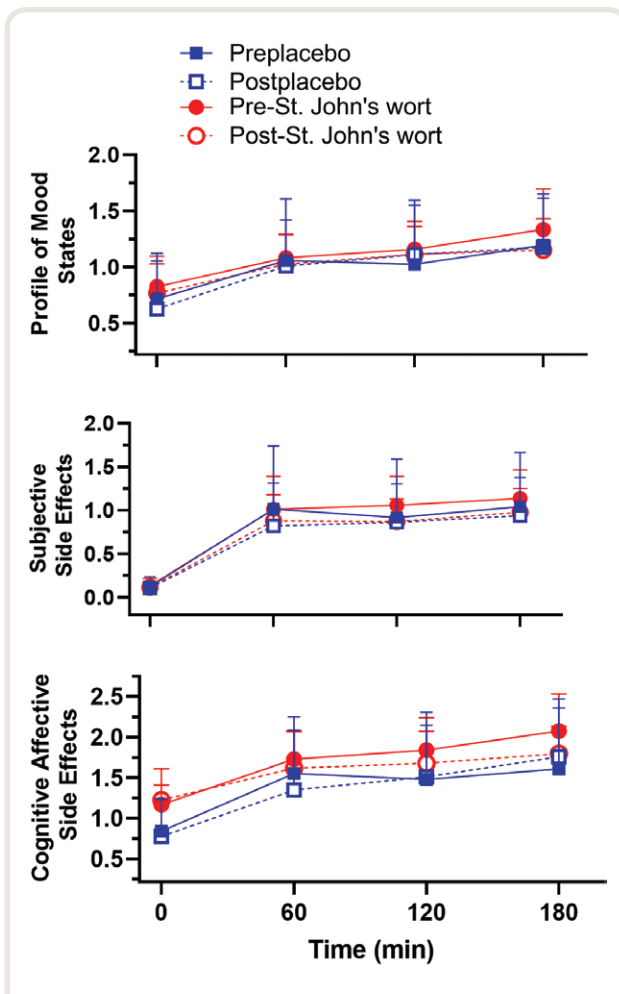
Fig. 6. Subjective side effects scales: the Somatic Side Effects scale for physical symptoms, Cognitive–Affective Side Effects scale for mental symptoms, and Profile of Mood States for negative mood state at baseline and the three testing periods during pharmacokinetically tailored intravenous infusion of fentanyl on pre- (*closed symbol*) and post- (*open symbol*) treatment days in the placebo (*blue squares*) and St. John's wort (*red circles*) groups (n = 8 in each). Results are mean ± SD. All three side effect scales showed a significant opioid effect (Somatic Side Effects scale, $P = 0.003$; Cognitive–Affective Side Effects scale, $P = 0.01$; Profile of Mood States–Short Form, $P = 0.005$), from baseline to fentanyl infusion with no further changes over time or by pre- and posttreatment.

thereby accelerating plasma clearance. Reported clearance of fentanyl varies over a large range. Results of our analysis are very similar to those previously reported by McClain and Hug; they indicate a high clearance of fentanyl that approaches liver blood flow.[40] As such, our findings that the systemic pharmacokinetics of fentanyl are unaffected by St. John's wort treatment is not entirely unexpected. Traditional pharmacokinetic theory predicts that increase in intrinsic metabolic clearance will have minimal impact on the hepatic clearance of high extraction ratio drugs

since it is rate-limited by liver blood flow. In this regard, it is noteworthy that treatment with two other strong cytochrome P450 enzyme inducers—rifampin and carbamazepine—have been reported to increase the respective clearance of transmucosal and IV fentanyl; a combination of increased intrinsic metabolic clearance and liver blood flow was hypothesized as the underlying mechanism, blood flow being the predominant effect.[42,43] We surmise that St. John's wort treatment most likely resulted only in an increase in intrinsic metabolic clearance of fentanyl and had no effect on liver blood flow. On another note, the lack of change in the systemic disposition of fentanyl presents an advantage from our study standpoint; any changes in pharmacodynamics could be attributed to either perturbation in transport of fentanyl across the blood–brain barrier or the central actions of the opioid.

Our second aim was to explore if a regimen of St. John's wort, known to increase intestinal and hepatic CYP3A and up-regulate intestinal P-glycoprotein expression and function, is also capable of up-regulating P-glycoprotein activity at the blood–brain barrier and effectively decreasing blood–brain barrier permeability of fentanyl, slowing the time course of onset of opioid response, accelerating offset of response during washout, and decreasing the apparent potency of fentanyl. Opioid-induced miosis was utilized as a convenient reporter of fentanyl access to the brain. We did not detect a change in the magnitude and time course of pupillary response, suggesting that St. John's wort treatment did not have a measurable effect on the efflux action of P-glycoprotein at the blood–brain barrier. Several explanations can be offered for the apparent lack of an effect on the brain uptake and pharmacodynamics of fentanyl after St. John's wort treatment.

First, circulating concentrations of the bioactive ingredient hyperforin, which is the principal P-glycoprotein inducer, may not have been high enough to activate pregnane X receptor at the blood–brain barrier. We used the manufacturer's recommended dose of 300 mg, three times a day, which has previously been shown to increase P-glycoprotein expression and activity at the intestinal mucosa.[7] In the only human study that has investigated the ability of St. John's wort to induce P-glycoprotein in systemic tissues beyond the intestinal mucosa and liver, a St. John's wort dose (1800 mg/day) twice that of the present study was shown to induce P-glycoprotein expression in circulating lymphocytes.[44] The reported *in vitro* EC_{50} of hyperforin as an agonist of pregnane X receptor is 12 ng/ml.[6] Our laboratory previously measured plasma hyperforin concentrations in a group of healthy volunteers (n = 17) during the recommended dosing schedule; a steady-state plasma hyperforin concentration of 76.8 ± 29.6 ng/ml (unpublished data) was observed. Although total circulating hyperforin concentrations appear sufficient for activation of gene transcription, the more relevant variable is the unbound or free concentration of hyperforin in
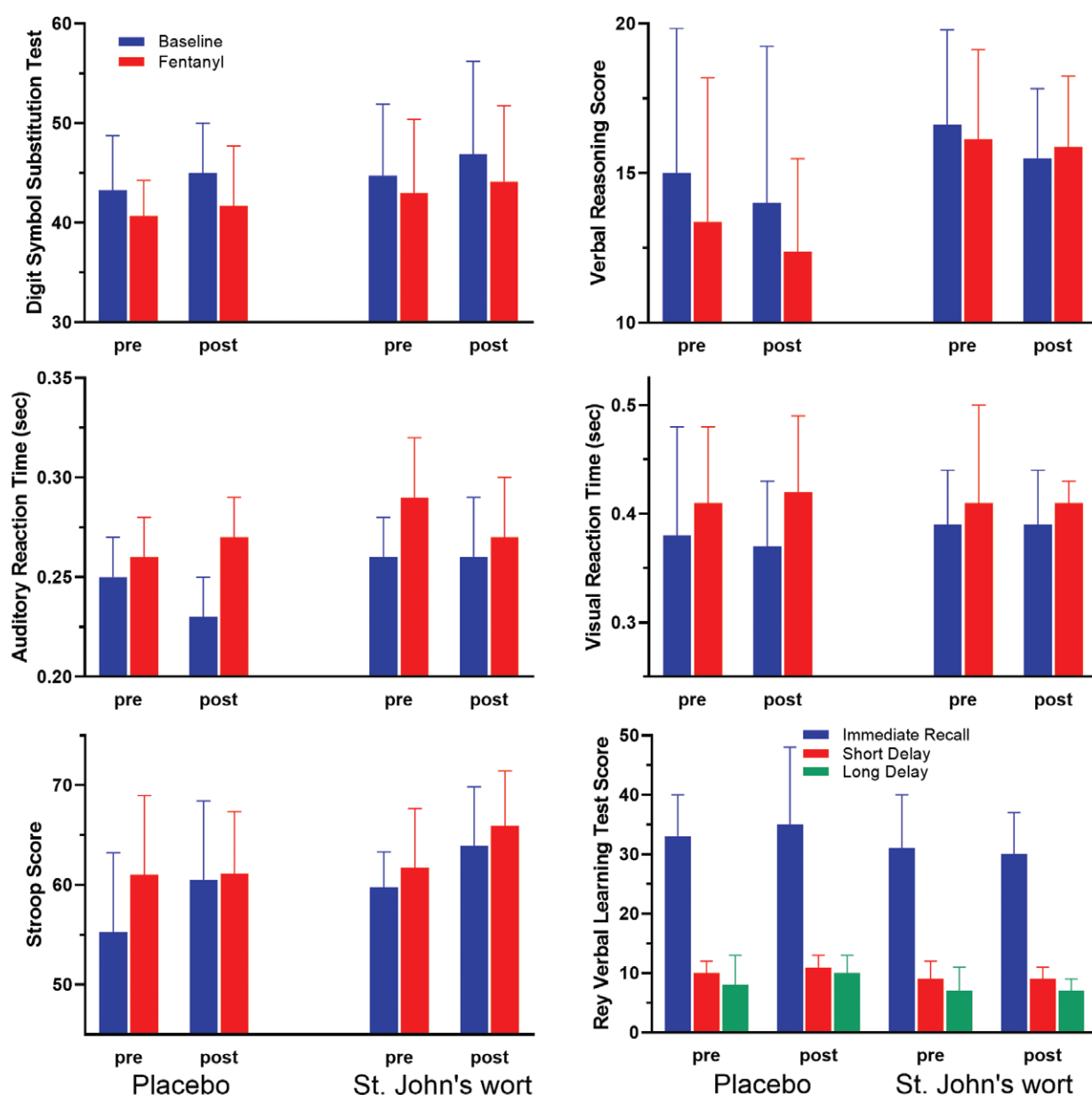
Fig. 7. Digit Symbol Substitution Test, Auditory Reaction Time, Stroop, Verbal Reasoning, and Visual Reaction Time before fentanyl (*blue*) and the average score of three test periods after the start of the pharmacokinetically tailored intravenous fentanyl infusion (*red*) on pre- and posttreatment days in the placebo and St. John's wort groups (n = 8 in each). Rey Verbal Learning Test scores are sum of words remembered during three trials: immediate (*blue*), short delay (*red*), and long delay (*green*). Pre results are from training day as test may only be given once a day. Verbal learning was not significantly affected by fentanyl. Results are mean ± SD.

plasma. Plasma protein binding of hyperforin has not been previously investigated. Preliminary study in our labora-tory using equilibrium dialysis indicates that hyperforin is indeed extensively bound to plasma proteins (greater than 96% at 1,000 to 3,000 ng/ml of hyperforin). Applying a free fraction of 0.04, would lower the effective circulating concentrations of hyperforin below the reported EC50 for activation of the pregnane X receptor. Additionally, there

may be differential tissue sensitivity to pregnane X recep-tor activation. An investigation, with another prototypal pregnane X receptor transcription activator and CYP3A inducer (rifampin) suggests that induction of blood–brain barrier P-glycoprotein does not occur readily at dose levels that elicit a robust induction of hepatic CYP3A.[45]

Second, there is some debate as to whether pregnane X receptor is expressed in the human brain capillary

endothelium. Early animal studies, as well as two recent human studies, did not detect expression of pregnane X receptor in whole brain homogenates.[46–48] However, more recent studies looking at brain micro vessels, as well as at the whole brain using more sensitive techniques have demonstrated pregnane X receptor gene expression at both the rodent and human blood–brain barrier.[47,49]

Third, it is possible that there was simultaneous induction and inhibition of P-glycoprotein by St. John's wort resulting in little or no net change in P-glycoprotein activity at the blood–brain barrier. There are a number of *in vitro* studies indicating that St. John's wort can inhibit P-glycoprotein.[51,52]

Finally, P-glycoprotein may not be a critical determinant of fentanyl uptake at the human blood–brain barrier as suggested by earlier *in vitro* study. It is possible that, despite P-glycoprotein induction, passive diffusion of fentanyl remains the predominant means by which fentanyl gains passage across the blood–brain barrier *in vivo*. Using primary cultured bovine brain microvessel endothelial cell monolayers, Henthorn *et al*. showed that in addition to being a P-glycoprotein substrate, fentanyl uptake was mediated by an active carrier mediated process. They concluded that in their endothelial cell culture system active P-glycoprotein mediated extrusion of fentanyl was overshadowed by an active inward transport process, mediated by an as of yet unidentified transporter.[14] Whether the same situation exists in humans *in vivo* remains an unknown. If true, it is possible to speculate that this unidentified transporter is also induced by St. John's wort and negates any effect St. John's wort has on P-glycoprotein induction.

The third aim of the study was to determine if treatment with St. John's wort attenuates fentanyl analgesia and side effects. The steady-state IV infusion study allowed us to assess whether there is a pharmacodynamic interaction between fentanyl and St. John's wort at the opioid receptor, the downstream signaling processes, and actions at the level of neural nociceptive or antinociceptive circuitries. Hyperforin is an inhibitor of monoamine reuptake by synaptosomes; it also activates central benzodiazepine receptors.[52,53] The crude extract, as well as, the flavonoid fraction of St. John's wort extract have been shown to elicit antinociceptive activity in mice.[54,55] In fact, the ability of St. John's wort extract to potentiate the antinociceptive action of morphine in the rat has been reported.[56] In our study, St. John's wort did not appear to have any intrinsic analgesic effect, given our observation that baseline pain tolerance to the Cold Pressor Test did not differ before and after chronic St. John's wort treatment at standard therapeutic levels.

We elected to study such pharmacodynamic interactions during a pharmacokinetically tailored IV fentanyl infusion, which allowed us to directly determine the effect of St. John's wort on the pharmacodynamics of fentanyl at the same plasma concentration within and between subjects and would presumably minimize the confounding influence of variable systemic pharmacokinetics on the assessments of analgesia and side effects. We did not find any evidence that St. John's wort interacts with fentanyl at the pharmacologic level based on no significant differences in analgesia or subjective reports or objective testing of side effects.

In summary, a 2-week treatment with the recommended daily dose of St. John's wort did not alter IV fentanyl pharmacokinetics or pharmacodynamics. Furthermore, the treatment did not affect the rate of effect site equilibration, nor did it affect the steady-state plasma concentration-effect relationship of fentanyl. From these results we surmise that there is no significant change in P-glycoprotein function at the blood–brain barrier and brain uptake of fentanyl, nor did we find an effect on hepatic clearance of fentanyl despite a known robust induction of CYP3A enzymes at the chosen therapeutic regimen of St. John's wort. Moreover, St. John's wort did not alter the analgesia or side effects of fentanyl during steady-state IV fentanyl infusion. Collectively, these results suggest that patients who are chronic users of St. John's wort will not respond differently to IV fentanyl whether used for anesthesia or analgesia.

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Competing Interests

The authors declare no competing interests.

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