STIMULUS REPORT

blood advances

Effect of chronic opioid therapy on pain and survival in a humanized mouse model of sickle cell disease

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Key Points

- Chronic morphine treatment leads to decreased survival in control mice, but not in sickle mice.
- Chronic morphine treatment leads to hyperalgesia in sickle mice, but does not lead to analgesic tolerance.

Introduction

Pain, a major comorbidity of sickle cell disease (SCD), has been associated with early mortality.[1] Chronic opioid therapy (COT) is the mainstay for analgesia in SCD.[2] COT has been associated with opioid-induced hyperalgesia (OIH)[3,4] and reduced survival in humans.[5-7] We found that chronic morphine treatment of transgenic mice with breast cancer significantly reduced survival.[8] Therefore, it is critical to know whether opioids influence survival and/or cause OIH in SCD. Clinical trials in SCD have remained challenging due to barriers including unpredictable pain episodes.[9] Transgenic homozygous BERK sickle mice show pain characteristics observed clinically in SCD, including increased sensitivity to mechanical and thermal stimuli and increased opioid requirement, and higher circulating substance P and tryptase compared with normal subjects/control mice.[10-17] Like female patients with SCD, female BERK sickle mice show more pain compared with males.[13,15,18,19] We therefore examined the effect of COT on analgesia and survival in humanized female BERK sickle mice using a randomized double-blind placebo-controlled trial.

Methods

Animals

Female transgenic homozygous mice at 5 to 6 months of age expressing >99% human sickle hemoglobin (HbSS; HbSS-BERK) or normal human hemoglobin A (HbAA; HbAA-BERK) were used.[20] This study focused on the effect of morphine on females because female SCD patients and female BERK sickle mice in our colony show higher pain when compared with males.[13,15,18,19] Obtaining large cohorts of 5- to 6-month-old male HbSS-BERK sickle mice in numbers required for a similar study was challenging because fewer male pups are born; they also have higher mortality in the early postnatal months compared with females.[21,22] HbSS-BERK feature a severe disease pathology that resembles human sickle cell anemia, involving hemolysis, reticulocytosis, anemia, extensive organ damage, reduced life span, and pain.[13,20] Mice were bred and phenotyped following established protocols as described.[22] Protocols 1603-33542A and 1406-31621A were preapproved by the University of Minnesota's Institutional Animal Care and Use Committee.

Drugs and treatment

Morphine sulfate (West-Ward Pharmaceuticals, Eatontown, NJ) or saline was injected daily subcutaneously, at a starting dose of 20 mg/kg, escalated to 25 mg/kg, 30 mg/kg, 35 mg/kg, and 40 mg/kg after weeks 12, 18, 28, and 30, respectively (Figure 1A), to mimic morphine dose escalation over time clinically for treating chronic pain in SCD.[23] The dose was maintained at 40 mg/kg until the end of the survival study.

Behavioral testing

All mice were weighed and tested biweekly before and after drug treatments for mechanical (von Frey)–, thermal (heat and cold)–, and musculoskeletal/deep (grip force)–hyperalgesia (supplemental Figure 2).[13,20] Behavioral tests were performed consecutively at a 5-minute interval between tests.[13]

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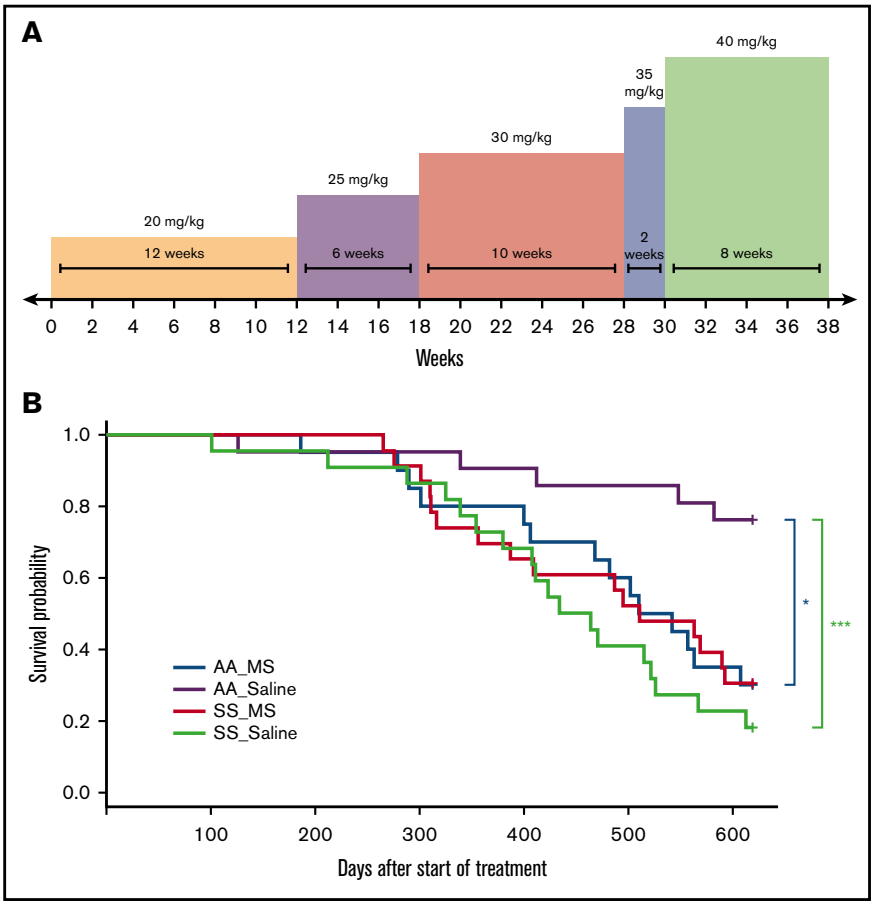

Figure 1. Treatment schedule and survival times in control and sickle mice treated with morphine and saline. (A) Schedule of morphine treatments. (B) Survival of control and sickle mice treated with morphine and saline. Survival was estimated using a Kaplan-Meier curve; the log-rank test was used to compare groups. Control morphine-treated, AA_MS, n = 23; control saline-treated, AA_Saline, n = 21; sickle morphine-treated, SS_MS, n = 22; sickle saline-treated, SS_Saline, n = 20; log-rank test with Sidak correction, $*P < .05$, $***P < .001$ compared with AA_Saline.

Mechanical hyperalgesia. Mechanical sensitivity was measured by applying a 1.0 g (4.08 mN) von Frey (Semmes-Weinstein) monofilament (Stoelting Co, Wood Dale, IL) to the plantar surface of each hind paw for 1 to 2 seconds. This procedure was repeated 10 times with a 5-second interstimulus interval, and paw withdrawal frequency (PWF) was recorded.

Thermal hyperalgesia. Heat sensitivity was obtained by applying a stimulus generated by a radiant bulb (Stoelting Co). The stimulus was applied to the plantar surface of the hind paw, and paw withdrawal latency (PWL), to the nearest 0.1 second, was recorded once the mouse withdrew its paw in response to the stimulus. Cold sensitivity was obtained by placing the mouse on a 4°C cold plate (Stoelting Co) and recording the PWF over a 2-minute period.

Grip force. Deep tissue/musculoskeletal hyperalgesia was assessed by peak forepaw grip using a computerized grip-force meter (SA Maier Co, Milwaukee, WI). Mice were made to pull on a wire-mesh gauge with their forepaws. The peak force exerted in grams was recorded.

Statistical analysis

Survival data were analyzed using Kaplan-Meier curves and the log-rank test with Sidak correction (SAS v. 9.3; SAS Institute, Cary, NC). Three-way repeated-measures analysis of variance (ANOVA) was performed but no significant 3-way interactions were present. Thus, a 2-way ANOVA with Bonferroni correction was used to compare hyperalgesia between time points among control and sickle mice (v 7.0c; GraphPad Prism Inc, San Diego, CA). The association of hyperalgesia and survival was tested using Cox proportional-hazards regression. $P < .05$ was considered significant. Data are presented as mean plus or minus standard error of the mean.

Results and discussion

Patients with SCD have reduced survival compared with healthy subjects.[24,25] We compared survival of sickle and control mice with saline or morphine treatment (Figure 1). We observed significantly decreased survival of sickle mice compared with control mice with saline treatment ($\chi^2 = 14.27$, $P = .0009$; Figure 1B). In control mice, morphine treatment compared with saline led to significantly reduced survival ($\chi^2 = 7.58$; $P = .035$), complementary to the observations in cancer-bearing mice and cancer patients.[6-8] However, no significant difference in survival was observed in morphine-treated sickle mice compared with saline (Figure 1B). No significant difference in body weight between treatments was observed (supplemental Figure 1). No association was found between hyperalgesia and survival in either control or sickle mice (data not shown).

Relatively higher doses of opioids are required to manage acute and chronic pain in SCD relative to other pain conditions.[23,26] Therefore, it is reassuring that morphine did not further impair survival of sickle mice in this study. However, this is in contrast to control mice in this study, as well as to mice/patients with cancer, both of which show reduced survival with morphine treatment.[6-8] In cancer, morphine may contribute to poor survival by its effect on cancer progression.[6-8] However, altered morphine metabolism and increased clearance in SCD may prevent the survival-impairing effect of morphine in sickle mice.[27-30]

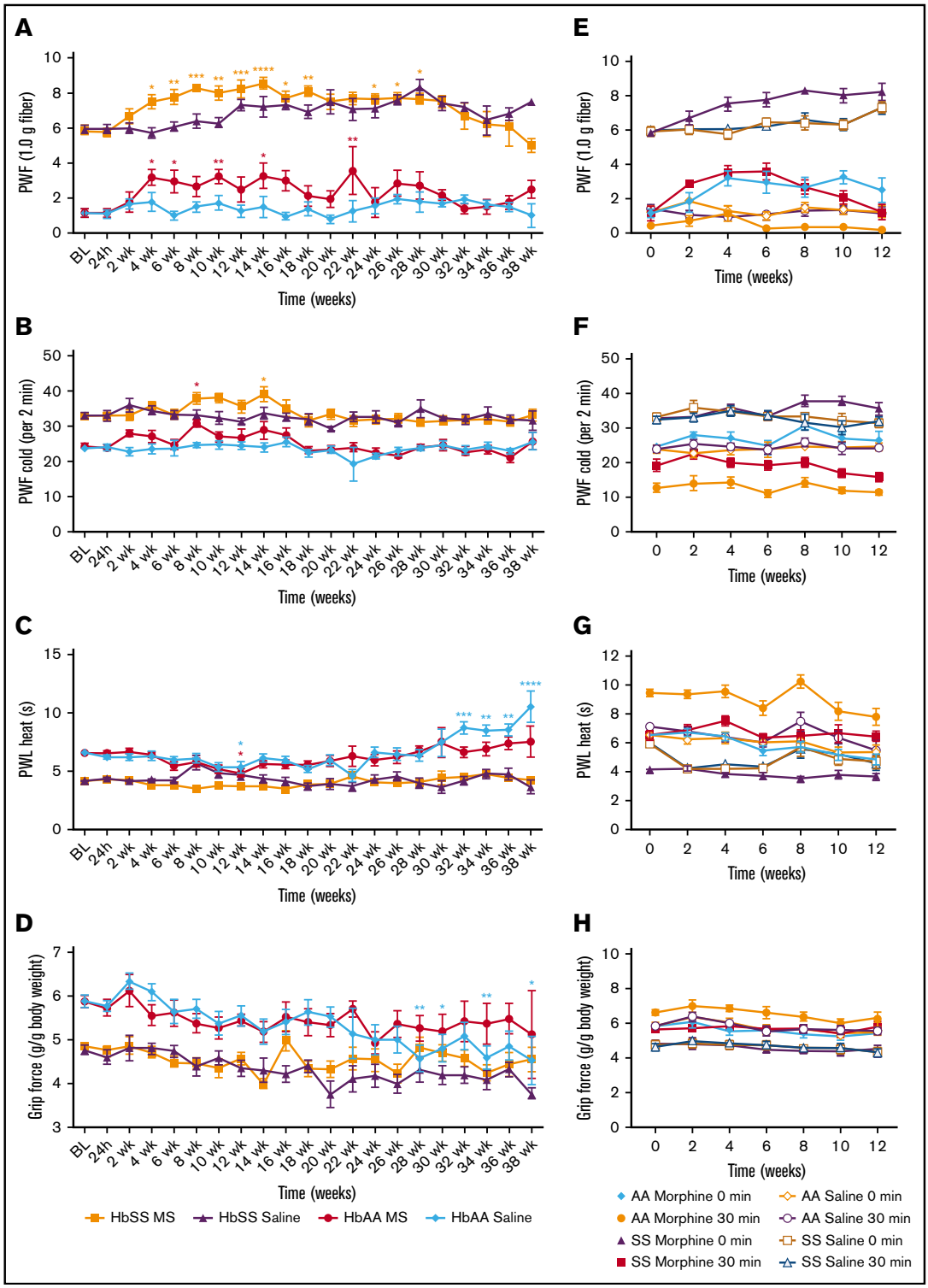

Figure 2. Influence on chronic morphine on pain. (A-D) Evaluation of morphine-induced mechanical, thermal, and musculoskeletal/deep hyperalgesia via preinjection measurement of pain over 38 weeks. (A) PWF in response to 1.0-g von Frey filaments preinjection of saline or morphine in control and sickle mice. (B) PWF in response to 4°C cold for 2 minutes prior to injection of saline or morphine in control and sickle mice. (C) PWL in response to heat stimulus preinjection of saline or morphine in control and sickle mice. (D) Grip force preinjection of saline or morphine in control and sickle mice. (E-H) Assessment of pain responsiveness to chronic morphine treatment over 12 weeks at a dose of 20 mg/kg. (E) PWF in response to 1.0-g von Frey filaments pre- and postinjection of saline or morphine in control and sickle mice. (F) PWF in response to 4°C cold for 2 minutes pre- and postinjection of saline or morphine in control and sickle mice. (G) PWL in response to heat stimulus pre- and postinjection of saline or morphine in control and sickle mice. (H) Grip force pre- and postinjection of saline or morphine in control and sickle mice. Female HbSS-BERK and HbAA-BERK mice between 5 and 6 months old at the beginning of the survival study. ANOVA 2-way analysis with Bonferroni correction, *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$, panels A-D: significance compared with baseline (BL).

Over time, we observed a significant increase in hyperalgesia in response to mechanical, cold, and heat stimuli in control mice treated with a constant dose of morphine, comparing baseline to weeks 4, 6, and 10 levels for mechanical (Figure 2A; $P = .0106$, $P = .0415$, $P = .0079$) and baseline to week 8 levels for cold (Figure 2B; $P = .0142$) and week 12 levels for heat hyperalgesia (Figure 2C; $P = .0411$), but not for deep hyperalgesia (Figure 2D). A similar increase in mechanical hyperalgesia was observed in sickle mice treated with a constant dose of morphine comparing baseline to weeks 4, 6, 8, 10, and 12 levels (Figure 2A; $P = .0433$, $P = .0076$, $P = .0002$, $P = .0014$, $P = .0005$), but no change was observed in thermal and deep hyperalgesia (Figure 2B-D). These results suggest the presence of OIH in both control and sickle mice.

In saline-treated sickle mice, there was no significant increase in mechanical, thermal, and deep hyperalgesia with age when compared with the baseline levels (Figure 2A-D; supplemental Figure 6). Saline-treated control mice showed a significant increase in hyperalgesia with increasing age between baseline and week 12 levels (Figure 2C; $P = .0285$) for heat hyperalgesia; baseline and weeks 28 to 30, 34, and 38 levels for deep hyperalgesia (Figure 2D; $P = .0080$, $P = .0377$, $P = .0026$, $P = .0410$); and baseline and weeks 14, and 28 to 38 levels for cold hyperalgesia (supplemental Figure 6). No increase with age was observed for mechanical hyperalgesia in saline-treated control mice (Figure 2A). The increase in heat hyperalgesia due to morphine in control mice may be age-dependent, but increased mechanical hyperalgesia due to morphine in control mice is independent of age as there was no change in mechanical hyperalgesia with age in saline-treated mice (Figure 2A).

Lastly, we observed no tolerance to chronic morphine treatment: the analgesic effect did not diminish over the 12 weeks of treatment at a constant dose in sickle mice (Figure 2E-H; supplemental Figures 3-7). OIH and increased pain with age were also observed throughout the 38-week study at escalating doses of morphine. Morphine-treated sickle mice showed OIH at weeks 14 to 18 and 24 to 28 for mechanical (Figure 2A), and week 14 for cold, hyperalgesia (Figure 2B). Morphine-treated control mice demonstrated OIH at weeks 14 and 22 for mechanical hyperalgesia (Figure 2A), and weeks 22 and 28 for cold hyperalgesia (supplemental Figure 6).

Morphine at 20 mg/kg was the lowest analgesic dose in sickle mice,[13] which is higher than the dose used in humans, perhaps due to differences in surface area, body mass, and metabolism in mice.[23,29,31] Unlike the continuous dosage schedule in humans, mice were treated with only 1 dose of morphine per day, which may have influenced opioid tolerance. Despite differences in dosing, these observations carry significant translational implications. A similar study in SCD patients may be extremely challenging because of diversity in disease severity and the influence of other factors, including environmental, cultural, and emotional, on analgesic response to opioids.[27,32] Because mice were treated under uniform conditions, the effect of chronic morphine treatment could be observed without the confounding factors.

We demonstrate that chronic morphine treatment provides analgesia in sickle mice without impairing survival. Acute analgesic effects of morphine are not lost with chronic treatment, however, following the resolution of this short-lasting effect, mice exhibit OIH, which may contribute to the vicious cycle of continuation of pain and increased opioid requirement in SCD. Therefore, alternative analgesic strategies are required to treat sickle pain more effectively.

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Authorship

Contribution: H.T. performed experiments and wrote and prepared the manuscript; V.S. analyzed and interpreted the data and wrote and prepared the manuscript for submission; W.L.S.-N. performed experiments and wrote the manuscript; Y.W. performed experiments and analyzed the data; A.M. wrote the manuscript, analyzed and interpreted data, and prepared the figures and manuscript for submission; Y.L. performed experiments; L.Z. performed data analyses; and K.G. conceived, designed, planned, and supervised the entire study, analyzed and interpreted data, and edited the manuscript.

Conflict-of-interest disclosure: K.G. is a consultant for Tau Tona Group and Novartis, but this does not conflict with the present work. The remaining authors declare no competing financial interests.

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