BUSPIRONE ATTENUATES TOLERANCE TO ANALGESIC EFFECT OF MORPHINE IN MICE WITH SKIN CANCER

ALIREZA MOHAJJEL NAYEBI*[1,2], HASSAN REZAZADEH[2] AND MOSTAFA NOURAFKAN[1,2]

[1]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran*
[2]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51664, Iran*

ABSTRACT

Adjuvant drugs that can delay tolerance to morphine analgesia may lead to improved management of pain in chronic disease such as cancer. This study was aimed to investigate effect of buspirone, as a partial agonist of 5-HT_{1A} receptor, on tolerance induced to morphine analgesic effect in animals with skin cancer. Study was carried on female Swiss albino mice. For skin tumorigensis, mice were treated with single dose of 7,12-dimethylbenz(a)anthracene (DMBA) and promoted by multiple dose of croton oil. Tolerance to morphine analgesia was induced by daily subcutaneous (sc) injection of morphine (5mg/kg for 30 days) and assayed by using the hot plate method. Results obtained from this study showed that pain threshold in mice with skin cancer were significantly lower. Tolerance to analgesic effect of morphine (5 mg/kg, sc) was appeared at day 15, whereas, in normal and skin tumor bearing mice co-treated daily with morphine (5 mg/kg, sc) and three different intraperitoneal (ip) doses of buspirone (5, 7.5 and 10 mg/kg) tolerance was observed at days 25 and 30. In conclusion our data indicate that concurrent use of morphine with buspirone may produce good cancer pain control and attenuate development of tolerance.

Keywords: Buspirone, morphine, tolerance, skin cancer.

INTRODUCTION

Opioids are the drug of choice for the control of severe clinical pains such as cancer patients. A major problem associated with chronic administration of opioids is tolerance to their analgesic effect which complicates the treatment of pain among patients (Arends *et al*., 1998; Cohen *et al*., 2008). The identification of adjuvant drugs that can delay the development of tolerance to opioids may lead to improved management of pain.

Neurotransmission systems that interact with opioidergic system offer a target for clinically useful strategies to block or delay opioid tolerance. According to the recent reports, N-methyl-D-aspartic acid (NMDA)-antagonists (Danysz *et al*., 2005; Bryant *et al*., 2006; Xu *et al*., 2007; Mendez and Trujillo, 2008; Xu *et al*., 2008) and nitric oxide synthase inhibitors (Santamarta *et al*., 2005; Abdel *et al*., 2006; Liu *et al*., 2006) attenuate development of tolerance to morphine in rodents. The exact biochemical mechanism(s) underlying this effect is not clearly described. There is concern over the potential adverse effects of these new pharmacological agents that may limit their clinical applicability as adjuvant in pain management (Trujillo and Akil, 1995). Also, NMDA-Antagonists and nitric oxide synthase inhibitors attenuate rather than completely inhibit the development of tolerance morphine, which suggest that other systems also play essential roles in the tolerance process.

**Corresponding author:* e-mail: nayebia@tbzmed.ac.ir

Studies suggest that alteration in serotonergic neurotransmission has a crucial role in mediating the analgesic action of morphine (Nemmani and Mogil, 2003; Dyuizen *et al*., 2004; Fang *et al*., 2005). It is well known that opioids establish part of their analgesic effect through stimulation of the serotonergic system (Arends *et al*., 1998). Acute morphine administration increases serotonin synthesis, release and metabolism (Arends *et al*., 1998), particularly in projection areas of the dorsal raphe nucleus (Jolas and Aghajanian, 1997; Nayebi and Charkhpour, 2006), but subsequent to chronic morphine administration, a decrease in the release of 5-HT from the nerve terminals is observed (Jolas *et al*., 2000). Fenfluramine attenuates tolerance development to morphine by modulating the pharmacological process (Arends *et al*., 1998). The presence of 5-HT_{1A} receptor in the brain areas involved in pain prosesses suggests a possible involvement of these receptors in pain modulation. Studies show that 5-HT_{1A} receptors are involved in mediating the 5-HT (serotonin) evoked antinociception (Xiao *et al*., 2005; Colpaert 2006). Therefore, it may be used as a new target in the search for new pharmacological approaches in the augumentation of analgesia. F 13640 is a high-efficacy 5-HT_{1A} receptor agonist that prolongs the development of tolerance to morphine in a rat model of trigeminal neuropathic pain (Deseure *et al*., 2004). According to our study, chronic injection of 5-HT_{1A} receptor agonist 8-hydroxy-2-[di-n-propylamino]tetralin (8-OH-DPAT) to dorsal raphe nucleus delays tolerance to morphine analgesia (Nayebi

and Charkhpour, 2006). Moreover, we have also shown that fluoxetine, as a specific serotonin reuptake inhibitor, prolongs development of tolerance to morphine antinociceptive effect in skin tumor bearing mice, suggesting that it may allow improvement of opioid therapy for persistent pain states, such as cancer pain (Nayebi *et al*., 2009). In the present study we investigated the effect of buspirone on tolerance induced to morphine analgesic effect in mice with skin cancer.

MATERIALS AND METHODS

Chemicals

All chemicals were obtained from Sigma Chemical Co. (USA) with the exception of morphine and buspirone that were purchased from Temad Co. (Tehran, Iran) and Heumann Co. (Germany) respectively.

Solutions were prepared fresh on the days of experimentation. Morphine sulfate and Buspirone were dissolved in physiological saline (0.9% NaCl) and 7,12-dimethylbenz(a)anthracene (DMBA) in acetone. Subcutaneous (sc) morphine (5mg/kg) and intraperitoneal (ip) buspirone (5, 7.5 and 10 mg/kg) were administered once daily for 30 days. Hot plate test was performed 30 min after drug injections.

Animals

The experiments were carried out on female Swiss albino mice weighing 25±2 g. Animals were housed in standard polypropylene cages, eight per cage (group), under a 12:12 h L/D schedule at an ambient temperature of 25 ± 2°C and were allowed food and water. The dorsal skin of the mice was shaved with electric clippers followed by the application of depilatory cream at least 2 days prior to cancer induction. Only mice that did not show signs of hair regrowth were used for experiments. All animals were accustomed to the testing conditions for 2 days before the behavioral experiment was conducted. Experiments were carried out in accordance with the guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No 85-23, revised 1985).

Cancer induction

For skin tumorigenesis, the mice were received a single topical application of DMBA 40 mg/100 mL acetone/mouse. After seven days, mice were promoted topically by applying 0.5 mg of croton oil twice weekly for a period of 30 weeks (Ashman *et al*., 1982; Rezazadeh *et al*., 2005). During this period, the mice were observed weekly for the incidence of tumors (papilomas). Only the mice which had a good yield of skin tumors (at least 3 tumors/mouse) were included for behavioral pain study (O'Connell *et al*., 1986; Nayebi *et al*., 2009).

Induction of tolerance

In order to induce tolerance to analgesic effect of morphine, mice were injected daily with morphine (5 mg/kg, sc). This dose has been found to cause profound analgesia without any side effects in mice (Drouin *et al*., 2001; Nayebi and Charkhpour, 2006). The responses to thermal stimuli 30 min after the morning dose were recorded daily by using hot plate test.

Hot plate test

Animals were individually placed on a hot plate maintained at a constant temperature (50 ± 0.5 °C). The latencies to first hind paw withdrawal during thermal stimulation were measured in seconds as an index of nociceptive threshold with cut off time of 50 sec. This index was referred to as the Pain Latency Time (Nayebi *et al*., 2009).

Statistical analysis

Descriptive statistics and comparisons of differences between each data set were calculated by use of SigmaStat software. The data were expressed as mean ± SEM, and were analyzed by one-way ANOVA in each experiment. Statistical significance was accepted at the level of $p<0.05$. In the case of significant variation ($p<0.05$), the values were compared by Tukey test.

RESULTS

1- The effect of skin cancer and morphine on pain latency time

In those group of mice which were suffering from skin cancer the pain latency time was significantly ($p<0.05$) less than normal saline-treated mice. In order to induce tolerance to morphine analgesic effect, daily sc injection of morphine was used during 30 days course. Results showed that the analgesic effect of morphine was decreased so that tolerance to its analgesic effect was appeared at the day fifteen (fig. 1).

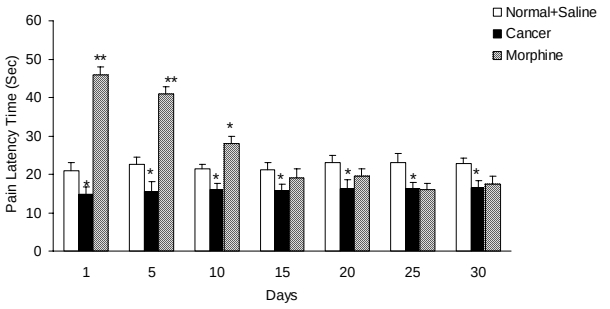

Fig. 1: Pain latency time in mice with skin cancer and daily injection of morphine (5mg/kg, sc). Each bar represents the mean±SEM of pain latency time (sec), n=8 mice for each group. *$p<0.05$ and **$p<0.01$ when compared with normal+saline group.

2- The analgesic effect of morphine in skin cancer bearing mice

Daily morphine (5mg/kg, sc) injection in mice with skin cancer caused analgesia ($p<0.05$) until the day of tenth, but its analgesic effect was decreased on latter days so that there was not any analgesic effect at day fifteen. This time the pain latency time reached to the level of saline treated-cancer bearing group (fig. 2).

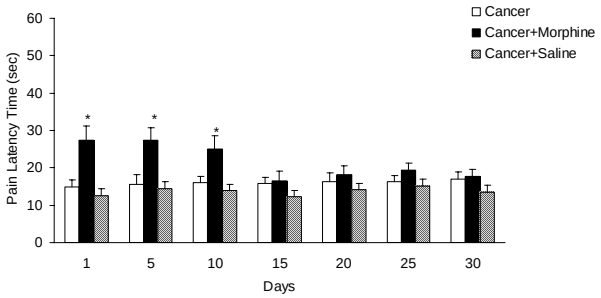

Fig. 2: The effect of daily morphine (5 mg/kg, sc) injection in mice with skin cancer. Each bar represents the mean±SEM of pain latency time (sec), n=8 mice for each group. *p<0.05 when compared with cancer and cancer+saline groups.

3- The effect of buspirone on morphine analgesia

Buspirone (5, 7.5 and 10 mg/kg, ip) was co-injected daily with morphine (5mg/kg, sc) in three groups of normal mice. As it has been shown in fig. 3, buspirone prolonged development of tolerance to morphine analgesic effect ($p<0.05$). In morphine (5 mg/kg, ip) treated mice tolerance was appeared at day fifteen, whereas in mice co-treated with morphine (5 mg/kg, ip) and 5, 7.5 and 10 mg/kg doses of buspirone tolerance occurred at days 25 and 30.

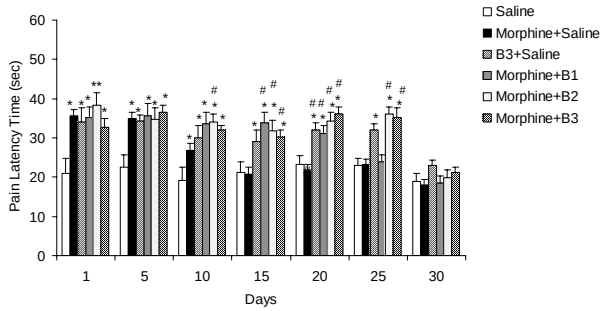

Fig. 3: The effect of buspirone (B1=5, B2=7.5 and B3=10 mg/kg, ip) on morphine (5mg/kg, sc) analgesia. Each bar represents the mean±SEM of pain latency time (sec), n=8 mice for each group. *p<0.05 when compared with saline group, #p<0.05 when compared with morphine+saline group.

4- The effect of buspirone on morphine analgesia in mice with skin cancer

Six groups of mice with skin cancer were received saline, morphine (5 mg/kg, ip) and saline, buspirone (10 mg/kg, ip) and saline, morphine (5 mg/kg, ip) and three different doses of buspirone (5, 7.5 and 10 mg/kg, ip). Results showed that buspirone increased morphine analgesic effect in a dose dependent manner, and delayed development of tolerance to morphine analgesia ($p<0.05$ and $p<0.01$). In group of mice co-treated with morphine(5 mg/kg, ip) and 5 mg/kg dose of buspirone tolerance was appeared at day 25, while in mice co-treated with morphine and 7.5 and 10 mg/kg doses of buspirone tolerance did not developed until day 30 (fig. 4).

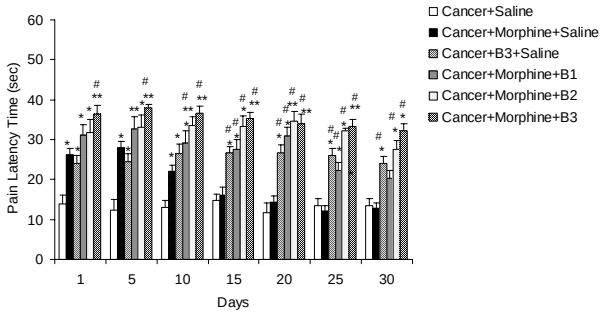

Fig. 4: The effect of daily co-injection of buspirone (B1=5, B2=7.5 and B3=10 mg/kg, ip) with morphine (5 mg/kg, sc) on pain latency time in mice with skin cancer. Each bar represents the mean±SEM of pain latency time (sec), n=8 mice for each group. *p<0.05 and **p<0.01 when compared with cancer+saline group, #p<0.05 when compared with cancer+morphine+saline group.

DISCUSSION

Opioid therapy is a major therapeutical approach for management of severe chronic pain in cancer patients (Mandalå *et al*., 2006; Cohen *et al*., 2008). Morphine has been used for many years in the treatment of moderate to severe cancer-induced pain (Hanks *et al*., 2001); however tolerance to its analgesic effect remains to be a major clinical problem. Our results show that pain threshold of mice with cancer is much less in comparison to normal mice. This confirms other study showing a tumor-induced hyperalgesia in experimental animals (Wacnik *et al*., 2003). Morphine induced analgesic effect during the first ten days in normal and skin tumor bearing mice. This analgesic effect was decreased and tolerance was appeared at day fifteen. Morphine-induced analgesia in mice with cancer was predominantly less than normal mice. This indicates that cancer may induce some pathological changes resulting supersensitivity to noxious stimuli. In general, persistent pain such as cancer-induced pain is thought to depend on the expression and release of factors such as prostaglandins (Fortier *et al*., 2008) and cytokines (Schάfers and Sorkin, 2008) at the injury site that sensitize the peripheral nerve and the subsequent sensitization at the spinal level, which leads to a potent facilitation of pain processing (Sindrup and Jensen, 1999; Woolf and Salter, 2000; Tsuda *et al*., 2004).

We observed that co-injection of morphine with buspirone increased analgesic effects of morphine and delayed development of tolerance to morphine analgesia, so that there was a shift in tolerance appearing time from day fifteen to day thirty. These results are in accordance with other studies that show 5-HT_{1A} receptor agonists prevents from morphine hyperallodynia induced by chronic morphine administration (Deseure *et al*., 2003; Xu *et al*., 2003; Deseure *et al*., 2004; Colpaert 2006; Colpaert *et al*., 2006) and also substantiate our previous study showing that intra-dorsal raphe injection of 8-OH-DPAT, as a 5-HT_{1A} receptor agonist, prolongs development of tolerance to morphine analgesic effect (Nayebi and Charkhpour, 2006). It has been reported that very-high efficacy 5-HT_{1A} receptor stimulation initiates inverse tolerance as a remarkable neuroadaptive action (Colpaert *et al*., 2002; Buritova *et al*., 2003). As analgesia decays when opioids are administered repeatedly, the analgesia produced by the selective 5-HT_{1A} agonist, F 13640, increases with repeated or chronic administration (Colpaert *et al*., 2002; Bruins Slot *et al*., 2003). Thus, we may suggest that delay in the development of tolerance to morphine analgesia results from neuroadaptation induced by 5-HT_{1A} receptor stimulation. In contrast to the earlier studies (Colpaert *et al*., 2002; Buritova *et al*., 2003), we did not observed inverse tolerance following chronic buspirone administration. This controversy might be due to differences between drugs, doses, analgesic tests and duration of treatment. It should be noted that buspirone is a partial agonist of 5-HT_{1A} receptors that has also D2 and α2-adrenoceptor blocking effects (Gower and Trickle-bank, 1988; Jadhav *et al*., 2008). Thus, it is necessary to consider the possibility of these receptors involvement in the observed effects.

Some studies have suggested that S100B, a calcium-binding protein, produced and secreted by astrocytes, is a putative target of buspirone in preventing from ethanol-associated reduction in raphe astroglia cells (Eriksen and Druse, 2001; Tajuddin *et al*., 2003). Since neuron-glia communication may lead to the development of tolerance to morphine-induced antinociception (Song and Zhao, 2001; Narita *et al*., 2004), therefore the roles of glial cells in this regard should not be ruled out.

Furthermore, Administration of buspirone to skin tumor bearing animals decreased development of tolerance to morphine analgesic effects, therefore we offer that buspirone, as a partial agonist of 5-HT_{1A}, may allow improvement of opioid therapy for cancer pain.

One of the most common psychiatric diagnosis among patients with advanced cancer is anxiety and depression (Sellick and Crooks 1999). Since 5-HT_{1A} receptor stimulants, as well as buspirone produce antinociceptive effect (Giordano and Rogers 1992; Deseure *et al*., 2003; Deseure *et al*., 2004; Xiao *et al*., 2005); therefore adjuvant therapy with buspirone may offer an appropriate strategy for prevention from development of tolerance to morphine analgesic effect, along with its anxiolytic effect.

In conclusion, our data suggest that buspirone attenuates development of tolerance to morphine analgesic effect in skin tumor bearing mice. In addition we suggest that investigation of a possible clinical application of the buspirone should be carried out to test it usefulness in diminishing tolerance to morphine. Further studies are needed to elucidate the exact mechanism of buspirone on brain neuronal systems which are responsible to development of tolerance to morphine.

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