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# Characterization of the antinociceptive and anti-inflammatory activities of riboflavin in different experimental models

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**Abstract**

Riboflavin, similar to other vitamins of the B complex, presents anti-inflammatory activity but its full characterization has not yet been carried out. Therefore, we aimed to investigate the effect of this vitamin in different models of nociception, edema, fever and formation of fibrovascular tissue. Riboflavin (25, 50 or 100 mg/kg, i.p.) did not alter the motor activity of mice in the rota-rod or the open field models. The second phase of the nociceptive response induced by formalin in mice was inhibited by riboflavin (50 or 100 mg/kg). The first phase of this response and the nociceptive behavior in the hot-plate model were inhibited only by the highest dose of this vitamin. Riboflavin (25, 50 or 100 mg/kg, i.p.), administered immediately and 2 h after the injection of carrageenan, induced antiedema and antinociceptive effects. The antinociceptive effect was not inhibited by the pretreatment with cadmium sulfate (1 mg/kg), an inhibitor of flavokinase. Riboflavin (50 or 100 mg/kg, i.p., 0 and 2 h) also inhibited the fever induced by lipopolysaccharide (LPS) in rats. Moreover, the formation of fibrovascular tissue induced by s.c. implant of a cotton pellet was inhibited by riboflavin (50 or 100 mg/kg, i.p., twice a day for one week). Riboflavin (10 or 25 mg/kg, i.p.) also exacerbated the effect of morphine (2, 4 or 8 mg/kg, i.p.) in the mouse formalin test. In conclusion, the study demonstrates the antinociceptive and anti-inflammatory activities of riboflavin in different experimental models. These results, associated with the fact that riboflavin is a safe drug, is approved for clinical use and exacerbates the antinociceptive effect of morphine, may warrant clinical trials to assess its potential in the treatment of different painful or inflammatory conditions.
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*Keywords:* Riboflavin; Inflammation; Pain; Nociception; Edema; Fever; Fibrovascular tissue

## 1. Introduction

B vitamins have been described as useful drugs to treat some pathological conditions, particularly painful disorders, not necessarily associated with their deficiency. Supplying pyridoxine can relieve the pain associated with neuropathic disorders and carpal tunnel syndrome (Folkers et al., 1984; Bernstein, 1990; Bernstein and Dinesen, 1993). It has also been demonstrated that the analgesic effect of nonsteroidal anti-inflammatory drugs is increased in patients simultaneously treated with the combination thiamine/pyridoxine/cyanocobalamin (Bruggemann et al., 1990; Kuhlwein et al., 1990). Clinical trials have also shown the utility of high doses of riboflavin in the prophylactic treatment of migraine (Schoenen et al., 1998; Sándor et al., 2000; Boehnke et al., 2004). Moreover, this vitamin has been used in the treatment of other painful disorders related to some metabolic diseases (Tanaka et al., 1997a,b; Napolitano et al., 2000). Some studies carried out in experimental animals have also demonstrated the antinociceptive effect of the combination thiamine/pyridoxine/cyanocobalamin (Bartoszyk and Wild, 1990; Zimmermann et al., 1990; França et al., 2001), although the mechanisms involved are not clear. These vitamins also enhance the antinociceptive effect of nonsteroidal anti-inflammatory drugs (Bartoszyk and Wild, 1989; Zimmermann et al., 1990; Reyes-García et al., 1999).

Recently, the anti-inflammatory effects induced by another B complex vitamin, riboflavin, were demonstrated (França et al., 2001; Granados-Soto et al., 2004; Toyosawa et al., 2004a,b;

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Table 1
Effect induced by riboflavin (50 or 100 mg/kg, i.p.) on the time mice spent on the rota-rod and on their exploratory behavior in the open field ($n=6$)

| Treatment | Time (s) spent in the rota-rod | | | Exploratory behavior in the open field (crossed rectangles) |
|---|---|---|---|---|
| | Basal | 30 min | 60 min | |
| Vehicle | 60±0 | 56±1 | 58±0 | 26±3 |
| Rf 50 | 59±4 | 60±0 | 59±2 | 25±4 |
| Rf 100 | 60±0 | 56±2 | 60±0 | 30±2 |

Kodama et al., 2005; Verdrengh and Tarkowski, 2005). Riboflavin presented antinociceptive activity in models such as abdominal constrictions induced by acetic acid and licking behavior induced by formalin (França et al., 2001). It also reduced the thermal hyperalgesia induced by carrageenan (Granados-Soto et al., 2004). Moreover, riboflavin inhibited the paw edema induced by formalin (França et al., 2001) and olive oil (Verdrengh and Tarkowski, 2005) in mice and by carrageenan in rats (Granados-Soto et al., 2004), suggesting that it also has anti-inflammatory properties. Supporting this hypothesis, it has been demonstrated that riboflavin reduces the lipopolysaccharide (LPS)-induced synthesis of the inflammatory cytokines tumor necrosis factor (TNF) α, interleukin (IL)-1 and IL-6 (Toyosawa et al., 2004a,b; Kodama et al., 2005).

Although some clinical studies evaluated the usefulness of riboflavin to alleviate migraine and also the pain associated with some metabolic disorders, we have not found any study that investigated if this vitamin may also be useful to alleviate the pain associated with tissue injury or inflammation, a common burden to many patients. Similarly, there is a reduced number of studies that evaluated the effect of riboflavin in experimental models of acute pain and inflammation. Thus, the aim of the present study was to investigate the effects induced by riboflavin in different experimental models of nociception and inflammation in order to provide further information that may contribute to better evaluate the potential usefulness of riboflavin in the treatment of painful or inflammatory conditions that are not necessarily associated with its deficiency. Such proposal is justified as riboflavin is already approved for clinical use and the incidence of side effects associated with its use is rather low (Tanaka et al., 1997a; Schoenen et al., 1998; Boehnke et al., 2004).

## 2. Materials and methods

### 2.1. Animals

Female Swiss mice (20–25 g) and female Wistar rats (200–250 g) were used. The animals had free access to food and water and were maintained in a room with a 12 h light–dark cycle. The experiments were carried out at a room temperature of 27 °C, which corresponds to the thermoneutral zone for rodents (Gordon, 1990). All experiments were conducted according to the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983) and approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais.

### 2.2. Evaluation of the motor activity and exploratory behavior of mice

The motor activity of the animals was evaluated in a rota-rod apparatus. The day before the experiment, the animals were trained on the apparatus. On the experiment day, the animals were placed on a rota-rod (12 rpm) and the time they remained on the apparatus was determined. The cut-off time was 1 min. After determination of the baseline values, the animals were treated with riboflavin and 30 min later they were again tested in the apparatus. To evaluate the exploratory behavior, mice were placed in a 40×33 cm plastic box with 17 cm-high walls. Four straight lines were drawn on the floor of the box dividing it in 9 rectangles (11×13.3 cm each). The number of crossed rectangles in 1 min was determined 30 min after treatment with vehicle or riboflavin.

### 2.3. Evaluation of the nociceptive response of mice in the hot-plate model

Thirty minutes after treatment with riboflavin, the animals were placed on a heated (55 °C) metal plate (20×20 cm with 18 cm-high walls) and the latency for a nociceptive behavior, e.g. lifting or licking the hindpaws, was determined. Mice were removed from the hot plate immediately after the response. The cut-off time was 30 s.

### 2.4. Evaluation of the nociceptive response in the mouse formalin test

In this model, formalin (2.5%, 20 μl) was injected via the subcutaneous (s.c.) route into the dorsum of the right hindpaw of mice 30 min after the intraperitoneal (i.p.) administration of riboflavin, morphine or the combination of these two drugs. Each mouse was placed under a transparent glass funnel (18 cm diameter, 15 cm-high) and the amount of time that the animal licked the injected paw was monitored between 0 and 5 min (first phase) and 15 and 30 min (second phase) after the injection of formalin.

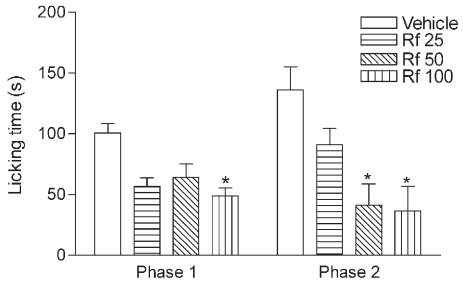


Fig. 1. Effect induced by riboflavin (25, 50 or 100 mg/kg, i.p.; −30 min) on the nociceptive response induced by formalin (2.5%; 20 μl; s.c.) in mice ($n=6$). * Significantly different from vehicle ($P<0.05$).

Table 2
Effect induced by riboflavin (25, 50 or 100 mg/kg, i.p.; −30 min) on the nociceptive response in the hot-plate model in mice ($n=8$)

| Treatment | Latency (s) |
|---|---|
| Vehicle | 9.6±1.4 |
| Rf 25 | 10.8±1.3 |
| Rf 50 | 10.8±1.1 |
| Rf 100 | 17.1±3.1[a] |

[a] Significantly different from vehicle ($P<0.05$).

### 2.5. Evaluation of mechanical allodynia induced by carrageenan in rats

Mechanical allodynia was measured with a 40 mN nylon filament (Sorri, Brazil) as previously described (Souza et al., 2002). Briefly, the rats were kept individually in Perspex boxes (20×20 cm with 18 cm-high walls) whose floor was a metal grid through which the filament was pressed on the plantar surface of the hindpaws with the strength just necessary to cause it to bend for approximately 1 s. The number of withdrawal reflexes was determined in a trial of 10 touches for each rat. The basal withdrawal frequency was determined before administration of any drug. After determination of the basal withdrawal frequency, the animals were divided in the experimental groups in such a way that the mean withdrawal frequencies of the different groups were similar. Riboflavin was administered immediately and 2 h after intraplantar (i.pl.) injection of carrageenan (500 μg, 50 μl). The withdrawal frequency was measured at different times after injection of the inflammatory stimulus. To evaluate the effect of cadmium on the antinociceptive effect induced by riboflavin, this cation was administered 24 h before the injection of the vitamin.

### 2.6. Evaluation of the edema induced by carrageenan in rats

Paw edema was measured with a plethysmometer (Model 7140, Ugo Basile, Italy). The basal volume of the right hindpaw was determined before administration of any drug. After determination of the basal volume, the animals were divided in the experimental groups in such a way that the mean volumes of the different groups were similar. Riboflavin was administered immediately and 2 h after i.pl. injection of carrageenan (500 μg, 50 μl). The paw volume was measured at different times after injection of the inflammatory stimulus. The results were presented as the paw volume variation in relation to the basal values.

### 2.7. Febrile response induced by lipopolysaccharide (LPS) in rats

Colonic temperature was measured with a digital thermometer (Cole Parmer, Model 8403) connected to a colorectal probe (YSI, Model 700), which was introduced 5 cm through the rectum. The basal temperature was determined as the mean of two measurements and the animals were divided in the experimental groups in such a way that the mean basal temperatures of the different groups were similar. LPS (50 μg/kg) was injected in one of the tail veins. Riboflavin was administered immediately and 2 h after intravenous (i.v.) injection of LPS. The colonic temperature was measured at different times after injection of the inflammatory stimulus. Fever index (expressed in °C h), i.e., the area under the curve over the 8-h monitoring period, was used for statistical analysis.

### 2.8. Fibrovascular tissue growth induced by s.c. cotton pellet implant in mice

Two cotton pellets (10 mg) were subcutaneously introduced into dorsum of mice anesthetized with ketamine (70 mg/kg) and xylazine (14 mg/kg). The treatment with riboflavin was initiated on the next day. Mice received one or two (12-h interval) i.p. injections of riboflavin for seven days. At the 8th day, the animals were euthanized in a $CO_2$ chamber. The cotton pellets

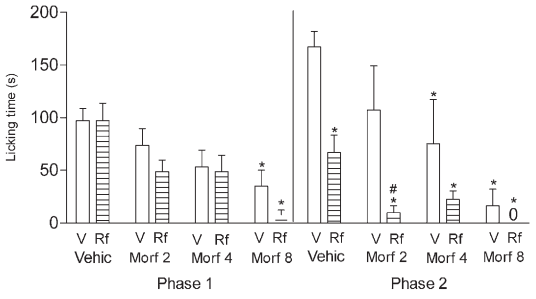


Fig. 2. Effect induced by morphine (2, 4 or 8 mg/kg; i.p.; −30 min), riboflavin (25 mg/kg; i.p.; −30 min) or the combination morphine–riboflavin in the mouse formalin (2.5%; 20 μl; s.c.) test ($n=6$). * and # Significantly different from vehicle+vehicle or the corresponding group treated with the same dose of morphine+vehicle, respectively ($P<0.05$).

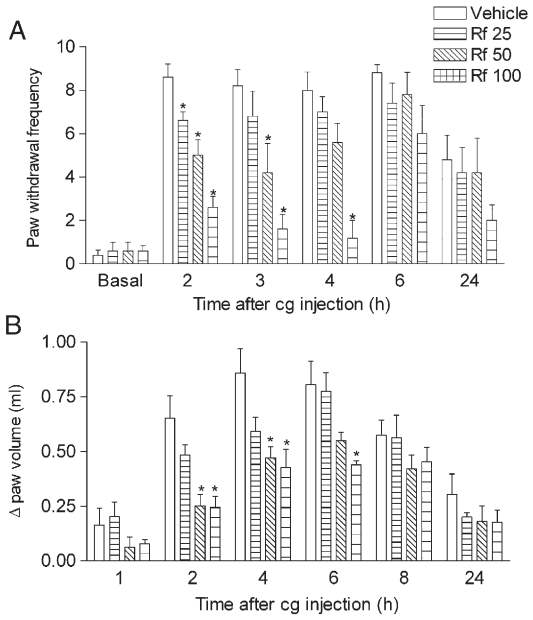


Fig. 3. Effect induced by riboflavin (25, 50 or 100 mg/kg; i.p.; 0 and 2 h) on the mechanical allodynia (A) and paw edema (B) induced by carrageenan (1%, 50 μl, i.pl.) in rats ($n=6$). The basal paw volume of the groups treated with vehicle, Rf 25, Rf 50 and Rf 100 were 1.20±0.04; 1.20±0.04; 1.23±0.04; 1.16±0.02 ml, respectively. * Significantly different from vehicle ($P<0.05$).

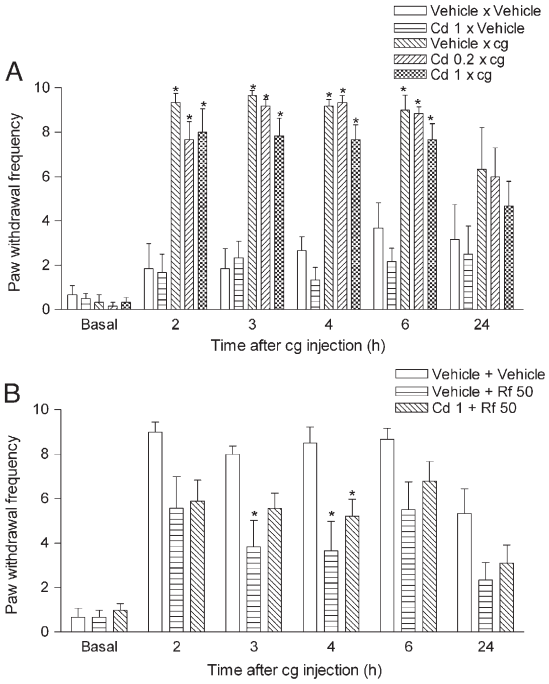


Fig. 4. (A) Effect induced by cadmium sulfate (0.2 or 1 mg/kg; i.p.; −24 h) on the paw withdrawal frequency and on the mechanical allodynia induced by carrageenan (1%, 50 μl, i.pl.) in rats ($n=5$). (B) Effect induced by riboflavin (50 mg/kg; i.p.; 0 and 2 h) on the mechanical allodynia in animals previously treated with cadmium sulfate (1 mg/kg; i.p.; −24 h) ($n=5$). * Significantly different from vehicle×vehicle (A) or vehicle+vehicle (B) ($P<0.05$).

with the surrounding fibrovascular tissue were removed, dried at 37 °C for 24 h and weighted. The results were expressed as the difference between the initial (10 mg) and the final dry weight.

### 2.9. Drugs

Riboflavin (Sigma, USA), λ-carrageenan (Sigma, USA), *E. coli* LPS 0127:B8 (Sigma, USA), formaldehyde (Ecibra, Brazil), cadmium sulfate (Sigma, USA), morphine sulfate (Cristália, Brazil), ketamine (Ketamina Agener®, Agener-União, Brazil), xylazine (Calmium®, Agener-União, Brazil) were used. Solutions and suspensions were prepared in saline immediately before the experiments. The volume of i.p. injection was 4 ml/kg (mice) or 2 ml/kg (rats).

### 2.10. Statistical analysis

The results, presented as mean±S.E.M., were analyzed by Student *t* test or one-way analysis of variance followed by Newman–Keul's post hoc test when the main effect was significant. A $P<0.05$ was considered significant.

## 3. Results

Table 1 shows that riboflavin did not alter the time mice spent in the rota-rod apparatus or the exploratory behavior evaluated in the open field model. The animals of all the experimental groups performed close to perfect in the rota-rod apparatus, indicating that the test may not be sufficiently challenging. However, the test, as carried out in the present study, allows the observation of the sedative effects induced by other drugs (Gavioli et al., 2003; Miyamoto, 2006). In addition, other studies have not shown any evidence of motor incoordination or muscle relaxing effects induced by riboflavin (França et al., 2001; Granados-Soto et al., 2004).

Fig. 1 shows that riboflavin inhibited both the first and second phases of the nociceptive response in the mouse formalin test. The first phase was inhibited only by the highest dose of riboflavin (100 mg/kg), while the second phase was inhibited to a similar extent by the doses of 50 and 100 mg/kg. The highest dose of riboflavin (100 mg/kg) also increased the latency for the nociceptive behavior in the hot-plate model (Table 2).

Fig. 2 shows that riboflavin exacerbated the antinociceptive activity of morphine. In this protocol, a fixed dose of riboflavin (25 mg/kg) was administered immediately before vehicle or different doses of morphine. The reduction of licking time induced by morphine in the animals that were not previously treated with riboflavin was 35, 55 and 90% for the doses of 2, 4 and 8 mg/kg, respectively. In the animals previously treated with riboflavin, these doses of morphine inhibited the nociceptive behavior by 95, 87 and 100%, respectively.

Riboflavin also inhibited the mechanical allodynia (Fig. 3A) and the paw edema (Fig. 3B) induced by carrageenan. The antinociceptive effect was dose-dependent and the duration of this effect was also related to dose. Riboflavin (25, 50 or 100 mg/kg) reduced the nociceptive response until 2, 3 and 4 h after carrageenan injection, respectively. Riboflavin also inhibited the paw edema induced by carrageenan in a dose

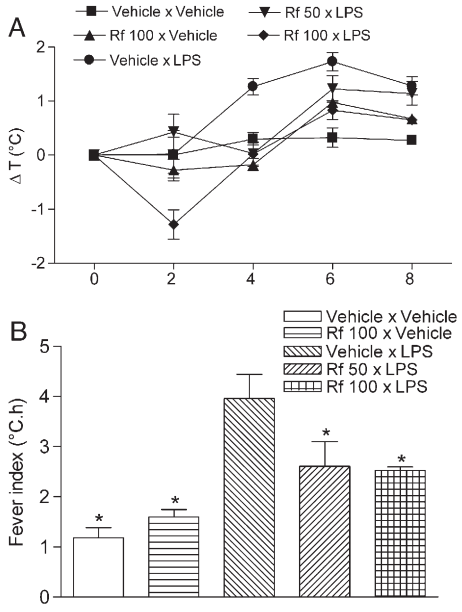


Fig. 5. (A) Effect induced by riboflavin (50 or 100 mg/kg; i.p.; 0 and 2 h) on the febrile response induced by LPS (50 μg/kg; i.v.) in rats ($n=6$). (B) Fever index. The basal temperature of the groups treated with vehicle×vehicle, Rf 100×vehicle, vehicle×LPS, Rf 50×LPS and Rf 100×LPS were 36.8±0.1; 36.8±0.1; 37.0±0.1; 37.0±0.1 and 37.1±0.1 °C, respectively. * Significantly different from vehicle×LPS ($P<0.05$).

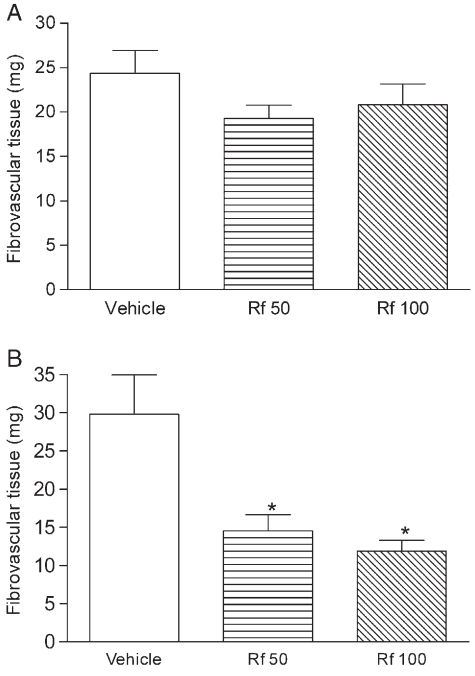


Fig. 6. Effect induced by one (A) or two (B) administrations of riboflavin (50 or 100 mg/kg, i.p., 7 days) on the formation of fibrovascular tissue induced by s.c. implantation of cotton pellet in mice ($n=10$). * Significantly different from vehicle ($P<0.05$).

dependent manner. The time course of the effect maintained a pattern similar to that observed in the model of mechanical allodynia. Administration of a single dose of riboflavin 30 min before injection of carrageenan, similarly to the protocol used in the nociceptive response induced by formalin, did not inhibit the mechanical allodynia (data not shown).

Next, the effect induced by cadmium, a cation that inhibits riboflavin conversion to flavin mononucleotide (FMN), on the antinociceptive activity of riboflavin was evaluated. Cadmium sulfate (0.2 or 1 mg/kg; i.p.; −24 h) altered neither the paw withdrawal frequency nor the mechanical allodynia induced by carrageenan (Fig. 4A). The antinociceptive effect induced by riboflavin (50 mg/kg) in this model was still observed in the animals previously treated with cadmium sulfate (1 mg/kg; i.p.; −24 h) (Fig. 4B).

Fig. 5 shows that riboflavin (50 or 100 mg/kg) inhibited the febrile response induced by LPS. In addition to its antipyretic effect, the highest dose of riboflavin reduced the colonic temperature of the animals 2 h after injection of LPS. Riboflavin (50 or 100 mg/kg) also inhibited the formation of fibrovascular tissue induced by a cotton pellet implant when administered twice a day (Fig. 6B). No reduction of the formation of fibrovascular tissue was observed after treatment with riboflavin (50 or 100 mg/kg) once a day (Fig. 6A).

## 4. Discussion

The present study demonstrated the antinociceptive and anti-inflammatory effects of riboflavin in different experimental models. The inhibition of the nociceptive behavior does not seem to result from sedative or muscle relaxant effects as riboflavin altered neither the time mice spent on the rota-rod nor the exploratory behavior in the open field.

Initially, riboflavin inhibited both the first and second phases of the nociceptive response induced by formalin. Riboflavin was more effective at inhibiting the second phase of this response. Both the doses of 50 and 100 mg/kg inhibited the second phase, while only the highest dose inhibited the first phase. This result may be due to the difference in pain intensity and nociceptive mechanisms between the first and second phases. We have previously demonstrated the antinociceptive activity of riboflavin in the mouse formalin test (França et al., 2001), although in that study only the second phase was inhibited. Inhibition of only the second phase of the formalin test in rats was also demonstrated by Granados-Soto et al. (2004). The explanation for these different results may be the doses of riboflavin used in the studies. The dose of riboflavin that inhibited the first phase of the formalin test in the present study (100 mg/kg) was higher than those used by França et al. and Granados-Soto et al. The highest dose of riboflavin (100 mg/kg), which inhibited the first phase of the formalin test, also enhanced the latency for the nociceptive response in the hot plate.

The nociceptive response in the formalin (first phase) and hot-plate models seems to result from direct activation of nociceptors by chemical (Tjϕlsen et al., 1992) and thermal stimuli (Le Bars et al., 2001) and are inhibited by drugs which act mainly at central sites (Loh et al., 1976; Mesdjian et al., 1983; Dubuisson and Dennis, 1977; Hunskaar and Hole, 1987). Although riboflavin inhibited these nociceptive responses, the dose necessary to induce such effects was higher than those that inhibited the second phase of the formalin test. This phase is attributed mainly to the development of an inflammatory reaction at the site of injection and increased synaptic transmission in the spinal cord. In addition to being inhibited by centrally acting drugs, the second phase is markedly inhibited by drugs which induce its effects mostly through anti-inflammatory mechanisms (Tjϕlsen et al., 1992). Thus, a marked inhibition of the second phase of the formalin test associated with a reduced inhibition of the first phase, only observed with higher doses, may give support to the hypothesis that the profile of riboflavin resembles more that of anti-inflammatory and less that of centrally acting drugs.

The effects induced by riboflavin on the inflammatory response induced by carrageenan were also evaluated. Administration of a single dose of riboflavin, 30 min before injection of carrageenan, did not inhibit the mechanical allodynia (data not shown). However, two administrations of this vitamin, immediately and 2 h after carrageenan, markedly inhibited both the mechanical allodynia and the paw edema. Since these responses present a long time course and the half-life of riboflavin is about 60 min (Christensen, 1969), a protocol with two administrations of this vitamin was used to demonstrate its effects. Similarly, Granados-Soto et al. (2004) demonstrated that riboflavin inhibits thermal hyperalgesia and paw edema induced by carrageenan, giving further support to our results.

Riboflavin also presented activity in a model of systemic inflammation, the febrile response induced by LPS. The

antipyretic effect induced by riboflavin was short lasting, being observed only in the fourth hour. Probably, this is related to the short half-life of riboflavin and a longer effect would require repeated administrations of the vitamin, more than the two used in the present study. Such approach is not very useful in the model of the febrile response induced by LPS, as the injection results in behavioral stress that may increase the colonic temperature. An unexpected finding was an early reduction of the colonic temperature presented by the animals treated with LPS and the highest dose of riboflavin (100 mg/kg). Although the reason for such effect is not clear, it can not be assumed as the explanation for the antipyretic effect induced by riboflavin. This conclusion is supported by the observation that a lower dose (50 mg/kg) of this vitamin did not reduce the colonic temperature, but markedly inhibited the febrile response induced by LPS.

We also investigated the effect induced by riboflavin on a more prolonged inflammatory response, the formation of fibrovascular tissue induced by s.c. implantation of a cotton pellet. Two, but not one, daily administrations of riboflavin markedly inhibited the formation of the fibrovascular tissue, indicating that this vitamin also inhibits the proliferative phase of the inflammatory response.

Altogether, the results already described give support to a proposal that the mechanisms of action of riboflavin resemble more those of anti-inflammatory drugs. However, there is a reduced number of studies that directly investigated the effect induced by riboflavin on the synthesis and action of different inflammatory mediators that are involved in the formalin test, the mechanical allodynia and edema induced by carrageenan, the febrile response induced by LPS and the formation of fibrovascular tissue induced by implantation of cotton pellet. Recently, Toyosawa et al. (2004a,b) and Kodama et al. (2005) demonstrated that riboflavin inhibits the synthesis of some inflammatory cytokines, including TNF-α, IL-1β, IL-6, interferon (IFN) γ, macrophage inflammatory protein-2 and monocyte chemotactic protein (MCP) 1, and also nitric oxide, induced by LPS in mice. Although these are the only studies that investigated the effect induced by riboflavin on the synthesis of inflammatory mediators, they may help in understanding the mechanisms associated with the antinociceptive, antiedema, antipyretic and antiproliferative activities of this vitamin.

There are evidences of the involvement of cytokines and chemokines, such as IL-1β, IL-6, TNF-α and MCP-1 (Cunha et al., 1992, 2000; Chen et al., 1994; Tanaka et al., 2004), and nitric oxide (Salvemini et al., 1996) in the inflammatory response induced by carrageenan. Cytokines (Oluyomi et al., 1994; Chichorro et al., 2004) and nitric oxide (Moore et al., 1991) have also been demonstrated to play a role in the nociceptive response induced by formalin. Some cytokines, mainly IL-1β (Long et al., 1990), IL-6 (LeMay et al., 1990) and IFNγ (Dinarello et al., 1984), are also considered endogenous pyrogens mediating the febrile response induced by LPS. Providing support to the pleiotropic activity of these inflammatory mediators, many cytokines have also been demonstrated to play important roles in the formation of the fibrovascular tissue (Scapini et al., 2004; Barcelos et al., 2005). Considering that riboflavin may also inhibit the production of inflammatory cytokines induced by the inflammatory stimuli used in our study, this may represent a putative mechanism to explain the different activities described.

Aiming to get further information about the mechanisms associated with the activities of riboflavin, we investigated the involvement of the coenzymes derived from riboflavin, FMN and flavin adenine dinucleotide (FAD). Animals were pretreated with cadmium sulfate, an inhibitor of flavokinase. This enzyme catalyzes the first reaction of riboflavin metabolism, its conversion to FMN. Defining a cadmium dose to be used in vivo is a difficult task, as it presents a high toxicity and there is a reduced number of in vivo studies in which this cation was used. The dose of 1 mg/kg of cadmium sulfate (which corresponds to 0.44 mg/kg of cadmium) was chosen because, in preliminary experiments, higher doses induced apparent toxic effects. As cadmium competes with riboflavin for the active site of the enzyme (Bandyopadhyay et al., 1997), an intermediate dose (50 mg/kg) of this vitamin was used to increase the likelihood of achieving a reversal of the antinociceptive activity. The pretreatment with cadmium did not alter the effect induced by riboflavin in the model of mechanical allodynia induced by carrageenan. However, the involvement of FMN and FAD in the activity of riboflavin in this model can not be wholly put aside since it is not possible to assure that the dose of cadmium inhibited flavokinase to an extent that could affect riboflavin activity. This result emphasizes the need of better pharmacological tools to evaluate in vivo the role of riboflavin conversion to FMN and FAD in the anti-inflammatory activities of this vitamin.

Riboflavin markedly enhanced the antinociceptive effect induced by morphine in the formalin test. The combination of an ineffective dose of morphine (2 mg/kg) to an effective dose of riboflavin (25 mg/kg) induced an effect similar to that induced by the higher dose of the opioid analgesic (8 mg/kg). Although the mechanisms that contribute to this positive interaction between riboflavin and morphine are not clear, they indicate that this vitamin, in addition to $\alpha_2$-adrenoceptor agonists (Goyagi et al., 1999), nonsteroidal anti-inflammatory drugs (Fletcher et al., 1997), NMDA receptor antagonists (Sevostianova et al., 2005), among others, may represent an alternative to reduce the doses of analgesic opioids and, consequently, their side effects.

In conclusion, the present study demonstrates the antinociceptive and anti-inflammatory activities of riboflavin in different experimental models, which probably result from multiple mechanisms. These results, associated with the fact that riboflavin is a safe drug, is approved for clinical use and exacerbates the antinociceptive effect of morphine, may warrant clinical trials to assess its potential in the treatment of different painful or inflammatory conditions.

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