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ALTERATIONS IN OPIOID INHIBITION CAUSE WIDESPREAD NOCICEPTION BUT DO NOT AFFECT ANXIETY-LIKE BEHAVIOR IN ORAL CANCER MICE

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Abstract—Widespread pain and anxiety are commonly reported in cancer patients. We hypothesize that cancer is accompanied by attenuation of endogenous opioid-mediated inhibition, which subsequently causes widespread pain and anxiety. To test this hypothesis we used a mouse model of oral squamous cell carcinoma (SCC) in the tongue. We found that mice with tongue SCC exhibited widespread nociceptive behaviors in addition to behaviors associated with local nociception that we reported previously. Tongue SCC mice exhibited a pattern of reduced opioid receptor expression in the spinal cord; intrathecal administration of respective mu (MOR), delta (DOR), and kappa (KOR) opioid receptor agonists reduced widespread nociception in mice, except for the fail flick assay following administration of the MOR agonist. We infer from these findings that opioid receptors contribute to widespread nociception in oral cancer mice. Despite significant nociception, mice with tongue SCC did not differ from sham mice in anxiety-like behaviors as measured by the open field assay and elevated maze. No significant differences in c-Fos staining were found in anxiety-associated brain regions in cancer relative to control mice. No correlation was found between nociceptive and anxiety-like behaviors. Moreover, opioid receptor agonists did not yield a statistically significant effect on behaviors measured in the open field and elevated maze in cancer mice. Lastly, we used an acute cancer pain model (injection of cancer supernatant into the mouse tongue) to test whether adaptation to chronic pain is responsible for the absence of greater anxiety-like behavior in cancer mice. No changes in anxiety-like behavior were observed in mice with acute cancer pain.

Key words: allodynia, cancer pain, anxiety, head and neck, widespread pain, nociception.

INTRODUCTION

Patients with oral squamous cell carcinoma (SCC) experience significant pain at the tumor site, which severely disrupts eating, drinking, and speaking (Hammerlid et al., 2001; Epstein et al., 2007; Viet and Schmidt, 2012). Prevalence of pain is estimated to be 75–90% among patients with advanced oral SCC (Epstein et al., 2007; van den Beuken-van Everdingen et al., 2007). Oral SCC pain is mediated, at least partially, by peripheral mechanisms (Schmidt et al., 2010). Central mechanisms, e.g., central sensitization, are not as well studied as the peripheral mechanisms. Central sensitization often yields pain in body regions distant from the site of injury (termed widespread pain) (Curatolo et al., 2006; Woolf, 2011). Widespread pain in non-cancer patients degrades quality of life and is a risk factor for poor response to pain treatment (Chen et al., 2012; Rabbitts et al., 2016). Sensory testing of healthy tissues distant from the cancer has never been performed in animal models. Accordingly, the presence or absence of widespread pain has not been documented in preclinical cancer models.

Central sensitization results from increased excitability and/or reduced inhibition in the central neural axis of the nociceptive pathway (Woolf, 2011). Endogenous opioids play an important inhibitory role in pain modulating mechanisms (Fields, 2004; Curatolo et al., 2006). Peripheral injury attenuates inhibitory mechanisms in the spinal cord by reducing the quantity of expressed opioid receptors; consequently the nociceptive signal transmitted to higher centers is amplified (Curatolo et al., 2006; Woolf, 2011). Opioid receptor agonists are the most common analgesics used to alleviate moderate to severe pain and are the mainstay of oral cancer pain treatment. Patients suffering from cancer pain generally require significantly higher doses of opioids than patients with inflammatory pain (Luger et al., 2002). Animal models demonstrate similar findings; the opioid dose required to block cancer pain-related behaviors in preclinical models

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is much higher than the dose required to block peak inflammatory pain behaviors (Luger et al., 2002). Cancer-induced reduction of endogenous opioid receptor activity and/or reduction in the quantity of receptors might explain the need for higher doses of exogenous opioids.

Central sensitization (induced by decreased opioid activity and/or receptor expression) might also contribute to psychological distress including anxiety. Many pain studies (not cancer related) in humans and in animals suggest that chronic pain and anxiety often co-occur (Zhang et al., 2014). The relationship between pain and anxiety seems to be reciprocal. Progressively greater pain leads to higher anxiety levels, while palliation of pain is associated with less anxiety (Sareen et al., 2005; Teh et al., 2009). Conversely, modulation of anxiety levels can alter pain levels (Heim and Oei, 1993; Chen et al., 2000; Delgado-Guay et al., 2009). Opioids attenuate both anxiety and pain in patients (Colasanti et al., 2011). A reduction in opioid tone may lead to widespread pain and anxiety.

In the current study we undertook the following: determine whether oral cancer generates widespread nociception; determine whether changes in widespread nociceptive behavior are caused by alterations in opioid receptor function or expression; and determine whether widespread nociception co-occurs with anxiety-like behaviors in a mouse model of oral cancer.

EXPERIMENTAL PROCEDURES

Oral cancer cell culture

HSC-3 (ATCC, Manassas, VA) derived from a human tongue SCC, was cultivated in supplemented Dulbecco's Modified Eagle's Medium (DMEM) at 37 °C with 5% CO_2, as previously reported (Ye et al., 2011).

Animal models of oral cancer

Animals. We used female mice in the present study because women are more likely to experience both widespread pain and anxiety compared to men (Hammerlid et al., 1999; Linden et al., 2012). Moreover, despite well-recognized sex differences in pain and anxiety, studies on female animals are rare (Mogil, 2009; Belviranli et al., 2012; Simpson and Kelly, 2012). Six- to 8-week-old female BALB/c nude mice and BALB/c albino mice (Charles River Laboratories) were housed in a temperature controlled room on a 12:12 light/dark cycle (6 AM to 6 PM), with ad libitum access to food and water. All procedures involving animals were approved by the New York University Institutional Animal Care and Use Committee (IACUC) under protocol # 160908-01, in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. Institute for Laboratory Animal Research (8th edition). For all experiments, animals were habituated to handling prior to testing. A total of 10 sets of animals (n = 222 BALB/c nude and n = 43 BALB/c albino) were used. No behavior data sets were combined for analysis to avoid experimental errors introduced by different experimenters.

Animal set 1: BALB/c nude mice with tongue cancer (n = 6) and sham mice (n = 6) were tested with facial von Frey in the morning followed by the dolognawmeter measurement at night at the baseline, and once per week after the day of HSC-3 inoculation (PID 0). At week 4 following the last dolognawmeter behavior measurement, all mice were euthanized, and spinal cords were dissected out for mRNA quantification of MOR, DOR, and KOR.

Animal set 2: BALB/c nude mice with tongue cancer (n = 10) and sham mice (n = 10) were tested for paw and tail withdrawal at the baseline, and on a weekly basis after cancer inoculation. At week 4 following tail flick measurement, mice were sacrificed, perfused with PBS followed by 4% PFA. Brain tissues were prepared for c-Fos staining. Two brain tissue samples from each group were excluded for c-Fos analysis due to sectioning problems and excessive background staining.

Animal set 3: At week 4, BALB/c nude mice with tongue cancer (n = 20) and sham (21) were tested for OF and EZM assays on PID 21 and PID 24, respectively. These post-injection day times were chosen because pain behaviors were stabilized at week 4; there was no difference in all nociceptive behavior indices measured between week 3 and week 4. In addition, since OF and EZM-induced anxieties are based on exposure to novel stimuli, to reduce the influence of one assay measurement on another assay measurement, a 3-day wash-out period was used. To determine the correlation of anxiety-like behaviors and nociceptive behaviors, the same set of cancer mice was then tested with paw withdrawal and tail flick assays on PID 27. The facial von Frey assay was conducted on PID 28 in the morning and the dolognawmeter at night. Two mice in the cancer group died before nociceptive behavior could be measured.

Animal set 4: Normal BALB/c albino mice were injected with either cancer supernatant (n = 16) or culture medium (n = 15) into the tongue and the animals were tested for anxiety-like behavior in the EZM assay one hour after anesthesia and supernatant injection. Due to the short-lasting effect of HSC-3 supernatant, and the two anxiety-like behavior assays cannot be tested within the same day, only the EZM assay was used.

Animal set 5: On PID 21 the group of BALB/c nude mice with tongue cancer were treated intrathecally with the opioid receptor agonists for MOR, DOR, KOR and vehicle (n = 8 per group) and compared with mice without cancer (n = 8). Mice were evaluated with paw and tail withdrawal assays one hour after opioid receptor agonist treatment.

Animal set 6: On PID 21 tongue cancer mice were treated intrathecally with the opioid receptor agonists for MOR, DOR, KOR and vehicle (n = 8 per group) and tested in the OF one hour after drug injection. Normal mice without cancer (n = 8) were administered intrathecal injection of PBS.

Animal set 7: On PID 24 another group of tongue cancer mice were treated intrathecally with the opioid receptor agonists for MOR, DOR, KOR and vehicle