Neuroscience Letters 619 (2016) 100–105

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Evaluation of heat hyperalgesia and anxiety like-behaviors in a rat model of orofacial cancer

Eder Gambeta, Caroline Machado Kopruszinski, Renata Cristiane dos Reis, Janaina Menezes Zanoveli*, Juliana Geremias Chichorro

Department of Pharmacology, Biological Sciences Building, Federal University of Parana, Curitiba, PR, Brazil

HIGHLIGHTS

- Rats with orofacial cancer developed heat hyperalgesia.
- Anxiety-like behavior was observed in rats with orofacial cancer.
- Lidocaine showed a transient anti-hyperalgesic effect, but did not affect anxiety-like behaviors.
- Midazolam reduced the anxiety-like behaviors, but did not modify heat hyperalgesia.

ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form 29 February 2016
Accepted 1 March 2016
Available online 4 March 2016

Keywords:
Hyperalgesia
Anxiety
Cancer
Lidocaine
Midazolam
Rats

ABSTRACT

Pain and anxiety are commonly experienced by cancer patients and both significantly impair their quality of life. Some authors claim that there is a relationship between pain and anxiety, while others suggest that there is not a direct association. In any case, there is indeed a consensus that anxiety impairs the pain condition beyond be under diagnosed and undertreated in cancer pain patients. Herein we investigated if rats presenting heat hyperalgesia induced by orofacial cancer cell inoculation would display anxiety-like behaviors. In addition, we evaluated if pain blockade would result in alleviation of anxiety behaviors, as well as, if blockade of anxiety would result in pain relief. Orofacial cancer was induced in male Wistar rats by inoculation of Walker-256 cells into the right vibrissal pad. Heat facial hyperalgesia was assessed on day 6 after the inoculation, and on this time point rats were submitted to the elevated plus maze and the light-dark transition tests. The influence of lidocaine and midazolam on heat hyperalgesia and anxiety-like behaviors was assessed. The peak of facial heat hyperalgesia was detected 6 days after cancer cells inoculation, and at this time point, rats exhibited increased anxiety-like behaviors. Local treatment with lidocaine (2%/50 μL) caused a marked reduction of heat hyperalgesia, but failed to affect the anxiety-like behaviors, while midazolam (0.5 mg/kg, i.p.) treatment failed to change the heat threshold, but induced an anxiolytic-like effect. Altogether, our data demonstrated that rats with orofacial cancer present pain- and anxiety-like behaviors, but brief heat hyperalgesia relief does not affect the anxiety-like behaviors, and vice-versa, in our experimental conditions.

1. Introduction

Head and neck cancer (HNC) encompasses a heterogeneous group of tumors originating from the tissues and organs of the head and neck that together comprise the seventh most frequent cancer worldwide [1,2]. Pain is commonly associated with HNC, as 85% of the patients report oral pain at the time of diagnosis [3,4]. The etiology of orofacial pain is multifactorial and may be related with the disease and/or the treatment.

Cancer pain during and following the treatment has been correlated with increased morbidity, impaired performance status, increased anxiety and depression with a reduction in the quality of life [5]. Of particular interest, there is evidence that anxiety plays an important role in modulating pain experience in cancer patients [6]. While some authors believe that there is a relationship between physical symptoms and the presence of anxiety, others did not find a correlation between these factors. In any case, it is widely accepted in the clinical setting that the anxiety is under

* Corresponding author.
E-mail addresses: janaina.zanoveli@ufpr.br, janainamz@hotmail.com (J.M. Zanoveli).

http://dx.doi.org/10.1016/j.neulet.2016.03.001
0304-3940/

recognized and consequently undertreated in cancer patients receiving palliative care [7–10].

In spite of the accumulating evidence that pain and anxiety are commonly experienced by cancer patients, few studies have evaluated both factors in HNC patients and the relationship between these factors have not been well studied. Consequently, the aim of this study was to investigate the presence of anxiety-like behaviors in rats presenting heat orofacial hyperalgesia induced by inoculation of Walker 256B tumor cells into the vibrissal pad. In order to explore a possible correlation between pain and anxiety we evaluated if pain blockade would result in alleviation of anxiety-like behaviors, as well as, if the blockade of anxiety would result in pain relief in rats with orofacial cancer.

2. Materials and methods

2.1. Animals

Experiments were conducted on male Wistar rats weighing 180–220 g, maintained five animals per cage at controlled temperature (22 ± 1 °C) under 12/12 h light/dark cycle (lights on at 07:00 h) with chow and water *ad libitum*. They were acclimatized in the laboratory for at least 48 h before use. Experimental procedures were conducted in accordance with the ethical guidelines of the International Association for the Study of Pain [11] and approved by UFPR's institutional Committee on the Ethical Use of Animals (authorization #848) and all efforts were made to minimize the number of animals used and their suffering.

2.2. Maintenance and inoculation of the tumor cells

Walker 256B-cells were used to induce orofacial cancer in rats, as previously described [12], with minor modifications. The cells were obtained by inoculating 1 × 107 (1 mL) tumor cells into the peritoneal cavity of the rats. Their maintenance was carried out by weekly passages through intraperitoneal inoculation at the same concentration. After 5–7 days, animals were euthanized and their ascitic fluids were collected in a solution of ethylenediaminetetraacetic acid (EDTA, 0.5 M, pH 8.0, 1:1). The viability of tumor cells was assessed by the Trypan blue exclusion method [13]. To induce the facial cancer, the animals were anesthetized intraperitoneally with xilazine (7.5 mg/kg) and ketamine (60 mg/kg) solution and 2 × 106 cells/100 μL were injected subcutaneously into the right vibrissal pad. Control animals received the same volume of vehicle (phosphate-buffered saline, PBS).

2.3. Heat stimulation

Thermal hyperalgesia on the orofacial area was measured as previously described [14]. Briefly, the animal was gently held by the experimenter and a radiant heat source was presented 1 cm from the surface of the right vibrissal pad. The response latency to display either head withdrawal or vigorous flicking of the snout was recorded (in s) using a stopwatch, and to prevent tissue damage, a 20 s cut-off time was stablished. Reaction to heat stimulation was assessed before (basal responsiveness) and on day 6 after inoculation of the cells or its vehicle.

2.4. Elevated plus-maze (EPM) test

The test was carried out as previously described [15] on day 6 after tumor inoculation, by placing the animal in the center of the apparatus followed by record of its behavior for 5 min. The time that each animal remained in the open arms, as well as the number of entries in each arm (open and enclosed) was evaluated. If the animal falls out of the open arm during the test, it was excluded.

2.5. Light-dark transition (LDT) test

The test was conducted according to Vicente and Zangrossi [16]. The animal was placed in the middle of the lit compartment facing the doorway separating the two compartments. After the first transition to the dark compartment, the behavior of the animal was recorded for an additional 5 min period. During this period, the total time spent in the lit compartment and the number of transitions between the two compartments was registered.

2.6. Drugs

Lidocaine and midazolam were obtained from Cristália Produtos Químicos Farmacêuticos (Itapira, SP, Brazil) and Hipolabor Farmacêutica (Sabará, MG, Brazil), respectively, and both were dissolved in sterile saline solution. The doses of lidocaine and midazolam were based on previous studies [17,18] and in a pilot study conducted in our laboratory (data not shown). Ketamine was obtained from Rhobifarma Ind. Farmacêutica (Hortolândia, SP, Brazil) and xylazine from Laboratórios König S.A. (Avellaneda, Argentina).

2.7. Experimental protocols

The response latency to facial heat stimulation was assessed before (pre) and 6 days after tumor cells inoculation, followed by rats treatment with a single injection of lidocaine (2%, 50 μL, s.c), midazolam (0.5 mg/kg, i.p.) or its corresponding vehicles. Heat hyperalgesia was evaluated at 30 and 15 min-intervals, respectively, up to 2 h after the treatments. In an independent group, 6 days after inoculation of tumor cells, rats were treated with lidocaine (2%, 50 μL, s.c), midazolam (0.5 mg/kg, i.p.) or its corresponding vehicles and 30 and 15 min after, respectively, the animals were submitted to the EPM and LDT tests.

2.8. Statistical analysis

All data are presented as mean ± S.E.M. (standard error of the mean). Two-way ANOVA with or without repeated measures followed by the Bonferroni post-hoc test was used to analyze the data, with condition (tumor or control) and/or drug treatment and/or time as the independent factors. When only condition factor was used as independent factor, Student *t*-test was applied. Results were considered statistically significant if $p < 0.05$.

3. Results

3.1. Local lidocaine treatment reduced heat hyperalgesia but failed to modify anxiogenic-like behaviors in tumor bearing rats

Tumor cells inoculation at the orofacial region induced the development of facial heat hyperalgesia, which was significantly reduced by local treatment with lidocaine (2%/50 μL) at 30 min after the treatment, compared with the control group (Fig. 1A, $p < 0.05$). Lidocaine treatment did not modify the response latency to the heat stimulus of animals inoculated with vehicle (Fig. 1A, $p > 0.05$). Tumor-bearing rats demonstrated an anxiogenic-like behavior (time spent on the lit compartment on LDT [condition factor: $F = (1,22) = 15.50$; $p < 0.05$], (Fig. 1B) and time spent in the open arm of EPM [condition factor: $F = (1,22) = 23.18$; $p < 0.05$]), (Fig. 1C) at the same point as heat hyperalgesia was detected (*i.e.* 6 days after cells inoculation). Local treatment with lidocaine failed to induce an anxiolytic-like effect evaluated 30 min after its injection (time in the lit compartment of LDT test [treatment