Iraqi Journal of Veterinary Sciences

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Interaction of meloxicam and phenylbutazone on the level of cyclooxygenase 2 in mice

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Article information

Article history:
Received January 25, 2022
Accepted May 8, 2022
Available online September 7, 2022

Keywords:
Cyclooxygenase-2
Isobolographic
Meloxicam
Mice
Phenylbutazone

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Abstract

The reason for the recent study was to inspect the therapeutic efficacy of meloxicam and phenylbutazone alone with their analgesic interaction and their subsequent inhibitory interaction at the level of cyclooxygenase-2 in mice. Meloxicam and phenylbutazone had the analgesic-median effective doses (ED50s) of 15.57 and 119.73 mg/kg, i.p., respectively, given once to mice separately as determined by the up-and-down procedure using a hot plate method. The estimated analgesic ED50s for meloxicam and phenylbutazone combination were at 12.84 and 98.75 mg/kg, i.p., correspondingly when given together at a ratio of 1:1 of their ED50s. The isobolographic analysis reveals that the analgesic interaction between meloxicam and phenylbutazone was antagonistic, as indicated by the interaction index of 1.65. The ELISA technique was used to estimate the cyclooxygenase-2 activity, reflecting that meloxicam or phenylbutazone significantly inhibited the cyclooxygenase-2 activity by 72 and 90%, respectively, compared to the control group. The combination composed of meloxicam and phenylbutazone has a lower limit of inhibition of the cyclooxygenase-2 activity (33%) in comparison to meloxicam or phenylbutazone. Meloxicam and phenylbutazone coadministration were significantly different from the control, meloxicam, and phenylbutazone groups concerning the cyclooxygenase-2 activity in mice. The sum of the data concluded that meloxicam and phenylbutazone have an excellent analgesic efficacy when administered alone. In contrast, the mixture of these two drugs has no benefit because of the antagonistic interaction at cyclooxygenase-2 in mice.

DOI: 10.33899/IJVS.2022.132859.2140,

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs), for instance, meloxicam and phenylbutazone, are of many benefits in human and veterinary medicine because of their analgesic, anti-inflammatory, and antipyretic properties (1-4). Meloxicam and phenylbutazone work by the non-selective mechanism of action by inhibiting the cyclooxygenase enzyme (prostaglandin-endoperoxide synthase) of both isoforms (1,2), which then decreases the production of prostaglandin E_2 (a chemical autacoids mediator which plays a vital role in inducing pain, inflammation, and fever) (5,6). Meloxicam is considered to have a more excellent selectivity among NSAIDs for inhibition of the inducible isoform of the cyclooxygenase-2 than the house-keeping cyclooxygenase-1, while phenylbutazone act by a similar degree of inhibition of both enzymes, which indicated a less potent activity on cyclooxygenase-2 than meloxicam (5,6). Meloxicam and phenylbutazone are considered drugs highly among NSAIDs that bound to plasma proteins approximately more than 99% (7-9), and this pharmacokinetic property may affect the

therapeutic efficacy and subsequent toxicity of each other through their possible interaction at the pharmacokinetic and pharmacodynamic levels. In addition, phenylbutazone can induce the microsomal enzymes, a vital component accountable for the phase-I of metabolism of own and other drugs given together (5,6).

The reason for the recent trial was to study the therapeutic efficacy of meloxicam and phenylbutazone with their kind and degree of pharmacodynamic interaction besides their inhibitory interaction on the level of cyclooxygenase-2 in mice.

Materials and methods

Laboratory animals and drugs preparation

Mice Albino Swiss from both genders weighing between 22-30 g were kept at 20 °C for 14 hours dark and 10 hours light routine besides consuming water and food were allowed freely. Experimental drugs comprising meloxicam (2%, Intracin Pharmaceuticals, India) and phenylbutazone (20%, Interchemie, Holland) were prepared by dilution in normal saline to acquire the anticipated dose which will be injected into mice intraperitoneally (i.p.).

Ethical consideration

Trials, including laboratory mice, were followed-up by the academic board of the Department of Physiology, Biochemistry, and Pharmacology at the University of Mosul's Veterinary Medicine College.

Assessment of the analgesic effect

The analgesic ED_{50s} of either meloxicam or phenylbutazone were assessed for each drug alone using the up-and-down technique (10-14). The technique is illustrated by giving an initial dose of meloxicam or phenylbutazone at 10 and 250 mg/kg, i.p. Both medications' dosages were lowered or increased by 30% (3 and 75 mg/kg, i.p., respectively), conferring the initial dose used (10). The thermal method was applied by using the hot-plate (Panlab, Spain) to evaluate the analgesic response of the two drugs mentioned. The hot-plate was fixed at 56 °C of temperature then, and the mice were separately sited at the center of the hot-plate and recorded for the pre-injection response times of pain, which were hind paw drawing, licking, or jumping. After 30 minutes once meloxicam or phenylbutazone treatment, the post-injection time response of pain was also documented. The induction of analgesic effect was then predicted if the post-injection time was beyond the pre-injection time. Mice may be left on a hot-plate for 20 seconds to avoid skin injury to paws (15).

Isobolographic analysis

The analgesic ED_{50}s for meloxicam and phenylbutazone jointly as 1:1 of their ED_{50}s be specified via the up-and-down procedure mentioned previously (10). The initial doses for meloxicam and phenylbutazone were 15.57 and 119.73 mg/kg, i.p., respectively, equal to their ED_{50}S found in the previous experiment. Mice were measured separately after 30 min of both drugs' administration using the hot-plate of the thermal method illustrated above. Later, dosages of the two drugs are decreased or elevated by 25% (3.90 and 29.93 mg/kg, respectively) from the initial dosage injected before conferring to the occurrence or absence of analgesia.

To assess the kind of analgesic interaction involving meloxicam and phenylbutazone administration in mice, meloxicam (15.57 mg/kg, i.p.), phenylbutazone (119.73 mg/kg, i.p.) which resemble their analgesic ED_{50}s be positioned on x- and y-axes. Straight-line is displayed to get isobolographic analysis amid the ED_{50}s doses for meloxicam besides phenylbutazone given separately, producing an analgesic effect in mice. A point beneath the straight line is a synergism, while a point over the straight line means an antagonism. The equation elucidating the interaction index is then produced as a Y character predicted by using the equation of da / Da + db / Db.

Da, Db means the analgesic ED_{50}s for meloxicam and phenylbutazone separately, whereas da, db resemble their collective ED_{50s}, correspondingly, illustrated in Table 2 and Figure 1. If the value of Y is equal to 1, this specifies additive interaction; less than one will indicates synergism, and if it is more than 1this will be pointed to antagonism (16,17).

Measurement of cyclooxygenase-2 activity

The experiment was designated to four groups of mice (5 mice / each group). The control group of mice was administered saline; the meloxicam group was treated at 31.14 mg/kg, i.p.; the phenylbutazone group was injected at 239.46 mg/kg, i.p., whereas the combined group consisted of meloxicam and phenylbutazone administration at 31.14 and 239.46 mg/kg, i.p., correspondingly. Subsequently, after 30 minutes for each treated group of mice, the blood was acquired to acquire serum to assess the cyclooxygenase-2 activity through Enzyme-Linked Immuno-Sorbent Assay (ELISA) using a specialized ELISA kit of cyclooxygenase-2 of the mouse (Cat No. MBS269104, USA). The ELISA method was demonstrated by determining the absorbance of cyclooxygenase-2 standards at 450 nm. The concentration of standards was made at 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10 ng/ml. The standard curve was then used to find the simple linear regression equation (which is y= a+bx) that was used for the calculation of the cyclooxygenase-2 activity in the serum samples of the groups mentioned above. The activity of cyclooxygenase-2 in the serum was determined by the ELISA technique through incubation of the serum (37 °C for 90 min) and washing, then adding biotinylated antibody and incubating (37 °C for 60 min). The working samples were then subjected to washing again, adding the enzyme-occupied solution, and incubating (37 °C for 30

min). Then, the color reagent solution with incubation (37 °C up to 30 min) is added and washed. Finally, we added the color reagent C and read through the microplate reader to quantify the absorbance of the working samples within 10 min at 450 nm (18,19).

Statistics

Parametric data of multiple groups of mice were analyzed using a one-way analysis of variance followed by the least significant difference (LSD) to relate the means of groups used in the study with the significant level at $P<0.05$ (20,21).

Results

The analgesic ED_{50s} for meloxicam and phenylbutazone

The dose value of meloxicam administered i.p. in mice resulted in the analgesic response in 50% of the mice was 15.57 mg/kg, and phenylbutazone was at 119.73 mg/kg (Table 1).

Isobolographic analysis

When given separately, the analgesic ED_{50} for meloxicam was 15.57 mg/kg, i.p. and for phenylbutazone was 119.73 mg/kg, i.p.,. The resulted analgesic ED_{50} values of meloxicam and phenylbutazone concomitant were at 12.84 and 98.75 mg/kg, i.p., respectively when given together at a ratio of 1:1 from their ED_{50}s. Table 2 shows the different results gained from this experiment. The value of the interaction index resembling Y is 1.65, which is greater than 1. According to the value measured, the pharmacological interaction between meloxicam and phenylbutazone is the antagonistic interaction (Table 2 and Figure 1).

Inhibition of the cyclooxygenase-2 activity

Meloxicam and phenylbutazone alone significantly inhibited the cyclooxygenase-2 activity by 72 and 90%, respectively, compared to the control group. The combination composed of meloxicam and phenylbutazone has a lower limit of inhibition of the cyclooxygenase-2 activity 33% in comparison to meloxicam or phenylbutazone. Meloxicam and phenylbutazone combination were significantly different from the control, meloxicam, and phenylbutazone group concerning the cyclooxygenase-2 activity (Table 3 and Figure 2).

Table 1: Analgesic ED_{50s} for meloxicam and phenylbutazone

Variables	Meloxicam	Phenylbutazone
ED_{50}= xf + (k × d)	15.57 mg/kg, i.p.	119.73 mg/kg, i.p.
The initial dosage	10 mg/kg	250 mg/kg
The last dosage (xf)	16 mg/kg	175 mg/kg
The table value (k) (Standard deviation of 0.61)	- 0.144	- 0.737
± Dosage (d)	3 mg/kg	75 mg/kg
Range of the dosages	19-10= 9 mg/kg	250-100= 150 mg/kg
Overall mice used	7 (OOOXXOX)*	6 (XXOXOX)*

*X means analgesia while O indicates no analgesia

Table 2: Isobolographic analysis between meloxicam and phenylbutazone

Variables	Meloxicam + Phenylbutazone (1:1)	
	Meloxicam	Phenylbutazone
ED_{50}= xf + (k × d)	12.84 mg/kg, i.p.	98.75 mg/kg, i.p.
The initial dosage	15.57 mg/kg	119.73 mg/kg
The last dosage (xf)	15.57 mg/kg	119.73 mg/kg
The table value (k) (Standard deviation of 0.61)	- 0.701	- 0.701
± Dosage (d)	3.90 mg/kg	29.93 mg/kg
Range of the dosages	15.57-11.67= 3.9 mg/kg	119.73-89.80= 29.93 mg/kg
Overall mice used	5 (XOXOX)*	
Interaction index (Y)= da/Da + db/Db = 1.65		

*X means analgesia while O indicates no analgesia. Da and Db indicate the analgesic values of ED_{50}s for meloxicam and phenylbutazone given separately. da and db means the analgesic ED_{50} values when meloxicam and phenylbutazone are given together.

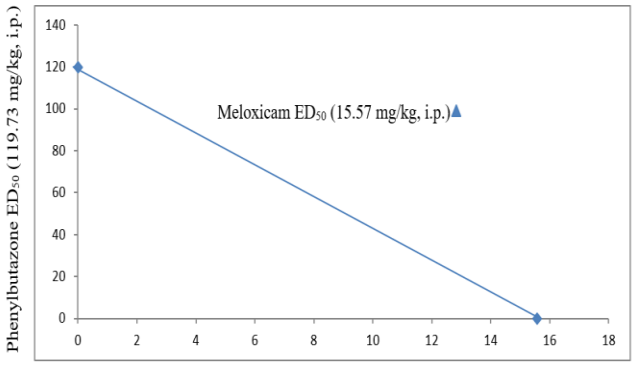

Figure 1: Isobolographic analysis between meloxicam and phenylbutazone

Table 3: Inhibition of cyclooxygenase-2 activity by meloxicam and phenylbutazone

Groups	Cyclooxygenase-2 activity (ng/ml)	Inhibition (%)[+]
Control	16.58 ± 2.97	0
Meloxicam	4.60 ± 0.94 [*]	72
Phenylbutazone	1.59 ± 0.32 [*]	90
Meloxicam and phenylbutazone	11.06 ± 1.73 [*,a,b]	33

Numbers categorized as Mean ± Std.E (5 mice / group). Mice treated with meloxicam (31.14 mg/kg, i.p.), phenylbutazone (239.46 mg/kg, i.p.) alone or together. * Significantly dissimilar than control group (at $p < 0.05$). [a] Significantly dissimilar than meloxicam group (at $p < 0.05$). [b] Significantly dissimilar than phenylbutazone group (at $p < 0.05$). [+] Inhibition (%)= Control group – treated group / Control group × 100.

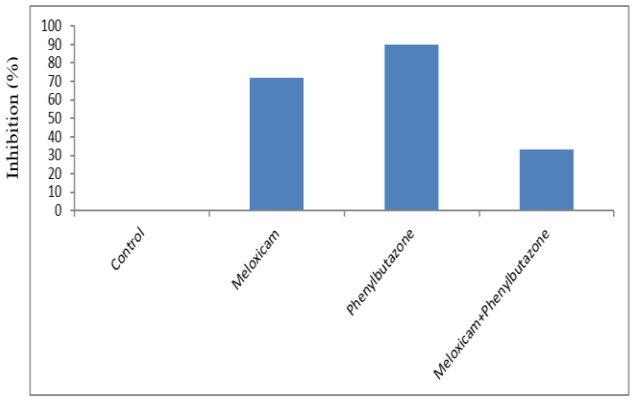

Figure 2: Percentages of inhibition in cyclooxygenase-2 activity by meloxicam and phenylbutazone alone or together in mice.

Discussion

The recent research assessed the therapeutic efficacy of meloxicam and phenylbutazone with their kind and degree of pharmacodynamic interaction besides their inhibitory interaction on the level of cyclooxygenase-2 in mice. The values of analgesic ED_{50}s concerning the meloxicam and phenylbutazone alone, which are found here in this study, were following previous studies on mice, respectively (22,23). Meloxicam and phenylbutazone are NSAIDs with many benefits and multiple usages in human and veterinary medicine due to their wide range of pharmacologic properties as an analgesic, anti-inflammatory, and antipyretic (1-4). They have a non-selective mechanism activity that leads to inhibiting both isoforms of the cyclooxygenase enzyme, especially the inducible one known as cyclooxygenase-2 responsible for the production of pain, inflammation, and fever through the formation of the prostaglandin E_2 (5,6) as found here in this study through the ability of meloxicam and phenylbutazone of successful inhibition of the cyclooxygenase-2. The variation in percentages of inhibition in the cyclooxygenase-2 activity caused by meloxicam and phenylbutazone may be assumed to be the variation in the selectivity between them toward cyclooxygenase-2 (5,6). Meloxicam and phenylbutazone are considered high among NSAIDs bound to plasma proteins with approximately 99% (7-9). This property leads to competitive antagonism on the binding sites at the plasma proteins (albumins). It changes the concentration of free drugs of each medication used in this study that reach their target site of action at the cyclooxygenase-2.

Consequently, they affect the therapeutic efficacy of each other through their possible interaction at the pharmacodynamic plane. This effect is revealed here in this study from the elevation of cyclooxygenase-2 activity when meloxicam and phenylbutazone are administered together compared to meloxicam and phenylbutazone given alone. The other possible interaction between meloxicam and phenylbutazone was the competition on the binding sites on the cyclooxygenase-2 at the metabolic criteria (phase I and II). This interaction is not far beyond because phenylbutazone is a cytochrome P_{450} inducer that enhances its conversion to a significant active metabolite known as oxyphenbutazone. Oxyphenbutazone is responsible for the pharmacological effects of phenylbutazone besides its ability to accelerate the metabolism of the other drugs administered simultaneously (24,25).

Conclusions

The sum of the data concluded that meloxicam and phenylbutazone have an excellent analgesic efficacy when administered alone. In contrast, mixing these two drugs has

no benefit because of the antagonistic interaction on the level of cyclooxygenase-2 in mice.

Acknowledgments

We would correspond to express our praise to the Veterinary Medicine Colleges belonging to the University of Mosul and Dohuk for providing the essential equipment for this research.

Conflict of interest

The authors declare there is no conflict of interest.

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تداخل الميلوكسيكام والفنيلبيوتازون على مستوى انزيم الأكسدة الحلقية-٢ في الفئران

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الخلاصة

هدفت الدراسة الحالية الى فحص الفعالية العلاجية للميلوكسيكام والفنيلبيوتازون كل على حدة مع تحديد التداخل الدوائي المسكن للألم بينهما وتداخلهما المثبط على مستوى أنزيمات الأكسدة الحلقية -٢ في الفئران. كانت الجرعة الفعالة الوسطية (الجف-٥٠) المسكنة للألم لكل من الميلوكسيكام والفنيلبيوتازون التي تم إعطاؤها كل على حدة في الفئران هي ١٥,٥٧ و ١١٩,٧٣ ملغم/كغم، عن طريق الحقن في الخلب على التوالي باستخدام طريقة الصعود والنزول وبطريقة اللوح الساخن.

بينما كانت قيم الجف-٥٠ المسكنة للألم عند إعطاء الميلوكسيكام والفنيلبيوتازون معا هي ١٢,٨٤ و ٩٨,٧٥ ملغم/كغم، عن طريق الحقن في الخلب على التوالي عند إعطائهما معا بنسبة ١:١ من قيم الجف-٥٠ لهما. بين تحليل الايزوبولوكرافيك أن نوع التداخل الدوائي المسكن للألم بين الميلوكسيكام والفنيلبيوتازون هو تداخل تضادي كما يتضح من مؤشر التداخل وهو ١,٦٥. استخدمت تقنية الاليزا لقياس نشاط أنزيم الأكسدة الحلقية-٢ وعكست أن كل من الميلوكسيكام والفنيلبيوتازون لوحدهما عملا على تثبيط نشاط إنزيم الأكسدة الحلقية-٢ وبشكل معنوي بنسبة ٧٢ و ٩٠٪ على التوالي مقارنة بمجموعة السيطرة بينما امتلك مزيج الميلوكسيكام والفنيلبيوتازون اقل نسبة تثبيط (٣٣%) في نشاط أنزيم الأكسدة الحلقية-٢ مقارنة بالميلوكسيكام او الفنيلبيوتازون. وأدى إعطاء المزيج المكون من الميلوكسيكام والفنيلبيوتازون إلى تغير معنوي مقارنة بمجاميع فئران السيطرة والميلوكسيكام والفنيلبيوتازون فيما يتعلق بنشاط أنزيم الأكسدة الحلقية-٢. خلصت هذه الدراسة إلى أن الميلوكسيكام والفنيلبيوتازون يمتلكان فعالية جيدة لتسكين الألم عند إعطاء كل منهما بمفرده بينما لا يعد مزيج هذين العقارين ذو فائدة بسبب التداخل الدوائي التضادي بينهما على مستوى إنزيم الأكسدة الحلقية-٢ في الفئران.