Comparative Medicine
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Vol 61, No 2
April 2011
Pages 119–130

Development of a Mouse Model for Assessing Fatigue during Chemotherapy

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Fatigue and disturbed sleep are common problems for cancer patients and affect both quality of life and compliance with treatment. Fatigue may be associated with cancer itself and with the treatment, particularly for therapies with neurotoxic side effects. To develop a model system for evaluation of chemotherapy-related fatigue, we studied mice treated with either a commonly used formulation of the chemotherapeutic agent paclitaxel (paclitaxel; Taxol), which is known to have neurotoxic properties, or a nanoparticle formulation of paclitaxel (nab-paclitaxel; Abraxane) that is reported to have greater potency and efficacy yet fewer side effects than does paclitaxel. Mice were treated with 1 of these 2 agents (10 mg/kg IV daily for 5 consecutive days) and were monitored from 1 wk before through 4 wk after treatment. Dependent measures included running wheel activity, locomotor activity on the cage floor, core temperature, sleep patterns, CBC count, serum cytokine and chemokine concentrations, and neurologic assessment. For both drugs, mice showed the most severe perturbations of activity during the first recovery week after drug administration. Mice treated with paclitaxel showed greater neutropenia and motor deficits than did mice treated with nab-paclitaxel. However, deficits had largely resolved by 4 wk after administration of either drug. We conclude that these measures provide an assessment of chemotherapy-related fatigue that potentially can distinguish toxicity associated with different formulations of the same agent.

Cancer and its associated therapies often cause sleep difficulties and fatigue that decrease quality of life and reduce compliance with planned treatment regimens.[17,28] Chemotherapy-associated fatigue is a multidimensional symptom that is characterized by unusual or extreme tiredness, feelings of weakness, unusual need for rest, and decreased physical performance. Fatigue has perhaps been studied most thoroughly with regard to breast cancer. As many as 90% of breast cancer patients report fatigue during chemotherapy or radiation, and fatigue is a key reason for patient discontinuation of treatment.[15] After completion of treatment, as many as 70% of breast cancer patients report continued fatigue, which has been documented to persist for up to 10 y.[2,3] Sleep problems often accompany this fatigue and affect between 30% and 60% of cancer patients in both short- and long-term time frames.[8]

The goal of the current project was to develop an in vivo mouse model that could be used for evaluation of fatigue related to chemotherapy. Taxanes are a class of drugs that exert their chemotherapeutic effects by stabilizing microtubules within cells.[21] This stabilization impairs the division of cancerous and other cells. However, another effect of microtubule stabilization is disruption of axonal transport in neurons, which results in peripheral neuropathy[18] and thereby could contribute to neuromuscular fatigue.

Because of these properties of taxanes, we selected the taxane agent paclitaxel for development of our model. Paclitaxel is available in 2 formulations. One formulation (Taxol) is administered as a solution of paclitaxel in 2 solvents—Cremophor:EL and ethanol—that are necessary to increase blood solubility of the drug. However, Cremophor:EL is known to cause severe hypersensitivity reactions.[9] A newer formulation of paclitaxel (nab-paclitaxel; Abraxane) binds the active drug to albumin, a natural blood transport protein, thus eliminating the need for Cremophor:EL. Animal and clinical studies have shown that nab-paclitaxel has a higher maximum tolerated dose, greater efficacy, and less myelosuppression than does Cremophor-based paclitaxel.[6,10] However, both drugs are associated with peripheral neuropathy, which could directly contribute to neuromuscular fatigue. Therefore, for development of our mouse model, we elected to compare these 2 different formulations of the same compound with regard to the side effect of fatigue.

Materials and Methods

Experimental animals. Young adult mice (n = 89) were purchased from Jackson Laboratory (Bar Harbor, ME) for use in these experiments. Female BALB/cJ mice were evaluated in all experiments. In the assessment of sleep, we also evaluated 13 male BALB/cByJ and 9 male C57BL/6J mice because of our experience in studying sleep in male mice of those strains.[33,34]

All mice used in these experiments were nontumor-bearing. Mice were used without tumors to avoid confounding toxicity of the drugs with effects related to tumors and antitumor actions of the drugs. This model therefore may mimic the clinical situation in which cancer patients undergo surgery to remove the primary tumor yet receive follow-up chemotherapy as a precautionary measure against metastasis.

All mice were free of known infections with mouse hepatitis virus, minute virus of mice, mouse parvovirus, mouse norovirus,

Received: 02 Oct 2010. Revision requested: 15 Nov 2010. Accepted: 11 Dec 2010.
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Theiler murine encephalomyelitis virus, epizootic diarrhea of infant mice virus, Sendai virus, *Mycoplasma pulmonis*, pneumonia virus of mice, reovirus 3, lymphocytic choriomeningitis virus, ectromelia virus, and endo- and ectoparasites, as monitored by using monthly testing of sentinel mice housed in the same room. Mice were housed singly on hardwood bedding, with food (Purina Laboratory Chow, PMI Nutrition International, St Louis, MO) and water available ad libitum. Mice were housed in environmentally controlled chambers that were maintained on a 12:12-h light:dark cycle at 22 ± 1 °C with 35% to 50% relative humidity. The Laboratory Animal Care and Use Committee at Southern Illinois University School of Medicine approved all animals and experimental procedures used in this study.

Drugs. Lyophilized nab-paclitaxel (Abraxane, Abraxis BioScience, Los Angeles, CA) was reconstituted in sterile endotoxin-free saline to a concentration of 2.5 mg/mL. Mice received 0.1 mL, which corresponds to a 10-mg/kg dose for a 25-g mouse. Paclitaxel was purchased as the formulation Taxol (6 mg/mL in Cremophor EL; Bristol-Myers Squibb Company, Princeton, NJ). The formulation was diluted with saline to provide 10 mg/kg paclitaxel in 0.1 mL for a 25-g mouse.

Mice were injected intravenously with 1 of 3 substances: saline, paclitaxel, or nab-paclitaxel. For administration of these substances, mice were anesthetized with isoflurane and injected by way of the retroorbital sinus. The retroorbital route was used because of the relative ease of obtaining an accurate intravenous injection at that site as compared with the tail vein. Injections were repeated daily for 5 sequential days; 5 daily injections were used because of the demonstrated efficacy of this regimen against breast cancer in female BALB/cJ mice.[35] Because drugs in the taxane family reportedly are most effective and least toxic when administered at 7 h after light onset,[20] mice were treated at that diurnal time (1400 h).

Surgery. For telemetric recording of locomotor activity and core body temperature, we implanted 51 mice (39 female BALB/cJ, 9 male C57BL/6J, and 13 male BALB/cByJ) with intraabdominal transmitters (Data Sciences International, St Paul, MN). Transmitters were gas-sterilized prior to implantation. Mice were anesthetized by subcutaneous injection of a mixture of ketamine (50 mg/kg) and xylazine (50 mg/kg) and were supplemented as needed with additional anesthetic during surgery. All surgery was conducted by using standard aseptic techniques. The abdomen was shaved by using a no. 40 clipper blade, and the skin was disinfected by using alternating scrubs of povidone idodine and alcohol. A midline incision of approximately 3 cm was made in the abdominal skin. The linea alba was identified and incised, exposing the abdominal cavity. The transmitter was implanted with the rounded end directed cranially. Sterile saline (1 mL) was added to the abdominal cavity to lubricate the transmitter and support hydration in the mouse. The abdominal muscles were closed in a simple continuous pattern with 4-0 Visorb (CP Medical, Portland, OR). The skin was closed with no fewer than 3 simple interrupted sutures of 4-0 Visorb. Mice recovered from anesthesia in a cage placed under a heating lamp. If still present, sutures were removed at 10 d after surgery.

During the same surgery, 32 (9 male C57BL/6J, 13 male C57BL.6J, and 10 female BALB/cJ) of the 51 mice that received telemetry receivers also were surgically implanted with electrodes for monitoring the electroencephalogram and electromyogram. Electroencephalogram electrodes consisted of 4 insulated stainless steel wires (Plastics One, Roanoke, VA) that were visually positioned parallel to and under the skull in bilateral frontal (approximately 1 mm anterior to bregma and 2 mm to the left and right of midline) and parietotemporal (approximately 3 to 4 mm posterior to bregma and 2 mm to the left and right of midline) positions. All electrodes were inserted into a pedestal that was secured to the skull by using dental acrylic. One of the electrodes was made continuous with cable shielding and served as a ground; this electrode was not used for data acquisition. Two of the remaining 3 electrodes were used in the combination that provided the best visual differentiation of 3 vigilance states (wakefulness, slow-wave sleep, and rapid-eye-movement sleep). Electromyogram electrodes (Plastics One) were placed subcutaneously overlying nuchal muscles of the left and right sides of the body.

After surgery, mice were housed in individual cages in a sound-shielded chamber under a 12:12-h light:dark cycle at 22 ± 1 °C and were allowed 14 d for surgical recovery. To provide analgesia, ibuprofen (1 mg/mL) was added to the drinking water from 1 d before through 7 d after surgery.[12] Recording was initiated at light onset after a minimal 14-d recovery period after surgery.

Experimental procedures. ***Assessment of locomotor and wheel running activity and core body temperature in association with chemotherapy.*** Female BALB/cJ mice implanted with intraperitoneal transmitters were placed in cages with running wheels and permitted time to acclimate to and master the use of the wheel. Running wheel data were analyzed for each mouse prior to drug administration to ensure that this behavior had been stable over the preceding 3 d. Mice that did not develop a stable pattern of running were eliminated from the study.

Mice that developed stable patterns of running were assigned randomly to receive either paclitaxel (n = 9) or nab-paclitaxel (n = 10). Drug was administered daily on 5 sequential days. For drug injection, mice were anesthetized with isoflurane, and a volume of 0.1 mL was injected intravenously through the retroorbital sinus to achieve an approximate dose of 10 mg/kg. Mice were evaluated for an additional 4 wk after the end of chemotherapy and then underwent euthanasia by exsanguination under isoflurane anesthesia. Horizontal locomotor activity and running wheel activity measures obtained during the dark (active) phase were averaged across days during baseline, chemotherapy, and the each of the 4 recovery weeks.

Revolutions of the wheel were counted and recorded every 10 min by using a software program (Data Sciences International software). Data were then averaged across every 2 h during the entire recording period. Intraperitoneal transmitters monitored horizontal locomotor activity and core body temperature by using emitted frequencies received by and location on a receiver (RPC1, Data Sciences International) positioned under each cage. Collected signals were processed through an analog converter. Locomotor activity was detected as cage crossings according to the transmitter's location above the receiver and was recorded as number of events per 10 min. Data were summarized for every 2-h interval across the entire recording period and analyzed by using by DQ3 software (Transoma Medical, St Paul, MN).

Ingestive behavior. To assess whether drug treatment caused general illness and debilitation, daily intakes of food, water, and saccharin (3% solution, a highly palatable substance) were measured in mice that received either paclitaxel or nab-paclitaxel (n = 7 per agent). Reduced intake of highly palatable substances by mice is a measure of anhedonia, which often is associated with

illness.[11,22,30] Anorexia (reduced food intake) and reduced water consumption also can reflect illness. Ingestion of food, water, and saccharin solution was estimated based on the weight of the food or volume of the fluids remaining in the cage each morning, with no attempt made to adjust for spillage. Mice that had access to saccharin solution also had untreated water available at all times. Body weight was measured on the middle day of each phase of the experiment.

Assessment of neurotoxicity. The 14 female BALB/cJ mice used to monitor ingestive behavior also were assessed for the development of vestibular and neurotoxic side effects that could interfere with locomotor activity. Before beginning the series of injections, mice were evaluated in 3 tests of motor function. The same evaluation was performed on days 5, 12, and 19 after termination of chemotherapy. After the final test, mice were euthanized by exsanguination under isoflurane anesthesia.

Three tests were used to assess potential vestibular and neurotoxic side effects of the chemotherapeutic drugs. In the 'inverted screen' test, the mouse was placed on top of a mesh screen that then was angled downward at an 80° angle. A normal response was defined as turning around to face upward. If the mouse fell off the screen or did not turn around within 30 s, the mouse was considered to have some degree of impairment. In the 'vertical pole' test, mice were placed on a rod (diameter, 2 cm; length, 40 in.) held horizontally. The pole then was moved slowly upward to a more vertical angle. A normal response was defined as remaining on the pole until it reached an angle of 60°. The 'wire hang' test assessed abnormalities in balance and grip strength. Mice were placed on top of a wire cage lid that was shaken gently 3 times, causing the mouse to grip the wire. The wire cage lid then was inverted. A normal response was defined as hanging on the wire for at least 60 s. All tests were quantitative by angle or time, providing an objective index of neuromuscular impairment.

Assessment of sleep. The effect of paclitaxel and nab-paclitaxel on sleep was evaluated in male BALB/cByJ, male C57BL/6J, and female BALB/cJ mice. Male BALB/cByJ and C57BL/6J mice were assessed because of their well-defined sleep patterns under normal conditions.[33,34] Sleep patterns of female BALB/cJ mice were assessed to allow comparison with measures of fatigue and neurotoxicity collected in female BALB/cJ mice.

For collection of electroencephalographic and electromyographic data, mice were tethered to a 6-channel electrical swivel by using a lightweight cable (Plastics One) that permitted unrestricted movement. Mice were acclimated to the tether for at least 2 d before data collection began. Throughout all recording sessions, the mice could move about freely in their cages and had continuous access to food and water.

Baseline patterns of sleep of each mouse were monitored for 24 h without experimental manipulation, beginning immediately after light onset. Each mouse therefore provided its own baseline values. Mice then were assigned randomly to receive either paclitaxel or nab-paclitaxel for 5 sequential days, as described earlier. Sleep was assessed on the third treatment day and on the fourth day after the end of the treatment regimen. At the end of the recording period, mice were euthanized by exsanguination under isoflurane anesthesia.

Sleep data were scored by assigning a specific vigilance state (slow-wave sleep, rapid-eye-movement sleep, or waking) to each 10-s epoch of the recording period by using a computer-assisted scoring method with custom software (Quality Software, Springfield, IL). Electroencephalographic tracings initially were examined visually to set a threshold value of delta-wave amplitude that was associated with slow-wave sleep for each mouse. Additional thresholds were set for electromyogram amplitudes associated with periods of movement and for theta-to-delta ratios associated with rapid-eye-movement sleep. The computer algorithm used these thresholds to score each mouse's vigilance states over the entire recording period. Mice were considered to be in slow-wave sleep whenever the delta-wave amplitude exceeded the slow-wave sleep threshold when a low-amplitude electromyogram signal was present. Rapid-eye-movement sleep was identified by concurrent low-amplitude electroencephalogram and electromyogram signals that occurred simultaneously with a high theta-to-delta ratio. At all other times, mice considered to be awake. All computer-based scoring was verified by visual inspection. For data reporting, time spent in slow-wave and rapid-eye-movement sleep was combined to obtain total sleep time.

Hematology and cytokine analysis. For measurements scheduled at specific time periods associated with drug treatment, 24 female BALB/cJ mice were injected with paclitaxel ($n = 8$), nab-paclitaxel ($n = 10$), or saline ($n = 6$) for 5 consecutive days as described earlier and were euthanized at 2 h after the last injection or at the end of recovery week 1. Whole blood was collected in EDTA-containing microphlebotomy tubes (Sarstedt, Newton, NC) and was used for assessment of CBC (Hemavet hematology instrument, CDC Technologies, Oxford, CT). Frozen serum aliquots were assayed for a 21plex panel of cytokines and chemokines (IL1α, IL1β, IL2, IL3, IL4, IL5, IL6, IL9, IL10, IL12[p40], IL12[p75], IL13, IL17, granulocytic colony stimulating factor, IFNγ, KC, monocyte chemoattractant protein 1, macrophage inflammatory protein 1α, macrophage inflammatory protein 1β, RANTES, and TNFα) by using a Luminex multiplex immunoassay kit (Millipore, St Charles, MO) on a Bio-Plex suspension array system with Bio-Plex manager 5.0 software (Bio-Rad laboratories, Hercules, CA) according to the manufacturers' instructions.

Statistics. For the 2 drug treatments, mixed-model ANOVA with repeated measures was used to compare running wheel and locomotor activity over time. A 2-factor repeated-measures ANOVA was used to compare running wheel activity with locomotor activity for each treatment group. To evaluate recovery of locomotor and running wheel activity, paired *t* tests were used to compare running wheel and locomotor activity during treatment and recovery weeks with baseline levels. Cytokine concentrations and CBC variables were compared across treatment groups by using one-way ANOVA, with follow-up comparisons performed by using the Tukey test for comparisons between means and Dunnett test for specific comparisons of treated groups with the saline-treated control group. Body weights and intakes of food, water, and saccharin were evaluated by using mixed-model analysis with follow-up paired *t* tests comparing treatment and recovery week values with baselines. Temperature data during the light and dark phases were analyzed separately by using generalized linear models. Paired *t* tests (baseline compared with experimental time) were used for follow-up comparisons. All data are presented as mean ± SEM for indicated sample sizes. A P value of less than 0.05 was considered to indicate a statistically significant effect, whereas a statistical trend was defined as $0.05 < P < 0.1$. The Statistical Package for the Social Sciences (SPSS, Chicago, IL) or SAS System software (SAS, Cary, NC) was used for all analyses.

Results

Development of a system for evaluation of chemotherapy-related fatigue in mice. Paclitaxel and nab-paclitaxel were used for model development to permit side-by-side comparison of the newer, purportedly less toxic albumin-bound formulation of paclitaxel with the standard formulation administered in Cremophor:EL. The dose used (10 mg/kg daily) was close to the maximum tolerated dose of paclitaxel in mice (13.4 mg/kg daily), whereas the maximum tolerated dose of nab-paclitaxel is 30 mg/kg daily.[7]

Because mice normally are relatively inactive during the light phase of the diurnal cycle, treatment-related changes in activity were assessed only during the dark phase. Two types of locomotor activity were measured: horizontal activity on the cage floor and spontaneous activity on a running wheel (Figure 1). Drug administration significantly reduced dark-phase locomotor activity during the treatment period ($P = 0.004$) and recovery weeks 1 ($P = 0.002$) and 2 ($P = 0.045$) after treatment. Mice treated with either drug formulation also showed significant ($P < 0.0001$) reductions in wheel running during the week of drug administration and all 4 recovery weeks (Figure 1). The effects of the 2 drugs were not significantly different with regard to either running wheel or locomotor activity.

To more closely assess activity as a measure of debilitation or fatigue, horizontal and running wheel activity were examined as a function of diurnal time across the light–dark cycle. During the first recovery week after drug administration, mice treated with either drug showed reduced locomotor and running wheel activity (locomotor activity: paclitaxel, $P = 0.0193$; nab-paclitaxel, $P < 0.0001$; running wheel activity: paclitaxel, $P < 0.0001$; nab-paclitaxel, $P = 0.0064$) and a flattening of the diurnal rhythms of activity, with a gradual resumption of the diurnal differences (Figure 2).

Values for running wheel activity and locomotor activity were calculated and compared for the first (hours 12 to 18) and second (hours 18 to 24) halves of the dark phase (Figure 3). For paclitaxel-treated mice, both running wheel activity and locomotor activity showed significant($P < 0.001$) effects of experimental week during both the early and late portions of the dark phase. Among these mice, locomotor activity and running wheel activity showed statistically significant ($P = 0.011$) differences from each other during hours 18 to 24, with a trend toward a significant difference ($P = 0.079$) during hours 12 to18. Similarly, mice that received nab-paclitaxel showed significant ($P < 0.001$) effects of experimental week on both running wheel activity and locomotor activity during both portions of the dark phase, with locomotor activity and running wheel activity showing significant differences from each other during hours 18 to 24 ($P = 0.023$) but not during hours 12 to 18 ($P = 0.454$).

Patterns of recovery of normal amounts of locomotor and running wheel activity were evaluated for each of the 2 drugs. Among mice that received paclitaxel, locomotor activity was significantly suppressed, as compared with baseline values, beginning during the period of drug administration, continuing through hours 12 to 18 of recovery week 2 ($P < 0.05$ for all comparisons), and returning to normal levels during hours 18 to 24 in recovery week 2. However, wheel running activity remained suppressed through recovery week 3 (hours 18 to 24, $P \leq 0.005$) and 4 (hours 12 to 18, $P \leq 0.035$). Among mice that received nab-paclitaxel, running wheel activity was significantly reduced, as compared with baseline values, through recovery week 4 ($P \leq 0.034$ for all comparisons), whereas locomotor activity recovered during hours 18 to 24 during recovery week 3. Therefore, mice treated with paclitaxel showed a more rapid resumption of normal activity than did mice treated with nab-paclitaxel.

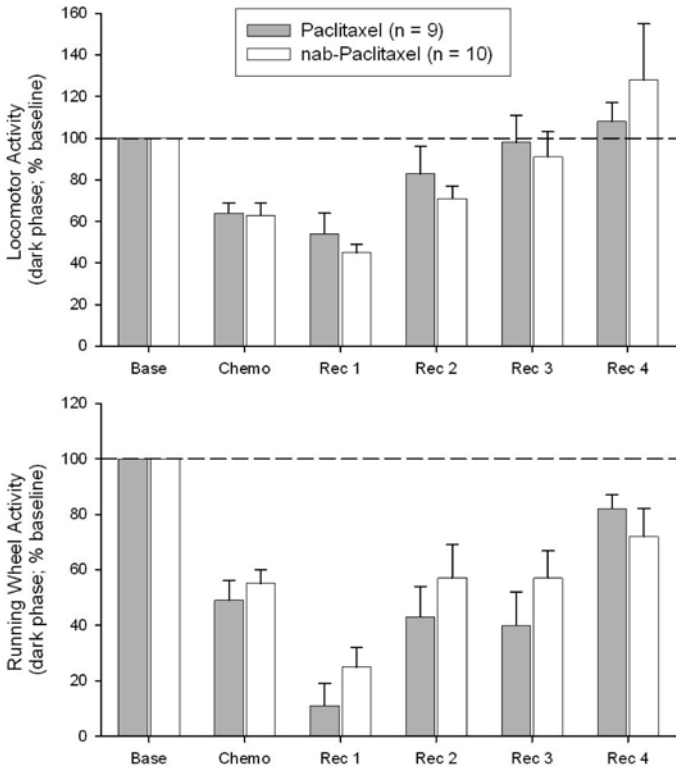

Figure 1. Locomotor and running wheel activity in mice treated with paclitaxel or nab-paclitaxel. Mice were evaluated for 1 wk under normal conditions (base), during a single5-d cycle of paclitaxel (shaded bars) or nab-paclitaxel (open bars; chemo; 10 mg/kg), and during recovery weeks 1 through 4 after treatment (Rec 1 through 4). The dotted horizontal line denotes the average values during the baseline period. Data (mean ± SEM) shown are for the 12-h dark phase only. For paclitaxel-treated mice, basal locomotor activity and running wheel activity were 18 ± 5 ($n = 9$) and 23 ± 3 ($n = 7$) counts per 10 min, respectively, compared with 14 ± 3 ($n = 10$) and 34 ± 5 ($n = 8$) counts per 10 min, respectively, for nab-paclitaxel-treated mice. Numbers of mice per group vary because of equipment failure of running wheels or transmitters for individual mice. ANOVA showed a significant effect of experimental week for both running wheel activity (paclitaxel, $P < 0.0001$; nab-paclitaxel, $P < 0.0001$) and locomotor activity (paclitaxel, $P = 0.0002$; nab-paclitaxel, $P = 0.0187$).

Assessment of potential causes of fatigue during chemotherapy. General debilitation was evaluated on the basis of body weight, food and fluid intakes, and core body temperature. Values for these measures all fell in association with experimental interval (ANOVA, $P < 0.001$), with no significant differences as a function of drug. Modest but statistically significant weight reductions developed during the 5 d of drug administration, but thereafter returned to normal (paclitaxel, $n = 10$; nab-paclitaxel, $n = 14$; $P < 0.001$; Figure 4). Intakes of food (paclitaxel, $n = 11$; nab-paclitaxel, $n = 13$), water (paclitaxel, $n = 12$; nab-paclitaxel, $n = 15$), and saccharin (paclitaxel, $n = 9$; nab-paclitaxel, $n = 9$) were reduced ($P < 0.0001$) as a function of time after administration of either drug.

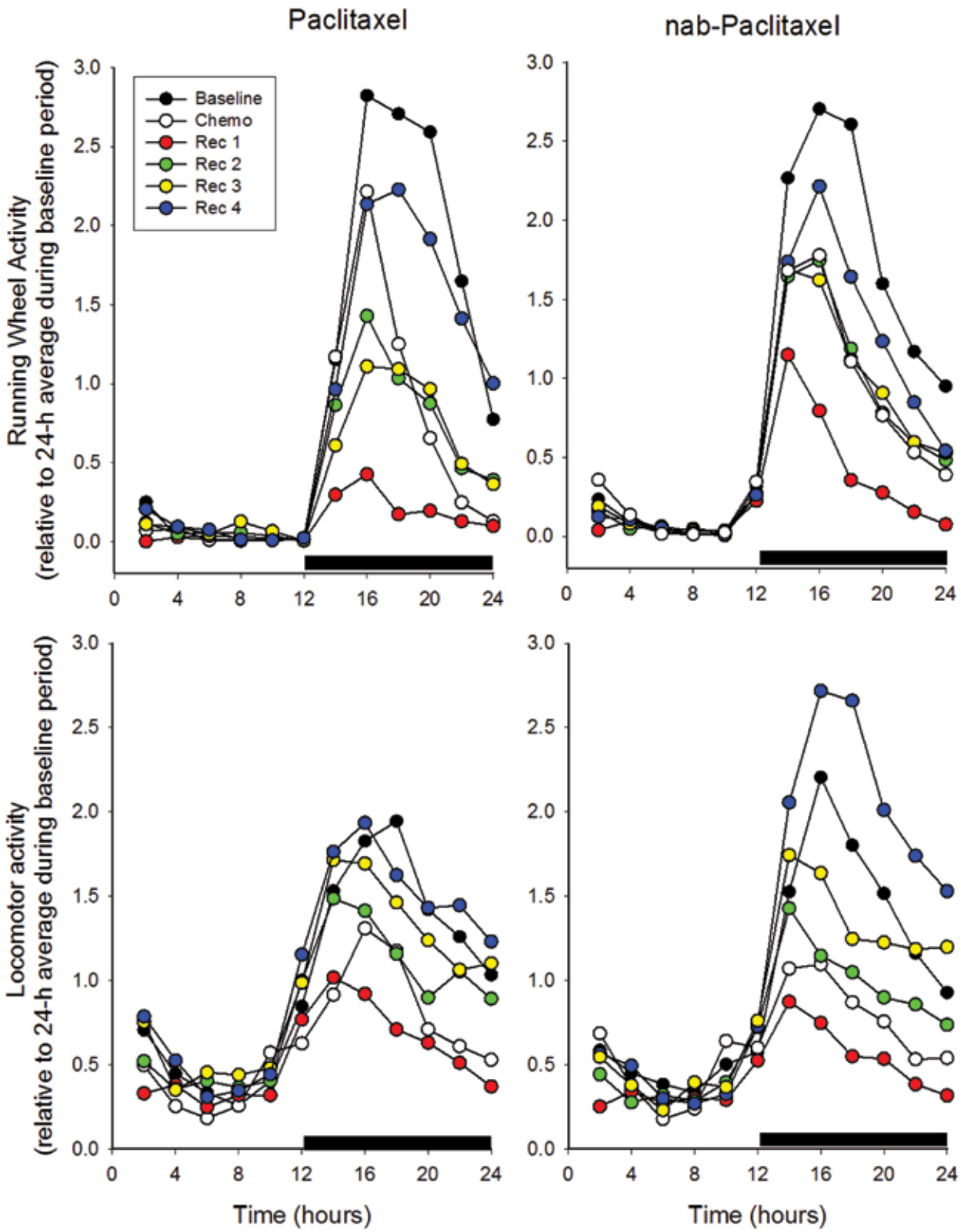

Figure 2. Diurnal pattern of activity over the course of the 24-h light-dark cycle. Locomotor activity and running wheel data from Figure 1 are expanded to include the light phase (hours 0 to 12) and illustrate the average activity values obtained during each 2-h interval across the entire 24-h cycle. Data are expressed as ratios of the average values obtained over the entire 24-h period during the baseline period (that is, before drug administration). Data points represent mean values; error bars are not illustrated because the large number obscures visualization of the plots and their relationships. ANOVA based on dark-phase values (hours 12 to 24, indicated by the dark bars on the abscissa) showed a significant effect of time during the dark phase (hour) for both running wheel activity (paclitaxel, $P < 0.0001$; nab-paclitaxel, $P = 0.0064$) and locomotor activity (paclitaxel, $P = 0.0193$; nab-paclitaxel, $P < 0.0001$).

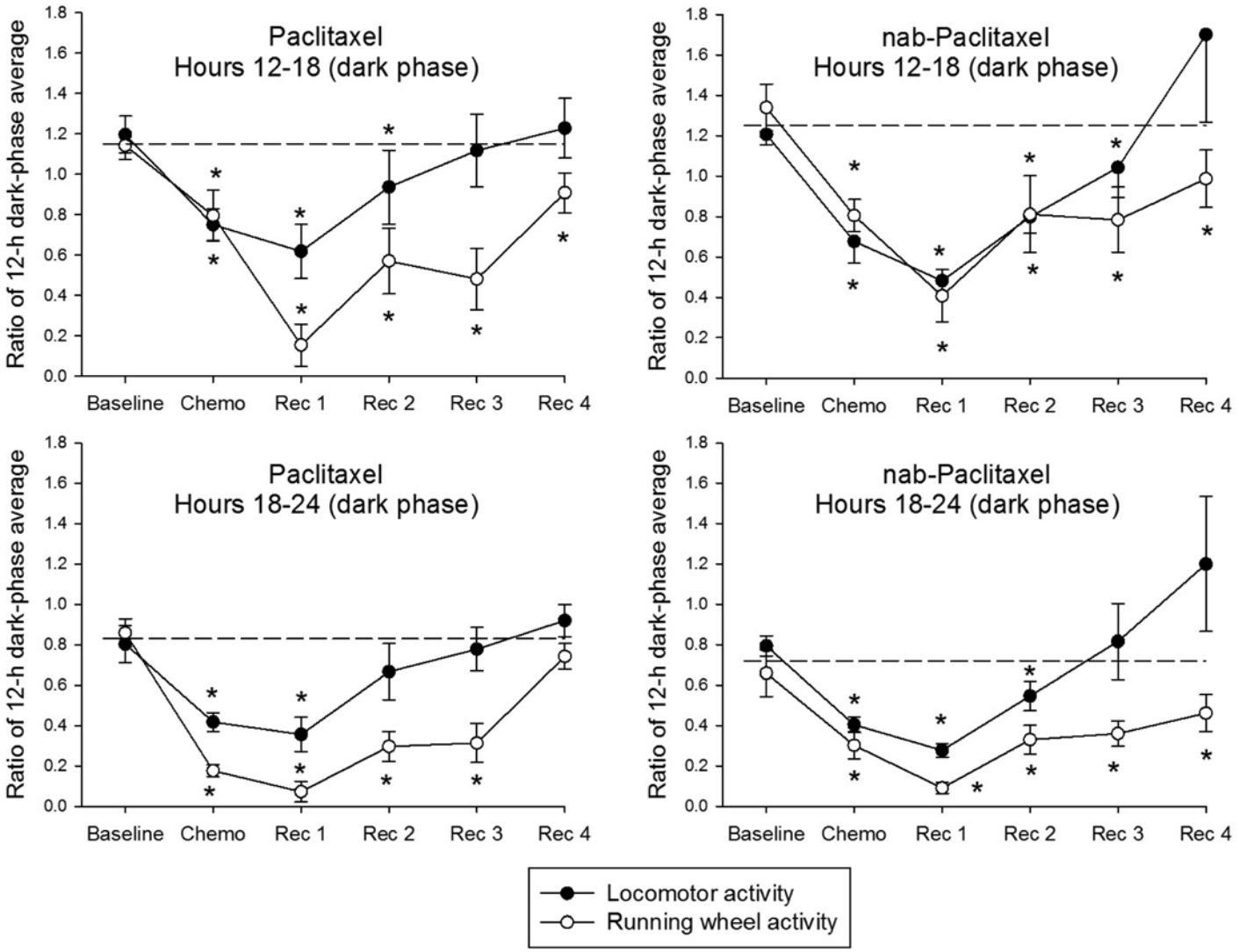

Figure 3. Activity during early and late portions of the dark phase. Data from Figure 2 are presented here as average values during the first (hours 12 to 18) and the second (hours 18 to 24) halves of the dark phase. Horizontal dashed lines provide a visual reference for values average values of both measures during the baseline period. Paired *t* tests were used to compare values from baseline values during week 1 to values during chemotherapy and recovery weeks 1 through 4 (Rec 1 through 4; *, $P < 0.05$).

Mice developed mild hypothermia during and after drug administration (paclitaxel, $n = 8$; nab-paclitaxel, $n = 10$; $P < 0.001$; Figure 4). This effect occurred during both light and dark phases of the diurnal cycle and did not vary significantly with regard to the specific drug administered.

Cancer patients often develop anemia, which can contribute to fatigue. Hematologic analysis performed during the first and third week after chemotherapy showed that hematocrit, hemoglobin, and numbers of RBC were not significantly different from values for saline-treated mice, indicating the absence of anemia (Figure 5). During the week after chemotherapy, mice treated with paclitaxel had significantly ($P < 0.05$) lower WBC counts than were present for other groups and time points (Figure 5).

Concentrations of 21 cytokines and chemokines were evaluated in serum from female BALB/cJ mice that were injected for a single 5-d cycle of paclitaxel ($n = 8$), nab-paclitaxel ($n = 10$), or saline ($n = 6$). Half of each of group was euthanized at 2 h after the last injection; the remaining mice were euthanized 1 wk after completing the series of injections. None of the analytes showed significant differences from saline-treated mice with regard to treatment group or time of measurement (data not shown). Concentrations in serum of saline-treated mice (pg/mL; mean ± SEM; $n = 6$) were as follows: IL1α, 19 ± 1; IL1β, 58 ± 5; IL2, 1.5 ± 0.3; IL3, 0.64 ± 0.20; IL4, 1.62 ± 0.15; IL5, 6.7 ± 0.5; IL6, 7.5 ± 1.6; IL9, 12 ± 3; IL10, 10 ± 3; IL12(p40), 51 ± 10; IL12(p75), 8.4 ± 1,1; IL13, 36 ± 7; IL17, 8.8 ± 1.8; granulocytic colony-stimulating factor, 7.8 ± 0.9; IFNγ, 56 ± 6; KC, 19 ± 1; monocyte chemoattractant protein 1, 59 ± 3; macrophage inflammatory protein 1α, 162 ± 10; macrophage inflammatory protein 1β, 11 ± 1; RANTES, 77 ± 6; and TNFα, 291 ± 36.

General observation of mice in the home cage revealed no obvious abnormalities in lacrimation, vocalization, coat condition, posture, or gait in association with administration of either drug (data not shown). Motor function was evaluated by using

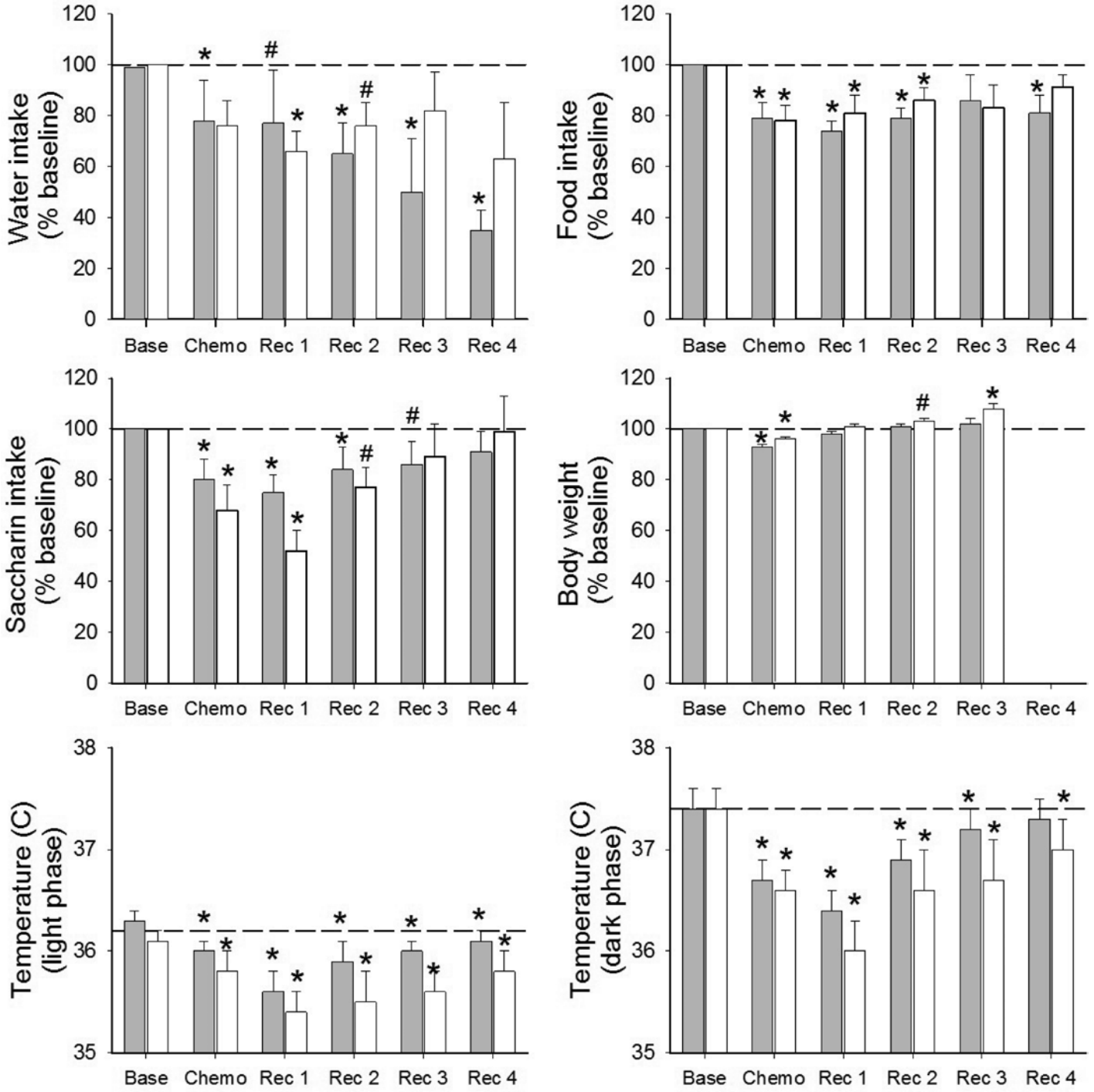

Figure 4. Fluid and food intakes, body weight, and temperatures in association with chemotherapy. In all panels, shaded and open bars denote paclitaxel- and nab-paclitaxel-treated mice, respectively (n = 7 per group). The dotted horizontal lines denote the average values measured during the baseline period. Bars represent the mean ± SEM. For body weights and ingestion, values are expressed as a percentage of those measured during the baseline period. Body weights and food and fluid intakes were measured daily under baseline conditions, during the administration of chemotherapy, and for 2 wk after termination of drug treatment. Body weights were measured at the midpoint of each phase of the experiment. Mean basal body weights and intakes of water, saccharin and food were 20.7 ± 1.4 g, 4.2 ± 0.9 mL, 6.4 ± 0.6 mL, and 7.2 ± 0.6 g, respectively, for paclitaxel-treated mice, and 21.9 ± 1.4 g, 3.7 ± 0.5 mL, 5.9 ± 0.6 mL, and 6.3 ± 0.4 g, respectively, for nab-paclitaxel-treated mice. Temperatures were measured every 10 min by using telemetry and were averaged over the 12-h light period and the 1-h dark period. *, $P < 0.05$; #, $0.05 < P < 0.1$.

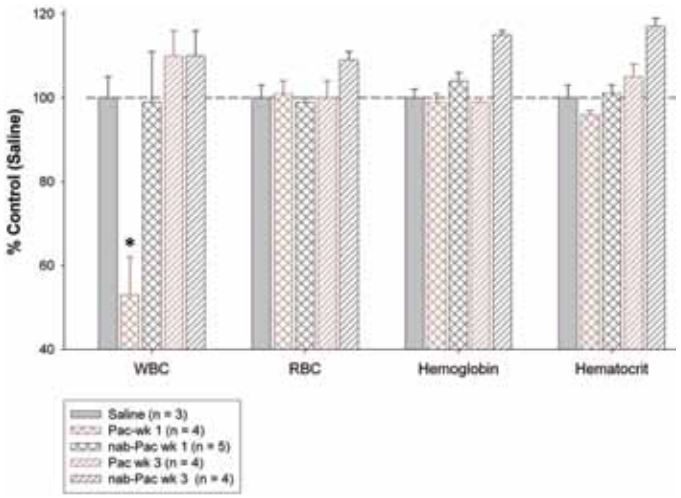

Figure 5. Hematologic analysis of mice treated with paclitaxel, nab-paclitaxel, or saline. Bars represent the mean ± SEM, expressed as a percentage of values measured in saline- (n = 3), nab-paclitaxel- (n = 5), and paclitaxel- (n = 4) treated mice at each time point. Mean basal values for WBC counts, RBC counts, hemoglobin, and hematocrit (packed cell volume) were 5.6 ± 0.3 × 10^3 cells/mm^3, 10.6 ± 0.4 × 10^6 cells/mm^3, 13.3 ± 0.3 g/dL, and 52.5 ± 1.4%, respectively. *, $P < 0.05$ as compared with saline-treated mice.

inverted screen, vertical pole grip, and wire hanging tests. Of these, only the wire hanging test, which evaluates coordination and muscle strength, revealed impairment. During the baseline period, all mice retained their grip for the entire 60-s duration of the test (data not shown). Mice were not tested during the week of treatment. However, during the first week after paclitaxel treatment, 4 of 7 mice fell, with an average hang time of 33 ± 10 s, whereas only 1 of 7 nab-paclitaxel-treated mice fell, with an average hang time of 53 ± 7 s (Table 1). During the second week after treatment, only one paclitaxel-treated mouse fell, with an average hang time of 58 ± 2 s, whereas all nab-paclitaxel-treated mice held on for 60 s. During the third week after treatment, none of the mice fell within 60 s. Therefore, significant (P = 0.036) impairment occurred only during the first week after treatment and only in mice that received paclitaxel, suggesting that this agent induced greater impairment than did nab-paclitaxel.

Fatigue can be accompanied by either excessive or disturbed sleep. Sleep was evaluated in C57BL/6J, BALB/cByJ, and BALB/cJ mice during the 24-h period before, on day 3 during, and on day 4 after administration of a 5-d cycle paclitaxel or nab-paclitaxel. Time spent asleep did not vary significantly as a function of drug treatment in any of the 3 mouse strains tested (Figure 6).

Discussion

Fatigue is a significant debilitating side effect of chemotherapy and is often associated with patient discontinuation of treatment and reduced quality of life. To create a model in which to evaluate chemotherapy-associated fatigue, we used BALB/cJ mice that were nontumor-bearing to mimic a clinical situation in which cancer patients are presumed to be largely tumor free (that is, have undergone surgical removal of a primary tumor mass) prior to beginning chemotherapy. Measures associated with chemotherapy-related fatigue were quantified and assessed. This model and the measured variables allow us to objectively test for fatigue,

Table 1. Neurologic assessment by wire hang test (n = 7 per group)

	Paclitaxel		Nab-paclitaxel	
Recovery week	No. of mice that fell	Hang time (s)	No. of mice that fell	Hang time (s)
1	4	33 ± 10[a]	1	53 ± 7
2	1	58 ± 2	0	>60 ± 0
3	0	>60 ± 0	0	>60 ± 0

Hang times represent the time (mean ± SEM) that mice were able to maintain their grip without falling (maximum, 60 s)
[a]Hang times were significantly (P = 0.036) shorter during week 1 for paclitaxel, whereas significant changes did not occur for nab-paclitaxel (P = 0.172).

which is a complex and multidimensional symptom that is difficult to define and manage even in people. We also evaluated potential underlying causes of behavioral fatigue in our mice. Mouse models, as compared with human studies, allow rigorous standardization of genetics and environment and eliminate the possible effect of concurrent disorders or treatments (for example, medications, caffeine). Furthermore, psychologic disturbances such as depression, anxiety, and general distress that accompany cancer diagnosis and treatment can contribute to feelings of fatigue in patients yet are not factors in mouse studies. Therefore, our model allows evaluation of fatigue associated with chemotherapy in the absence of concurrent disease and psychologic responses to cancer and cancer treatment.

In our model, mice were evaluated under baseline conditions, during one 5-d cycle of nab-paclitaxel or paclitaxel administration, and during the 4-wk period of recovery after completion of treatment. Activity on the cage floor was reduced in response to administration of either drug, both during the course of drug administration and to an even greater degree during the first week after treatment. Wheel running was reduced to a greater degree and for a greater duration, indicating that voluntary wheel running may be a more sensitive measure of fatigue or impairment than is horizontal locomotor activity. After receiving chemotherapy, mice generally continued to show increased activity early in the dark phase, as they did in the baseline period, but reduced their activity sooner than normal as the dark phase progressed. This pattern is consistent with the physical ability and initial desire to be active, coupled with the onset of behavioral fatigue late in the normal active phase. Therefore, both drugs reduced activity in mice, consistent with fatigue present during the first week after treatment and persisting for as long as 4 wk. As compared with that during the period of drug administration, both paclitaxel and nab-paclitaxel were associated with an even greater degree of behavioral suppression during the first week after treatment. We speculate that this exacerbation was related to a cumulative drug effect and time-dependent neuronal damage. Because exercise is reported to speed recovery from chemotherapy,[13] access to the running wheel may have promoted a faster recovery after chemotherapy for both treatment groups. This possibility remains to be tested.

A summary of our general findings regarding potential causes of fatigue in taxane-treated mice is provided in Table 2. The reductions in activity after taxane administration were not attributable to anemia, elevated levels of proinflammatory cytokines, or altered patterns of sleep. However, during the first week after treatment, mice treated with either of the 2 drugs showed mild

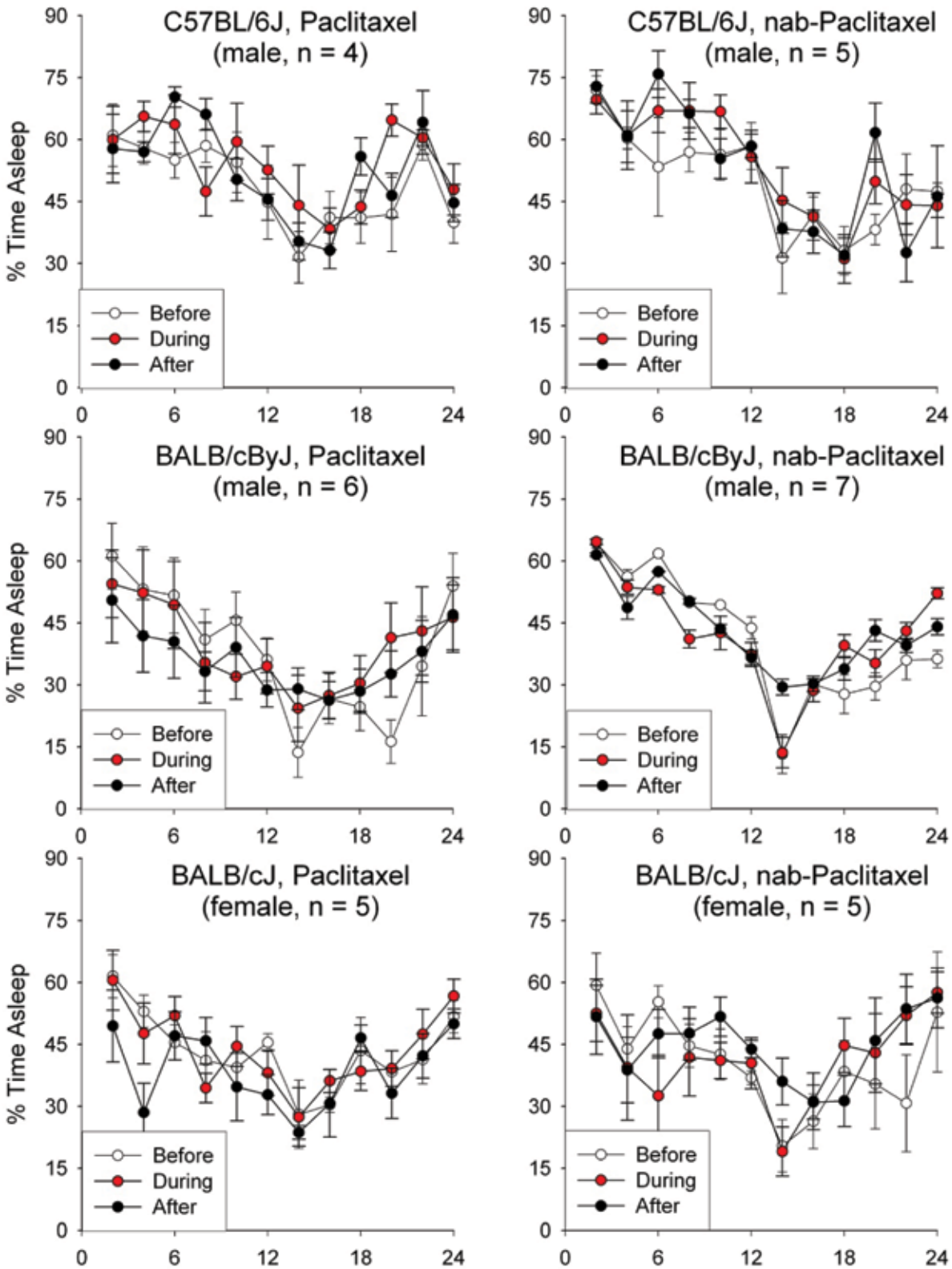

Figure 6. Diurnal patterns of sleep over the course of the 24-h light-dark cycle. Time spent asleep (that is, the sum of time spent in slow-wave sleep and rapid-eye-movement sleep) was determined on the day before drug administration (before, open circles), on day 3 of the 5-d regimen of drug administration (during, red circles), and on day 4 during the first week after drug administration (after, black circles). Data are presented as the mean ± SEM of values obtained during each 2-h interval across the entire 24-h cycle for the number of mice indicated. Hours 12 to 24 constituted the dark phase. ANOVA revealed no significant effects of drug, mouse strain, or treatment phase on sleep patterns.

Table 2. Summary of results on likely mechanisms of fatigue

Paclitaxel			Nab-paclitaxel	
Recovery Week 1	Recovery Week 3	Dependent measure	Recovery Week 1	Recovery Week 3
Yes	No	Decreased locomotor activity (Figures 1 through 3)	Yes	No
Yes	Yes	Reduced running wheel activity (Figures 1 through 3)	Yes	Yes
No	No	Altered concentrations of circulating cytokines or chemokines (or both; data not shown)	No	No
No	No	Anemia (Figure 5)	No	No
Yes	No	General debilitation (Figure 4)	Yes	No
Yes	No	Neuromuscular impairment (Table 1)	No	No
No	ND	Disrupted sleep (data not shown)	No	ND

ND, not done
Yes and No refer to whether a significant effect was detected relative to baseline values.

general debilitation (reduced food and water intakes, weight loss, and hypothermia). Paclitaxel-treated mice also showed neutropenia and mild neuromuscular impairment during recovery week 1, whereas mice that received nab-paclitaxel did not. Several considerations led us to expect that fatigue and other side effects would be greater in mice treated with paclitaxel as compared with nab-paclitaxel. First, the dosage we used (10 mg/kg) is closer to the maximum tolerated dose for paclitaxel (13.4 mg/kg as a single dose) than for nab-paclitaxel (30 mg/kg).[14] We opted to compare the 2 drugs administered at the same dose (10 mg/kg) to generate different toxicity profiles in the mice. If both drugs were administered at equivalent doses relative to the maximum tolerated dose, we would not expect a difference in drug-related side effects, and obtaining such a difference was important to testing our model. Second, as compared with nab-paclitaxel, paclitaxel is hydrophobic and has low solubility (0.3 to 1.2 mg/mL for paclitaxel compared with 2 to 10 mg/mL for nab-paclitaxel). Because of its low solubility, paclitaxel requires the use of Cremophor:EL as a drug vehicle. Cremorphor:EL contains a polyoxyethylated castor oil and ethanol and has numerous common and well-described toxicities.[6,24] As compared with paclitaxel and its Cremophor:EL vehicle, nab-paclitaxel generates lower plasma levels, higher tissue levels, more rapid and wider distribution, slower metabolism, 29-fold less toxicity than the vehicle Cremophor:EL, and 59-fold less toxicity than paclitaxel.[6]

Assessment of several possible causes of fatigue revealed mild general debilitation and neuromuscular impairment during the first week after treatment. Potential factors contributing to fatigue in humans during cancer and chemotherapy include other preexisting disorders, medication, depression, anxiety, stress, and perhaps genetic predisposition to fatigue or its causes.[1, 28] These many potential contributory factors complicate identification of the exact cause of fatigue during chemotherapy. The use of mice allows experimental control of several of these variables (for example, genetic background, medication, concurrent illness), thereby supporting more specific focus on other potential causes, such as elevated proinflammatory cytokines, anemia, general debilitation, neuromuscular impairment, and sleep disruptions.[4,5]

Proinflammatory cytokines influence sleep, appetite, depression, and many other processes that could interact to cause fatigue.[4,26] Paclitaxel is reported to increase circulating concentrations of IL6, IL8, IL10, IFNγ, and TNFα in cancer patients.[25,27,39] A review of the literature on cancer-related fatigue and cytokines in human serum revealed that fatigue correlated significantly with circulating IL6 but not with IL1β or TNFα.[29] In contrast to these reports, the proinflammatory cytokines IL1β, IL6, IL10, and TNFα were at low levels in taxane-treated mice in our study both during and after drug treatment. Several factors could contribute to this lack of response. First, because cytokines and chemokines have short half-lives in serum, our sample collection times may not have been optimal for detecting transient changes. Second, cytokines generated in the brain, rather than those present in the serum, may mediate fatigue.[26,29,31] Finally, paclitaxel may bind the Toll-like receptor 4,[36] which would increase cytokine production. TLR4 physically associates with MD2, a molecule that gives TLR4 its responsiveness to lipopolysaccharide. Mouse MD2 is involved in paclitaxel signaling, whereas human MD2 is not.[16] Differences in these interactions and relationships could account for species-specific differences in response to paclitaxel.[16]

Taxane-type drugs exert their anticancer effects by stabilizing microtubules in the G2–M phase of the cell cycle, thereby interfering with the formation and function of mitotic spindles and cytoskeleton. By stabilizing mitotic spindles, taxanes slow the division of hematopoietic stem cells, leading to leukopenia and anemia. In a large clinical trial, neutropenia was less severe and less common in breast cancer patients that received nab-paclitaxel than in those who received paclitaxel.[10] Similarly, we observed leukopenia in mice treated with paclitaxel but not in those treated with nab-paclitaxel. Anemia often develops in response to chemotherapy, worsens with treatment time, and can be associated with fatigue. However, the short duration of chemotherapy we tested did not cause anemia in mice treated with either paclitaxel or nab-paclitaxel, indicating that anemia does not contribute to fatigue in our model. Taxane-induced stabilization of microtubules also impairs axonal transport and causes other neuronal abnormalities, resulting in peripheral neuropathy.[19] One study[10] found a 10% incidence of grade 3 sensory neuropathy in patients treated with 250 mg/m^2 nab-paclitaxel as compared with 2% in patients that received 175 mg/m^2 paclitaxel[10] or a 32% incidence in patients receiving 250 mg/m^2 of paclitaxel in another study.[37] In our study, mice treated with 10 mg/kg paclitaxel had greater motor deficits than did those treated with the same dose of nab-paclitaxel (Table 1).

Mice treated with either nab-paclitaxel or paclitaxel lost weight during chemotherapy and reduced their intakes of food, water, and saccharin during the week of and week after chemotherapy. Weight loss and anorexia may not be totally interdependent, in that increasing caloric intake may not reverse weight loss during

cancer.[32] Instead, some proportion of the weight loss could be due to cachexia, which causes loss of both adipose tissue and skeletal muscle.[38] After chemotherapy, mice also became hypothermic, a response that often is associated with illness in mice. Side effects such as these are common and well documented after treatment with nab-paclitaxel and paclitaxel in humans and in mice. These signs of general debilitation could be accompanied by or contribute to fatigue.

Sleep disruptions such as hypersomnia, insomnia, and nonrestorative sleep are common factors in fatigue.[1] Patients with cancer often report poor sleep quality that is not significantly related to the type of cancer therapy used, and metastasis and advanced disease are often correlated with the worst sleep quality.[23] However, our study did not reveal changes in sleep in tumor-free mice during or after treatment with a single 5-d day cycle of taxane administration. This lack of effect is consistent with our finding of normal levels of serum cytokines, given that elevated levels of proinflammatory cytokines would be expected to promote sleep. Our findings may indicate that mice develop less of a proinflammatory response to taxanes than do humans or that the short duration of chemotherapy was not sufficient to disrupt sleep. Alternatively, sleep deficits primarily might result from tumor pathology or tumor-drug interactions, with chemotherapeutics alone having less effect.

In conclusion, our model allows objective assessment of a common, distressing, and long-lasting side effect of chemotherapy—fatigue. Prior animal models of fatigue have lacked features that distinguish locomotor impairment from fatigue.[4] Our model system identified deficits in both locomotor and running wheel activity, detected different rates of recovery of these 2 measures, and evaluated factors (including neuromuscular impairment) that could contribute to fatigue. Assessment of these markers is useful both for comparing drug toxicity profiles and as a screening tool for assessing pharmacologic interventions to reduce fatigue. This model therefore could provide an easy, fast, objective, and informative method of preclinical screening of chemotherapeutic agents.

Acknowledgments

This work was supported by the McElroy Foundation (RAT), NIH grant R011-AI080576 (LAT), and the SIU School of Medicine. We thank Lisa Cox, Christine Bosgraaf, and Lisa Volk for excellent technical assistance.

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