Randomized Animal Trial

Increased Thermal Pain Sensitivity in Animals Exposed to Chronic High Dose Vicodin® but Not Pure Hydrocodone

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Disclaimer: The study was sponsored by the Department of Otolaryngology – Head and Neck Surgery, Loyola University Medical Center, Maywood, Illinois, and grant funds from the Crown Family Philanthropies. Conflict of interest: Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (i.e., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

Manuscript received: 01-08-2014

Revised manuscript received:

03-12-2014

Accepted for publication: 03-17-2014

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Vicodin, the combination drug of acetaminophen and the opioid hydrocodone, is one of the most prescribed drugs on the market today. Opioids have demonstrated the ability to paradoxically cause increased pain sensitivity to users in a phenomena called opioid-induced hyperalgesia (OIH). While selected opioids have been shown to produce OIH symptoms in an animal model, hydrocodone and the combination drug Vicodin have yet to be studied. The purpose of this study was to explore the effect of exposure to chronic high dose Vicodin or its components on the sensitivity to both thermal and mechanical pain. Animals were randomly divided into 4 groups, Vicodin®, acetaminophen, hydrocodone, or vehicle control, and administered the drug daily for 120 days. Rats were subsequently tested for thermal and mechanical sensitivity. The rats in the Vicodin group displayed a significant decrease in withdrawal time to thermal pain. The rats receiving acetaminophen, hydrocodone, and vehicle showed no statistically significant hypersensitivity in thermal testing. None of the groups demonstrated statistically significant hypersensitivity to mechanical testing. The data suggests Vicodin produces signs of OIH in a rodent model. However, increased pain sensitivity was only noted in the thermal pathway and the hypersensitivity was only seen with the opioid combination drug, not the opioid alone. The results of this study both support the results of previous rodent opioid studies while generating further questions about the specific properties of Vicodin that contribute to pain hypersensitivity. The growing use of Vicodin to treat chronic pain necessitates further research looking into this paradoxical pain response.

Key words: Vicodin, acetaminophen, hydrocodone, opioid-induced hyperalgesia, thermal sensitivity, mechanical sensitivity, pain, hypersensitivity

Pain Physician 2014; 17:353-357

In the 1870s and 1880s, doctors observed increased pain sensitivity in their patients that were prescribed opioids (1). This phenomenon was first published in 1943 and animal studies in the 1970s renewed interest in the phenomena (2,3). This paradoxical response to painful stimuli has been defined as opioid-induced hyperalgesia (OIH). OIH is a decreased pain threshold from baseline after nociceptor stimulation. Evidence-

based research has confirmed OIH in rodents, but a definite mechanism has yet to be elucidated (1,4). Firm evidence of OIH in humans is lacking due to the many confounding variables involved with studying opioids in humans (5).

Rodent studies have elicited OIH mostly through the thermal and/or mechanical nociceptive pathway. The effects of morphine, DAMGO, fentanyl, heroin,

and alfentanil on pain sensitivity have all been studied in rodents (3,6-10). One opioid absent from the literature is hydrocodone. In February 2013, Vicodin®, the combination drug of acetaminophen and the opioid hydrocodone, was the most commonly prescribed drug option for chronic pain in the United States (11). Hydrocodone most notably binds to mu opioid receptors, but also acts on delta and kappa receptors (12). Although human and rats have different isoforms of the cytochrome CYD2D6 isozyme which is necessary to metabolize (O-demethylated) hydrocodone, both have the capability to ketone reduction hydrocodone to its active form, hydromorphone in the liver by the Cytochrome P450 system (13). Hydromorphone has a stronger mu-receptor affinity but it is unclear whether hydrocodone or hydromorphone is producing the analgesic effect (11). The primary effects of hydrocodone reside in the brain, spinal cord, and peripheral and sensory autonomic nerves (12). The purpose of this study was to explore whether Vicodin could produce pain hypersensitivity following chronic treatment.

METHODS

Design

All protocols were approved by Edward Hines Jr. VA Hospital Institutional Animal Care and Use Committee. Animals were housed in pairs on a 12 h/12 h light/dark cycle, followed a standard diet, and were provided water ad libitum. Thirty-two female Sprague Dawley (200 g) were randomly placed into one of 4 treatment groups: control, Vicodin (acetaminophen/hydrocodone), hydrocodone, and acetaminophen. The drugs were suspended in yogurt and administered once daily via oral gavage at a dose of 5 mg/kg hydrocodone and/or 200 mg/kg acetaminophen. The control group was given the yogurt vehicle. Thermal and mechanical responsiveness testing was performed on day 120. One researcher who was not blinded to the treatment groups randomized the rats in these behavioral tests while 3 independent researchers scored the animals in a blinded fashion.

Thermal Responsiveness Test

Rats were placed in a microprocessor-controlled infrared Ugo Basile Thermal Plantar Analgesia Instrument (Stoelting Co., Wood Dale, IL, USA). Rats were given 5 minutes to acclimate in the cage in a temperature-controlled room ($23 \pm 2^\circ\text{C}$). The high-intensity heat source was placed beneath the glass focused onto

the plantar surface of the right hind paw. The nociceptive endpoint in the radiant heat test was lifting or licking of the hind paw. The time from onset of radiant heat to endpoint was the paw withdrawal latency and was measured to the nearest 0.1 s for 3 independent trials. The radiant heat intensity was adjusted to obtain basal paw withdrawal latency of 12~15-s (70 IR Intensity), and kept constant thereafter. An automatic 25-s cutoff was used to prevent tissue damage. Each animal was tested 3 times and the latencies averaged to determine the final withdrawal latency of the pain response (14).

Mechanical Responsiveness Test

Responsiveness was semi-quantified by Dixon's Up-Down method using logarithmically calibrated Semmes Weinstein monofilaments (Stoelting Co.) (14). Rats were tested in wire bottom cages and allowed to acclimate for 5 minutes. The hind paw was introduced to monofilaments perpendicular to the plantar surface for 6 – 8 s with enough force for the monofilament to slightly buckle. Withdrawal of the paw was considered a positive response. Monofilaments were applied in ascending strength starting with 2, and continuing with 4, 6, 8, 10, 15, and 26 g force. Each monofilament was tested 10 times with greater than 50% positive response indicating threshold.

Statistics

Significant changes were determined with a one-way analysis of variance with repeated measures followed by post hoc analysis of all pair wise comparisons, using the Holm-Sidak method to correct for multiple comparisons. Statistical significance was determined by $P < 0.05$. Data was recorded as mean $\pm$ SEM of N observations.

RESULTS

After 120 days of exposure, a one-way ANOVA revealed significant amongst the treatment groups ($P = 0.013$). The Holm-Sidak multiple comparisons tests revealed that the Vicodin group demonstrated a significantly shorter withdrawal latency (10.09 ± 0.36 ; $N = 6$; $P < 0.05$) in the thermal responsiveness test (Fig. 1) compared to compared to the acetaminophen, hydrocodone, and control groups which expressed thermal withdrawal threshold sensitivities of 13.06 ± 0.35 ($N = 5$), 14.05 ± 1.11 ($N = 7$), and 13.37 ± 1.14 ($N = 5$), respectively.

Mechanical responsiveness testing did not reveal

Fig. 1. Paw withdrawal latencies (sec) were recorded after 120 days of drug exposure following a thermal insult. Data represented as mean $\pm$ SEM (control, N = 5; acetaminophen, N = 5; hydrocodone, N = 7; Vicodin, N = 6). The Vicodin group had a statistically significant shorter withdrawal latency compared to the other 3 groups (* $P < 0.05$, one-way ANOVA with repeated measures followed by post hoc Holm-Sidak multiple comparison analysis).

Fig. 2. Withdrawal thresholds (g) were recorded after 120 days of drug exposure following mechanical stimulus. Data represented as mean $\pm$ SEM (control, N = 5; acetaminophen, N = 5; hydrocodone, N = 7; Vicodin, N = 6). None of the groups recorded statistically significant differences in withdrawal threshold.

any significant difference between any of the groups when the rats were tested after 120 days of exposure (Fig. 2). Tactile withdrawal threshold sensitivity was

17.33 $\pm$ 4.3 (N = 6) for Vicodin, 11.6 $\pm$ 4.15 (N = 5) for acetaminophen, 7.43 $\pm$ 0.62 (N = 7) for hydrocodone, and 12.5 $\pm$ 3.20 (N = 6) for control.

Discussion

This was the first study to explore the effects of chronic exposure to Vicodin on with pain hypersensitivity in an animal model. Previous animal studies defined chronic opioid exposure as 3 – 12 days of opioid exposure (3); however, the animals in this study were exposed to Vicodin for 120 days. Consistent with the literature, the results showed the rats displayed hypersensitivity to noxious thermal stimuli while on chronic, high dose opioids. It was interesting to note that Vicodin produced hypersensitivity to thermal testing while hydrocodone alone did not. This could possibly be due to some synergistic effect between acetaminophen and hydrocodone or possibility chronic exposure to acetaminophen destroyed the Cytochrome P450 enzymes needed to metabolize hydrocodone (12). Potentially, the Vicodin group would have unmetabolized hydrocodone in their system while the metabolite, hydromorphone, would be the active form in the hydrocodone group.

While this study was performed with a limited sample size over an abbreviated time period, the sample size was similar to previous studies and the time course was much longer than similar studies in the literature (3). Ideally, thermal and mechanical responsiveness testing would have been performed multiple times throughout the treatment course to track and document sustained differences in pain thresholds. Future studies will address this concern and potentially determine the time course for when Vicodin switches from producing the analgesic effect to a hyperanalgesic effect.

No definitive mechanism has been established for OIH; however, exploring how opioids work in pain relief may lead to potential candidates. Centrally, opioids bind in the periaqueductal gray matter (PAG) which allows “off-cells” in the rostral ventromedial medulla to be turned on and blunt afferent nociceptive signals in the spinal cord. In the periphery, opioids decrease pain transmission through phosphorylating NMDA receptors

in the dorsal horn of the spinal cord (15-17). Rats exposed to Vicodin only demonstrated hypersensitivity to thermal stimuli. TRPV1 is acknowledged as the primary transduction channel for noxious heat stimuli. Nociceptors can also modulate the pain pathway by releasing substances from their central terminals (18). Perhaps Vicodin created alterations in these receptors resulting in hypersensitivity to thermal stimuli.

Conclusion

Vicodin is currently the most prescribed drug in the United States. This study is the first to show pain hypersensitivity due to thermal noxious stimuli in rodents chronically exposed to high doses of Vicodin. This study has important clinical implications for the development of new treatment paradigms for patients dealing with chronic pain.

Acknowledgments

Author Contributions

Eileen Foecking, PhD, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Eileen Foecking, Ph.D., and Gregory Matz, MD, designed the study protocol. Tom O’Connell managed literature searches and summaries of previous work and wrote the first draft of the manuscript. Eileen Foecking, Ph.D., provided revision for intellectual content and final approval of the manuscript. Carpenter, Caballero, Putnam, and Steere all participated in the completion of the experiments.

Funding/Support:

The authors wish to disclose and thank the sponsors of this study. The study was sponsored by the Department of Otolaryngology – Head and Neck Surgery, Loyola University Medical Center, Maywood, Illinois, and grant funds from the Crown Family Philanthropies.

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