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Pharmacology, Biochemistry and Behavior 83 (2006) 230–238
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Antinociceptive and behavioral effects of ribavirin in mice

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Received 29 September 2005; received in revised form 16 January 2006; accepted 31 January 2006
Available online 24 March 2006

Abstract

The antinociceptive effect of ribavirin, an antiviral drug, was studied after systemic injection using several pain tests in mice. In the hot-plate test of thermal pain, capsaicin-induced chemogenic pain, formalin test and abdominal stretching assay induced by the i.p. injection of 0.6% acetic acid, ribavirin produced a dose-related reduction in nociceptive responses. The visceral antinociceptive effect of ribavirin was unaffected by co-treatment with yohimbine, atropine or theophylline, but partially reversed by naloxone. Antinociception by ribavirin was augmented by treatment with prazosin, doxazosin, propranolol, guanethidine, glibenclamide, baclofen, indomethacin or cysteamine. Further, the ribavirin induced antinociception was enhanced by D2 receptor antagonists haloperidol, sulpiride, clozapine or domperidone and by the dopamine D2 receptor agonist bromocryptine. Ribavirin did not exhibit depression-like effect, nor it influenced the effect of amitriptyline in the forced swimming test. It did not impair cognitive performance in the Morris water Maze test. The present data demonstrate that ribavirin administered via systemic route possesses visceral and thermal anti-nociceptive properties. The ribavirin analgesic effect was partially reversed by naloxone, an opioid antagonist.

Keywords: Ribavirin; Thermal pain; Visceral pain; Capsaicin-induced pain; Formalin test; Mice

1. Introduction

Ribavirin (1-β-d-ribofuranosyl-1,2,4, triazole-3 carboxamide) is a broad-spectrum antiviral drug. It prevents replication of a large number of RNA and DNA viruses by inhibiting the enzyme inosine monophosphate dehydrogenase, which is required for the synthesis of guanosine triphosphate. The final step in this chain of events is lethal mutagenesis of the RNA genome (Cameron and Castro, 2001). Ribavirin has been used alone or more commonly in combination with interferon-alpha for the treatment of patients with chronic hepatitis C virus (Fontaine et al., 2000). Ribavirin decreases necroinflammation in chronic hepatitis C without causing virological clearance (Hoofnagle et al., 2003) and enhances the efficacy of interferon-alpha (Collier and Chapman, 2001), most likely by virtue of its ability to decrease the synthesis of proinflammatory cytokines (e.g., IFN-gamma) (Meier et al., 2003; Barnes et al., 2004).

Ribavirin induces a number of side effects including reversible hemolysis, mild fatigue and headaches (Hoofnagle et al., 1996; Bizollon et al., 1997). Central nervous system effects, including depression, mood changes and cognitive impairment have been described; however, these effects are usually seen only in patients receiving concurrent interferon-alpha for treatment of hepatitis (Trask et al., 2000; Kraus et al., 2005).

Ribavirin is being used in the treatment of a broad spectrum of viral infections (Prince, 2001). Despite its wide spread use, data on possible other pharmacological actions are essentially lacking. More recently, however, ribavirin was shown to down regulate the process of reactive astrogliosis after brain injury (Pekovic et al., 2005) and to suppresses tumor growth of human squamous cell carcinoma in vivo (Kentsis et al., 2004).

The present study describes the effects of ribavirin in several experimental pain models in mice: the hot-plate test, a model of supraspinal analgesia; the capsaicin-induced chemogenic pain, the formalin test and the acetic acid-induced writhing response, a model of visceral inflammatory pain (chemonociception). Moreover, the behavioral effect of ribavirin on locomotor activity and possible interaction with antidepressant drugs was evaluated. Ribavirin was administered by i.p. route so as to ensure adequate and rapid absorption of the drug. In rats and rhesus monkeys, ribavirin given intramuscularly or i.p. was effectively absorbed (Ferrara et al., 1981; Narayana et al., 2002).

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doi:10.1016/j.pbb.2006.01.010

2. Materials and methods

2.1. Animals

Swiss male albino mice 20–22 g of body weight was used. Standard laboratory food and water were provided ad libitum. Experiments were performed between 9 am and 3 pm. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), the UK Animals Scientific Procedures Act 1986 or the European Communities Council Directive of 24 November 1986 (86/609/EEC). Equal groups of 6 mice each were used in all experiments. The doses of ribavirin used in the study were based upon the human dose after conversion to that of rat according to Paget and Barnes (1964).

2.2. Hot plate assay

The hot plate test was performed by using an electronically controlled hot plate (Ugo Basile, Italy) heated to 52 °C (± 0.1 °C). The cut-off time was 30 s. Groups of mice ($n=6$/group) were given ribavirin at doses of 30, 60 or 120 mg/kg, i.p., or saline (control), 30 min prior to testing. The experimenter was blind to dose. Latency to lick a hind paw or jump out of the apparatus was recorded for the control and drug-treated groups.

2.3. Capsaicin-induced hind paw licking

Ribavirin (30, 60, 120 or 240 mg/kg) or saline (control) was given i.p., 30 min, before injection of capsaicin (1.6 μg/paw; 25 μl) under the skin of the dorsal surface of the right hind paw. Observation started after capsaicin injection and lasted for 5 min. The time (s) the animals spent licking the injected paw was determined using a stopwatch (Sakurada et al., 1992).

2.4. Formalin test

Mice were placed in the observation chamber for an initial 20-min accommodation interval to familiarize them with their surroundings. Mice received ribavirin (30, 60, 120 or 240 mg/kg, i.p.) or saline (control), 30 min prior to the test. Mice were injected with 20 μl of 2.5% formalin solution in normal saline subcutaneously into the plantar surface of the left paw with a 26-guage needle fitted to a microsyringe. Pain behavior was quantified by counting the number of flinch behaviors from 0–10 min and 10–30 min after the injection. This includes lifting, shaking and overt flinching that manifests as a ripple over the haunch (Corrêa and Calixto, 1993).

2.5. Acetic acid-induced writhing

Separate groups of 6 mice each were administered vehicle and/or drug (30–240 mg/kg, i.p.). After 30 min pretreatment interval, an i.p. injection of 0.6% acetic acid was administered (Koster et al., 1959). Each mouse was then placed in an individual clear plastic observational chamber, and the total number of writhes made by each mouse was counted for 30 min after acetic acid administration. In another series of experiments, the effects of indomethacin (5 mg/kg, i.p.) on anti-writhing induced by ribavirin (120 mg/kg, i.p.) were examined. Indomethacin was administered alone or combined with ribavirin 30 min prior to the abdominal constriction assay.

Further experiments were designed in an attempt to elucidate the mechanisms by which ribavirin exerts its anti-nociceptive effect. The dose of 120 mg/kg of ribavirin was selected to be used in the subsequent experiments.

Thus, the effect of co-administration of the alpha-1 adrenoreceptor antagonists prazosin (2 mg kg, i.p.) and doxazosin (16 mg/kg, i.p.), the alpha-2 adrenoreceptor antagonist yohimbine (4 mg/kg, i.p.), the beta adrenoreceptor antagonist, propranolol (2 or 4 mg/kg, i.p.), the adrenergic blocker, guanethidine (16 mg/kg, i.p.), the muscarinic acetylcholine receptor antagonist atropine (2 mg/kg, i.p.), the non-selective opioid receptor antagonist naloxone (5 mg/kg, i.p.), the non-selective adenosine receptor antagonist theophylline (20 mg/kg, i.p.), the GABA agonist baclofen (5 mg/kg, i.p.), and the potassium channel blocker glibenclamide (5 mg/kg, i.p.) were examined on antinociception caused by ribavirin. Furthermore, the effect of the centrally acting dopamine D2 receptor antagonists, sulpiride (5 or 10 mg/kg, i.p.) and haloperidol (0.4, 1 or 2 mg/kg, i.p.), the peripherally acting D2 receptor antagonist domperidone (5 or 10 mg/kg, i.p.) or D2 receptor agonist bromocryptine (1.5 or 3 mg/kg, i.p.), the D2 and D4 receptor antagonist clozapine (1.5 or 3 mg/kg, i.p.) was examined. All drugs were administered 30 min prior to the abdominal constriction assay. In addition, the effect of pretreatment with cysteamine (300 mg/kg, p.o.), which depletes the tissues somatostatin (Szabo and Reichlin, 1981), on antinociception caused by ribavirin was examined. Cysteamine was administered 2 h prior to ribavirin.

2.6. Rotarod testing

Motor performance was measured as the latency to fall from an accelerating rotarod located over plates connected to an automatic counter (Ugo Basile, Varese, Italy). Mice were trained to remain on a rotating rod for 2 min as the rod rotated toward the animal. After the 2-min training period, the mice were administered vehicle (saline) or drug (60–120 mg/kg, i.p.) and 30 min later placed on the rotating rod as it accelerated from 4 to 40 rpm over 5 min and the time that they could remain on the accelerating rod was noted (Millan et al., 1994). The cutoff time was 600 s. The time was measured from the start of the acceleration period. The test was repeated 2 h after vehicle or drug injection. Six animals were used per dose and for the controls.

2.7. Porsolt's forced-swimming test

Each mouse was placed individually in a glass cylinder (diameter 12 cm, height 24 cm) filled with water at a height of 12 cm. Water temperature was maintained at 22–23 °C.

The animal was forced to swim for 6 min and the duration of immobility was measured. The mouse was considered as immobile when it stopped struggling and moved only to remain floating in the water, keeping its head above water. The floating time, which is used as the measure of despair (Porsolt et al., 1977), was recorded after treatment with saline, ribavirin (30–120 mg/kg, i.p.), imipramine (15 mg/kg, i.p.), amitriptyline (15 mg/kg, i.p.) or co-administered ribavirin (120 mg/kg, i.p.) and amitriptyline (15 mg/kg, i.p.).

2.8. Cognitive testing

The Morris water Maze (MWM) was performed to test spatial learning and memory. The MWM is a paradigm that requires the rat to use spatial memory to find a hidden platform just below the surface of a pool of water, and to remember its location from the previous trial (Morris, 1984). Therefore, the rat must use distal cues to effectively locate it. Accurate navigation is rewarded by escape from the pool. The maze consisted of a glass tank, narrowed to 20 cm wide, 40 cm in height, 70 cm in length, filled to a depth of 21 cm with water maintained at 25 °C. The escape glass platform was hidden from sight, submerged 1 cm below the surface of the water at the end of the tank (Dunnett et al., 2003). The effect of ribavirin (120 mg/kg, i.p.) on working memory was studied in mice pretreated with scopolamine (1 mg/kg, i.p.) to induce cognitive impairment (Smith et al., 2002). Mice were pre-treated with scopolamine alone (1 mg/kg, i.p.) or in combination with ribavirin (120 mg/kg, i.p.) ($n=6$/group) 60 min prior to testing. Mice rapidly learn to swim directly to the escape platform and climb out. Once the mice reached the platform, it remained there for 15 s (trial 1; reference memory or acquisition trial). At the end of each trial, the rat was towel dried, returned to its home cage (where a heat lamp was available), and 3 min elapsed before the next trial (trial 2; working memory or retrieval trial), which used the same platform location and start position as trial 1. The latency to find the platform (s) is assessed with a stopwatch.

3. Drugs

Ribavirin (Virazole, October Pharma, Cairo, ARE), guanethidine, propranolol hydrochloride, yohimbine hydrochloride, naloxone hydrochloride, capsaicin (Sigma, St. Louis, USA), imipramine hydrochloride, bromocryptine (Novartis Pharma, Cairo, ARE.), amitriptyline hydrochloride, haloperidol, indomethacin (Kahira Pharm and Chem. IND Co., Cairo, ARE), glibenclamide (Hoechst Orient, Cairo, ARE), atropine sulphate, baclofen (Misr Pharm Co., Cairo, ARE), domperidone (Janssen-Cilag, Switz), clozapine (APEX Pharma, Cairo, ARE) were used. Analytical-grade glacial acetic acid (Sigma, St. Louis, USA) was diluted with pyrogen-free saline to provide a 0.6% solution for i.p. injection. All drugs were dissolved in isotonic (0.9% NaCl) saline solution immediately before use. Indomethacin was dissolved in a 5% solution of sodium bicarbonate. Stock solutions of capsaicin (10 mg/ml) contained 10% ethanol, 10% Tween 80, 80% saline solution.

4. Statistical analyses

Data are expressed as mean ± S.E. The effects of different drugs used in the abdominal constriction assay are also expressed as percent inhibition (%) compared to the control value. Differences between vehicle (control) and treatment groups were determined by using one- and two-way ANOVA followed by multiple comparison by the Tukey's honestly significant difference. A probability value less than 0.05 was considered statistically significant.

5. Results

5.1. Antinociceptive effects of ribavirin

5.1.1. Hot plate assay

The mean reaction time on the hot plate was significantly delayed at 30 min and 1 h after the administration of ribavirin (30, 60 or 120 mg/kg, i.p.), compared with basal values, denoting decreased nociception ($P<0.05$, one-way ANOVA) (Fig. 1). The anti-nociceptive effect of the agent was produced with 30 mg/kg and maximal increase in hot-plate latency by 87.1% 30 min after injection at the dose of 120 mg/kg. Compared with basal values hot plate latency increased at 30 min and 1 h after drug injection by 60.7 and 41.5% after 30 mg/kg ribavirin; by 69.5 and 42.4% after 60 mg/kg ribavirin and by 87.1% and 37.1% after 120 mg/kg ribavirin, respectively.

5.1.2. Capsaicin-induced hind paw licking

The duration of paw licking following intraplantar capsaicin injection was significantly reduced by 59.1, 75.2% and 86.9% after 60, 120 or 240 mg/kg ribavirin, respectively (Fig. 2).

5.1.3. Formalin test

There was a dose-dependent effect of ribavirin treatment to decrease the nocifensive responses after formalin injection into

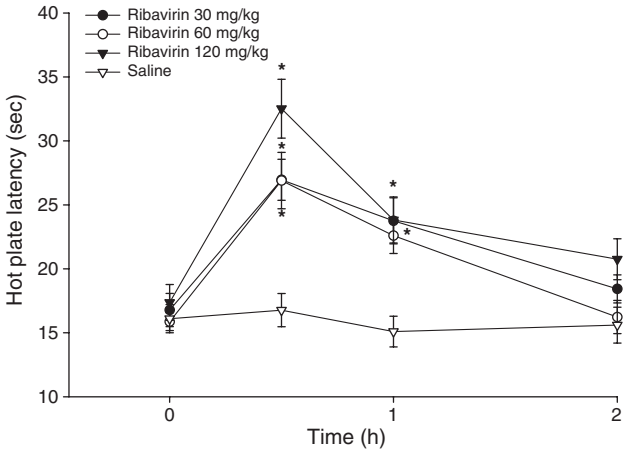

Fig. 1. Hot plate latency (s) of saline (control, $n=6$) and ribavirin-treated mice. Ribavirin (30, 60 or 120 mg/kg, i.p, $n=6$/group) or saline (control) was administered 30 min prior to testing. Data expressed as mean ± S.E. $*p<0.05$ (vs. control). $+p<0.05$ (vs. 30 mg/kg ribavirin).

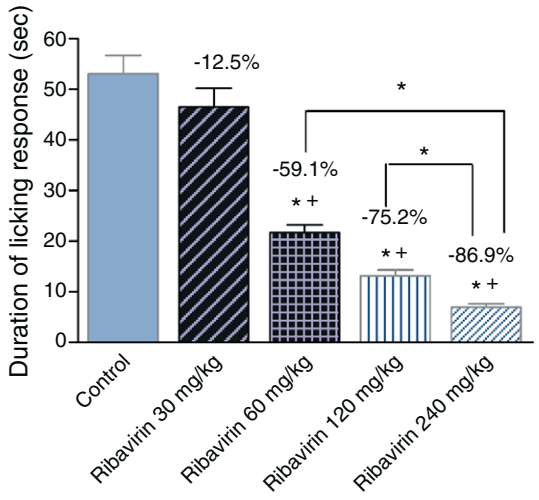

Fig. 2. Effect of ribavirin on the duration of licking response to capsaicin injection in mice. Mice received saline (control, $n=6$) or ribavirin (30, 60, 120 or 240 mg/kg, i.p., $n=6$/group) 30 min prior to the test. Data expressed as mean±S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (vs. control). $+p<0.05$ (vs. ribavirin 30 mg/kg).

the hind paw with the two highest doses of the drug (120, 240 mg/kg) significantly different from control at both phase 1 and phase 2 (Fig. 3).

5.1.4. Acetic acid induced writhing

Ribavirin (30–240 mg/kg, i.p.) dose-dependently inhibited the number of writhes induced by i.p. acetic acid in mice by 17.8–96.8% (Fig. 4A). When indomethacin (5 mg/kg, i.p.) was administered in combination with ribavirin (120 mg/kg, i.p.), an additive effect was noted (Fig. 4B).

Fig. 5 shows that the analgesic effect of ribavirin on writhing was unaffected by co-administration of the alpha (2)-

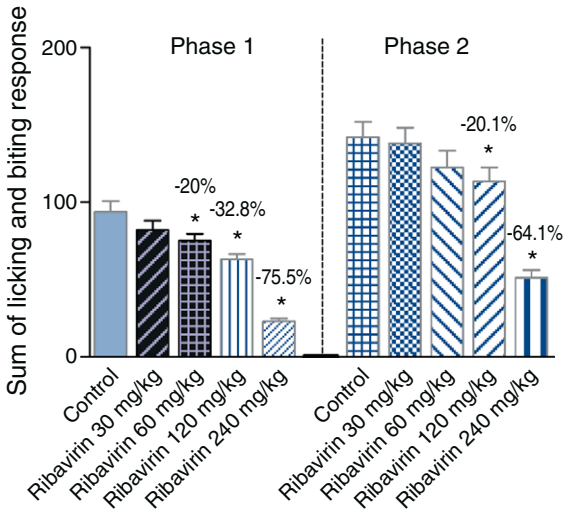

Fig. 3. Effect of ribavirin on the formalin-induced nociceptive responses. Mice received saline (control, $n=6$) or ribavirin (30, 60, 120 or 240 mg/kg, s.c., $n=6$/group) 30 min prior to the test. Flinching behaviors after formalin injection were summarized as phase 1 (0–10 min) and phase 2 (10–30 min). Data expressed as mean±S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (vs. control).

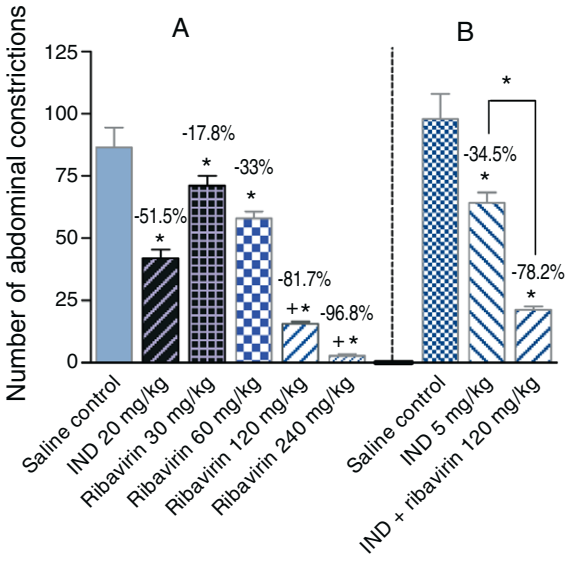

Fig. 4. (A) Effect of ribavirin (30, 60 or 120 mg/kg, i.p., $n=6$/group) or indomethacin (IND; 20 mg/kg, i.p., $n=6$) on abdominal constrictions caused by i.p. injection of 0.6% acetic acid in mice. Drugs were administered 30 min prior to acetic acid injection. Data expressed as mean±S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (vs. control), $+p<0.05$ (vs. indomethacin-treated group). (B) Effect of indomethacin (IND; 5 mg/kg, i.p., $n=6$) alone or co-administered with ribavirin (120 mg/kg, i.p., $n=6$) on abdominal constrictions caused by i.p. acetic acid in mice. Drugs were administered 30 min prior to acetic acid injection. Data expressed as mean±S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (compared to control and between the IND and IND+ribavirin-treated groups).

adrenoreceptor antagonist yohimbine (4 mg/kg i.p.), the unselective muscarinic receptor antagonist atropine (2 mg/kg, i.p.) or theophylline (20 mg/kg, i.p.), a non selective adenosine receptor antagonist. Yohimbine or theophylline administered

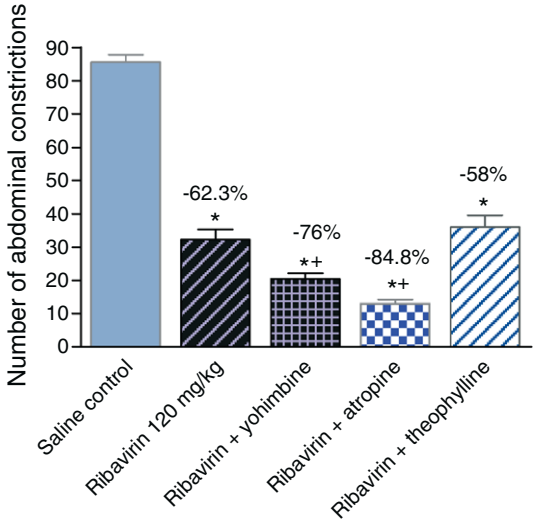

Fig. 5. Effect of yohimbine (4 mg/kg, i.p., $n=6$), atropine (2 mg/kg, i.p., $n=6$) or theophylline (20 mg/kg, i.p., $n=6$) on antinociception caused by ribavirin (120 mg/kg, i.p.) in the mouse abdominal constriction assay. Ribavirin was administered alone or in combination yohimbine, atropine or theophylline, 30 min prior to the test. Data represent mean±S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (compared to control). $+p<0.05$ (vs. ribavirin alone or ribavirin+theophylline-treated groups).

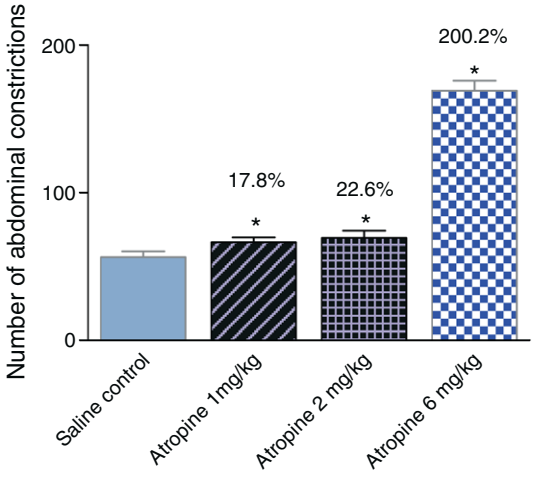

Fig. 6. Effect of atropine (1, 2 or 6 mg/kg, i.p., $n=6$/group) on abdominal constrictions caused by i.p. injection of 0.6% acetic acid in mice. Data expressed as mean±S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (vs. control).

alone increased the nociceptive response (data not shown). Fig. 6 shows that atropine increased visceral pain in a dose-dependant manner. Meanwhile, co-treatment with the alpha (1)-adrenoreceptor antagonists prazosin (2 mg/kg, i.p.) or doxazosin (16 mg/kg, i.p.) enhanced antinociception by ribavirin (Fig. 7). The co-administration of glibenclamide (5 mg/kg, i.p.), an ATP-gated sodium channels blocker or baclofen (5 mg/kg, i.p.), a GABA-ergic drug enhanced the effect of ribavirin (Fig. 7). Beta adrenoreceptor blockade with propranolol at 2 mg/kg, i.p., failed to influence the effect of ribavirin, but a higher dose of 4 mg/kg enhanced the effect of ribavirin in the abdominal constriction assay. Propranolol itself

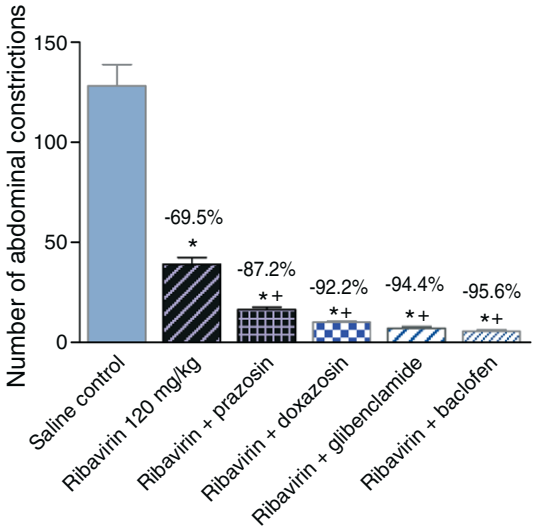

Fig. 7. Effect of prazosin (2 /kg, i.p., $n=6$), doxazosin (16 mg/kg, i.p., $n=6$), gibenclamide (5 mg/kg, i.p., $n=6$) or baclofen (5 mg/kg, i.p., $n=6$) on antinociception induced by ribavirin (120 mg/kg, i.p., $n=6$) in the abdominal constriction assay. Drugs were administered 30 min prior to the test. Data represent mean±S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (compared to control and between different groups as shown in the figure). $+p<0.05$ (vs. the ribavirin alone-treated group).

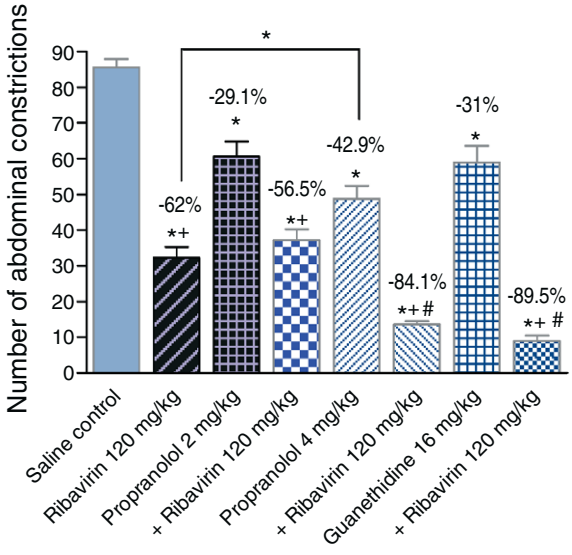

Fig. 8. Effect of propranolol (2 or 4 mg/kg, i.p., $n=6$/group) or guanethidine (16 mg/kg, i.p., $n=6$) on antinociception caused by ribavirin (120 mg/kg, i.p., $n=6$) in the mouse abdominal constriction assay. Drugs were administered 30 min prior to the test. Data represent mean±S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (compared to control and between different groups as shown in the figure). $+p<0.05$ vs. propranolol (2 mg/kg) or guanethidine-treated groups. The (#) sign indicates significant difference from the ribavirin alone-treated group.

exerted dose-dependent inhibition of the writhing response (Fig. 8). The antinociceptive effect of ribavirin was in addition enhanced by co-administration of the adrenergic neurone

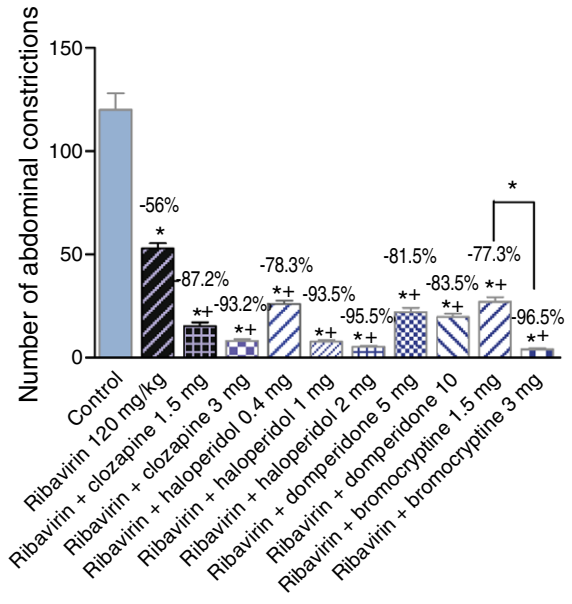

Fig. 9. Effect of dopamine D2 receptor antagonists haloperidol (0.4, 1 or 2 mg/kg, i.p., $n=6$/group), domperidone (5 or 10 mg/kg, i.p., $n=6$/group), the D2 and D4 receptor antagonist clozapine (1.5 or 3 mg/kg, i.p., $n=6$/group) ,and the D2 agonist bromocryptine (1.5 or 3 mg/kg, i.p., $n=6$) on antinociception caused by ribavirin (120 mg/kg, i.p., $n=6=6$) in the mouse abdominal constriction assay. Drugs were administered 30 min prior to the test. Data represent mean±S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ compared to control. $+p<0.05$ (vs. ribavirin alone-treated group).

blocker guanethidine (16 mg/kg, i.p.). In this test paradigm, guanethidine reduced the number of abdominal constrictions when administered alone (Fig. 8).

The ribavirin induced antinociception was also enhanced by co-treatment with the centrally acting dopamine D2 receptor antagonists haloperidol (0.4, 1 or 2 mg/kg) or sulpiride (10 or 20 mg/kg, i.p.), by the peripherally acting D2 receptor antagonist domperidone or by bromocryptine a dopamine D2 receptor agonist (1.5 or 3 mg/kg). In addition, clozapine; a centrally acting dopamine D2 and D4 receptor antagonist enhanced antinociception by ribavirin (Figs. 9 and 10A). Opioid receptor blockade with naloxone (5 mg/kg, i.p.) reduced the antinociception caused by ribavirin in the abdominal constriction assay (Fig. 10B). As shown in Fig. 11, cysteamine, which depletes tissue somatostatin augmented antinociception caused by ribavirin.

5.1.5. Rotarod testing

Ribavirin (30–120 mg/kg) did not produce any significant changes on the rotarod performances of the mice. Both controls and ribavirin-treated mice remained on accelerating rotarod during the acceleration period (5 min) and for 5 min thereafter (data not shown).

5.1.6. Porsolt's forced-swimming test

The floating time, was not significantly changed in mice treated with ribavirin (30–120 mg/kg, i.p.), but significantly reduced by 63.4% in rats given imipramine (15 mg/kg, i.p.) (Fig. 12A). Ribavirin (120 mg/kg, i.p.) given concurrently with amitriptyline (15 mg/kg, i.p.) had no significant effect on the antidepressant activity of the latter (Fig. 12B).

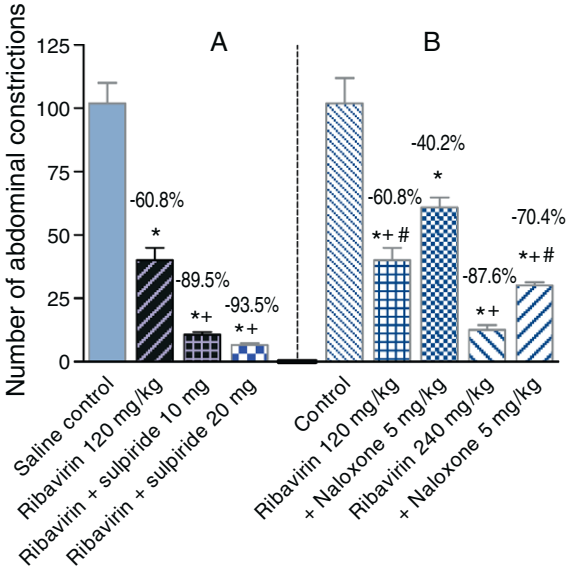

Fig. 10. (A) Effect of dopamine D2 receptor antagonist sulpiride (5 or 10 mg/kg, i.p., $n=6$/group) on antinociception caused by ribavirin (120 /kg, i.p., $n=6$) in the mouse abdominal constriction assay. Drugs were administered 30 min prior to the test. Data expressed as mean ± S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (compared to control). $+p<0.05$ (vs. ribavirin alone-treated group). (B) Effect of the non-selective opioid receptor antagonist naloxone on antinociception caused by ribavirin (120 or 240 mg/kg, i.p., $n=6$/group) in the mouse abdominal constriction assay. Drugs were administered 30 min prior to the test. Data expressed as mean ± S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (compared to control). $+p<0.05$ (vs. the naloxone-treated group). The sign (#) indicates significant difference from the ribavirin (240 mg/kg)-treated group.

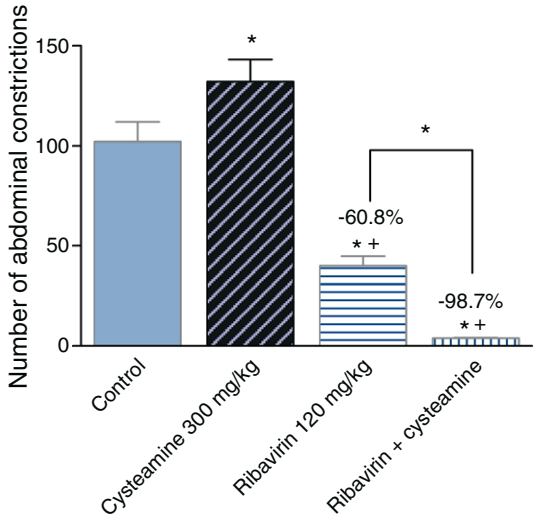

Fig. 11. Effect of cysteamine (300 mg/kg, i.p., $n=6$) on antinociception caused by ribavirin (120 mg/kg, i.p., $n=6$) in the mouse abdominal constriction assay. Cysteamine was administered 2 h prior to ribavirin injection. Acetic acid injection was carried out 30 min later. Data expressed as mean ± S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (compared to control). $+p<0.05$ (vs. cysteamine-treated group).

5.1.7. Cognitive testing

Scopolamine (1 mg/kg, i.p.) impaired cognitive performance leading to higher latencies to locate the plateform. Ribavirin (120 mg/kg, i.p.) co-administered with scopolamine (1 mg/kg, i.

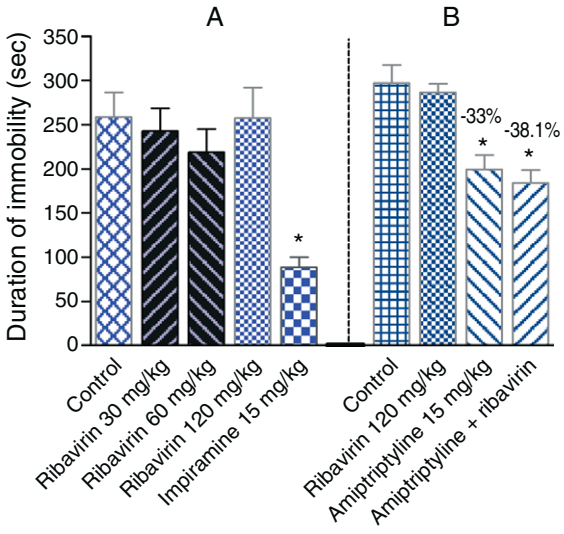

Fig. 12. (A) Effect of ribavirin (30, 60 or 120 mg/kg, i.p., $n=6$/group) or imipramine (25 mg/kg, i.p., $n=6$) on the duration of immobility in Porsolt's forced-swimming test. (B) Effect of ribavirin (120 mg/kg, i.p., $n=6$), amitriptyline (15 mg/kg, i.p., $n=6$) or ribavirin (120 mg/kg, i.p., $n=6$) combined with amitriptyline (15 mg/kg, i.p., $n=6$) on the duration of immobility in Porsolt's forced-swimming test. Data expressed as mean ± S.E and percent inhibition (%) compared to the control animals. $*p<0.05$ (compared to control).

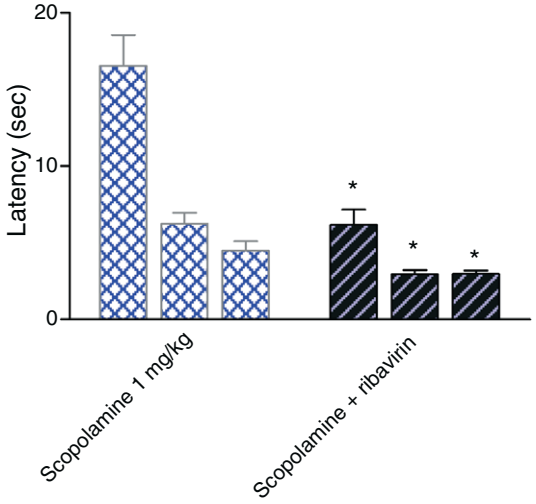

Fig. 13. Effect of scopolamine (1 mg/kg, i.p., $n=6$) alone or or ribavirin (120 mg/kg, i.p., $n=6$) co-administered with scopolamine (1 mg/kg, i.p., $n=6$) on the latency to locate the submerged platform in the Morris water Maze test. Data expressed as mean±S.E. $*p<0.05$ (vs. scopolamine-treated group).

p.) resulted in lower latencies to locate the submerged platform, thereby reducing the cognitive impairing effects of scopolamine (Fig. 13).

6. Discussion

Data from the present study suggest for the first time that the antiviral drug ribavirin possesses antinociceptive properties. Responses to noxious chemical and thermal stimuli were determined in the acetic acid-induced abdominal constriction assay, formalin- and capsaicin-induced licking, and hot-plate tests, four models of acute pain. The present study indicates that systemic administration of ribavirin attenuated the nociceptive response to thermal stimulation and reduced pain behavior caused by formalin or capsaicin injection. In addition, ribavirin exerted a marked analgesic effect on visceral pain caused by i.p. injection of acetic acid in mice. Taken together, these data suggest that ribavirin may modulate inflammatory pain. Ribavirin at doses, which caused effective anti-nociception, did not impair mouse performance evaluated by the rotarod test, thus ruling out the confounding influence of a possible sedative effect. The mechanism by which ribavirin modulates pain transmission is likely to involve opioid sensitive pathways. This is because the action of ribavirin in the abdominal constriction assay was reduced by prior treatment with naloxone, a non-selective opioid receptor antagonist.

In the present study an attempt was made elucidate other possible mechanisms by which ribavirin exert its analgesic effect. Other neurotransmitter systems, such as purinergic, cholinergic, catecholaminergic, GABAergic, dopaminergic were also evaluated. Glibenclamide, an ATP-gated sodium channels blocker and cysteamine, a selective depletor of somastostatin were tested as well. Adenosine is an inhibitory neuromodulator that can increase nociceptive thresholds in response to noxious stimulation (Sawynok, 1999; Wu et al., 2005) and blockade of adenosine receptors by theophylline, a non-selective adenosine receptor antagonists at A_1 and A_2 receptors, was shown to induce hyperalgesia (Paalzow, 1994). The possible contribution of adenosine receptor mechanism was ruled out by the finding that theophylline, failed to alter the ribavirin antinociception.

Muscarinic acetylcholine receptors modulate nociception at the level of the spinal cord (Li et al., 2002). Spinally administered muscarinic receptor agonists or acetylcholinesterase inhibitors can produce effective analgesia (Naguib and Yaksh, 1994). Evidence also indicates that primary sensory neuronal M2 receptors may represent a viable peripheral target for the treatment of pain and inflammation (Dussor et al., 2004). In the mouse acetic acid writhing test, M1-muscarinic agonists increased the pain threshold (Bartolini et al., 1992), whilst atropine, a cholinergic muscarinic antagonist administered at high doses of 5 mg/kg, resulted in hyperalgesia (Ghelardini et al., 1990). In the present study, blockade of muscarinic acetylcholine receptors with atropine (2 mg/kg, i.p.) reduced nociceptive threshold in this model of visceral pain, but failed to alter antinociception by ribavirin.

The involvement of alpha (2)-adrenoreceptor mechanism in antinociception induced by ribavirin is unlikely, since the alpha (2)-adrenoreceptor antagonist, yohimbine, did not reduce the antinociception induced by ribavirin. Co-treatment with, the alpha (1)-adrenoreceptor antagonists, prazosin or doxazosin, however, augmented the ribavirin response. In this test paradigm, alpha (1)-adrenoreceptor antagonists displayed antinociceptive properties (Korzeniewska-Rybicka and Plaznik, 2001 and present study).

The results of the present study also indicated that the non-selective beta-adrenoreceptor antagonist, propranolol (2 or 4 mg/kg, i.p.) and the adrenergic neurone blocker guanethidine (16 mg/kg, i.p.) enhanced antinociception by ribavirin. Behavioral, neurophysiological and clinical evidence showed that most forms of pain arising from the gastrointestinal tract are mediated by activity in visceral afferent fibres running in sympathetic nerves and that the afferent innervation of the gut mediated by parasympathetic nerves is not primarily concerned with the signalling and transmission of gastrointestinal pain (Cervero, 1988). Pain perception depends on the presence of functional voltage-gated sodium channels. Voltage-gated sodium channel (Nav1.7) is found predominantly in sensory and sympathetic neurons (Wood et al., 2004). Beta adrenoreceptor antagonists e.g., propranolol and metoprolol have been shown to reduce visceral pain caused by i.p. injection of acetic acid in rat (Korzeniewska-Rybicka and Plaznik, 2001). Propranolol is able to negatively modulate trigeminal nociception through antagonism of β_1 receptors on thalamocortical neurons (Shields and Goadsby, 2005). Propranolol, injected into the dorsal periaqueductal gray have ‘anxiolytic’ effects in the plus maze (Audi et al., 1991). Propranolol also possesses a membrane stabilizer activity (Pearce et al., 1983). This latter property is being potentially relevant to antinociceptive activity.

Sympathetic block relieves visceral pain e.g., relief of visceral pain caused by carcinoma of the pancreas, stomach, gall bladder or liver by coeliac plexus block (Brown et al., 1987; Eisenberg et al., 1995). Pain relief may result from the interruption of afferent

nociceptive fibres, which accompany the sympathetic nerves. The efferent viscero-visceral reflexes are also interrupted so that ischemia and spasm are relieved. Chemical sympathectomy attenuates visceral nociceptive responses and spontaneous activity of sacral spinal cord neurons, without effect on convergent cutaneous inputs, both under physiological and inflammatory conditions (Kalmari et al., 2001). Guanethidine, which impairs the release of norepinephrine from presynaptic sympathetic neurons, has been shown to reduce the number of abdominal constrictions induced by acetic acid in mice (Duarte et al., 1988). Guanethidine also has local anesthetic activity (Brock and Cunnane, 1988), which might be relevant to its observed antinociceptive effect.

Antinociception by ribavirin is also enhanced by baclofen, a prototypical agonist for $GABA_B$ receptors. Baclofen alters nociception at the level of the spinal cord by acting on $GABA_B$ receptors located on primary afferent terminals and is known to produce analgesia in man and animals (Dirig and Yaksh, 1995; Hara et al., 2004).

Dopamine D2 receptors are involved in modulation of nociceptive responses (Rooney and Sewell, 1989; Frussa-Filho et al., 1996; Taylor et al., 2003). The rostral agranular insular cortex, a cortical area that receives a dense dopaminergic projection and is involved in descending antinociception and there is evidence to suggest that dopamine may acts tonically in the cortex to inhibit nociception, an effect mediated through descending nociceptive inhibition of spinal neurons (Burkey et al., 1999). Therefore, the involvement of the dopamine receptors in antinociception induced by ribavirin was investigated. Findings in the present study indicated that the inhibition of the acetic acid-evoked abdominal constriction responses by ribavirin was augmented by a number of drugs acting on dopamine D2 receptors. Haloperidol and sulpiride, both are centrally acting dopamine D2 receptor antagonists, clozapine a centrally acting dopamine D2 and D4 receptor antagonist as well as bromocryptine, a centrally acting dopamine D2 receptor agonist, all appeared to enhance antinociception by ribavirin. These data suggest an interaction of ribavirin at the level of dopamine D2 receptors.

In the mouse writhing test, the D2 dopaminergic agonist bromocriptine decreases abdominal constrictions; this analgesic effect being attenuated by haloperidol (Frussa-Filho et al., 1996). Although dopamine D2 antagonists exert antinociceptive effects, they also potentiate the analgesic action of other agents. Haloperidol (2 mg/kg), for example, increased the antinociceptive effect of morphine in tail flick and in writhing test in mice (Petrov, 1987). Similarly, in the mouse tail immersion test, both D-2 receptor agonists and dopamine D-2 receptor antagonists potentiated opioid-induced antinociception (Rooney and Sewell, 1989).

Studies indicated a clinical association of neuropsychiatric symptoms, depressive episodes, cognitive impairment and the use of combination therapy of ribavirin and interferon-alpha in patients with hepatitis C virus infection (Chutaputti, 2000; Bonaccorsoa et al., 2000; Collier and Chapman, 2001; Fried et al., 2002). Therefore, in the present study, the effect of ribavirin was investigated in animal models related to depression and memory impairment. The forced swim test developed by Porsolt et al. (1977) is the most widely used tool for detecting novel antidepressants. Rats and mice, when placed in an inescapable cylinder of water initially make energetic attempts to escape, but their activity thereafter decreases, and they assume an immobile posture. The duration of immobility, which is a measure of despair, is reduced by antidepressants. In the present study, ribavirin did not display depressant-like activity in the swimming test. The immobility time was not significantly different from that of the saline control group, but was markedly reduced by the tricyclic drugs imipramine and amitriptyline. Ribavirin, in addition, did not reduce the anti-depressant effect of amitriptlyine when both agents were administered together. In the working memory version of the Morris water Maze, mice were pretreated with scopolamine to elicit a cognitive deficit and assessed for their ability to find a hidden platform. The present study indicated that ribavirin administration did not impair cognitive performance. Furthermore, the present results suggest that ribavirin rather enhanced the ability of mice to learn the spatial memory task. The mechanism by which ribavirin exert this effect is not clear, but its worthy to mention in this respect that ribavirin effectively cross the blood brain barrier and has been used successfully in the treatment of a number of viral infections (Ferrara et al., 1981; Smee et al., 1981). Ribavirin decreased the number of reactive astrocytes after adult brain injury and formation of glial scar (Pekovic et al., 2005). Results of the present study indicate that ribavirin can modulate acute pain. It is suggested that ribavirin might act within the central nervous system to enhance descending antinociceptive pathways. In this same way, changes in central adrenergic, serotonergic and opioid pathways may be involved in the effect of ribavirin on cognitive learning.

In summary, the present study indicates that ribavirin can be effectively absorbed after systemic administration so as to modulate nociception and behavior.

Acknowledgement

The author acknowledges Wael Zedan and Mohamed Abd-Elaziz for their technical assistance in the experiments.

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