Synergistic interaction between trimetazidine and ketoprophen in mice

Interacţiunea de tip sinergic dintre trimetazidină şi ketoprofen la şoarece

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Abstract

Recent studies have demonstrated the antinociceptive, anti-inflammatory, and gastric protective effects of trimetazidine (TDZ) on various models in rats. The present study proposes to demonstrate the antinociceptive action in mice, evaluated in conditions of inflammatory pain, together with the determination of the type of pharmacodynamic interaction between trimetazidine and ketoprofen (KETO). In this study we used as experimental model of nociception the abdominal constrictive response (writhing test), induced with Zymosan A in mice, and for the study of the interaction we used as quantitative evaluation method the method of binary combinations in fixed proportions. The experimental results allowed the demonstration of the ED 50 for ketoprofen (DE50 = 0.606 ± 0.108 mg/kg) and the demonstration of the synergism for the associated substances. (Zadd = 1.818 ± 0.326 mg/kg, Zmix = 0. 458 ± 0.101 mg/kg, γ = 0.251, Tc = 4.928, Tt = 3.84, P< 0.05). The results demonstrate that for the same level of activity (50%), smaller doses of each substance can be used, compared to the doses of the substances administered alone, which might contribute to a reduction in the number or severity of adverse effects. on the other side, the demonstration of the synergism might contribute to the clarification of the action mechanisms. The experiments presented below were made in agreement with the rules and regulations concerning the work with lab animals.

Rezumat

Studii recente au pus în evidenţă acţiunea antinociceptivă, antiinflamatoare şi efecte de protecţie gastrică a trimetazidinei (TMD) pe diferite modele, în condiţii experimentale la şobolan. Studiul de faţă îşi propune demonstrarea acţiunii antinociceptive a trimetazidinei la şoarece, evaluată în condiţii de durere inflamatorie şi determinarea tipului de interacţiune farmacodinamică între trimetazidină şi ketoprofen (KETO), antiinflamator nesteroidian utilizat in durerea postoperatorie sau alte situaţii care implică durere şi inflamaţie. În această lucrare se utilizează ca model experimental de nocicepţie, testul răspunsului constrictiv abdominal indus cu Zymosan la şoarece iar pentru studiul interacţiunii se utilizează ca metodă de evaluare cantitativă, metoda combinaţiilor binare în proporţie fixă. Datele experimentale au permis demonstrarea valorii DE50 a ketoprofenului (DE50 = 0.606 ± 0.108 mg/kg) şi demonstrarea sinergismului pentru substanţele asociate (Zadd = 1.818 ± 0.326 mg/kg, Zmix = 0.458 ± 0,101 mg/kg, γ = 0.251, Tc = 4.928, Tt = 3.84, P< 0.05). Rezultatele obţinute demonstrează că se pot utiliza în asociere, doze mai mici din fiecare substanţă pentru acelaşi nivel de activitate (50%) comparativ cu dozele administrate pentru substanţele ca atare ceea ce ar putea contribui şi la o reducere a numărului de reacţii adverse. Pe de altă parte, demonstrarea sinergismului poate contribui la elucidarea mecanismelor de acţiune în unele cazuri. Experimentele din prezentul studiu s-au efectuat în acord cu legislaţia în vigoare în ceea ce priveşte lucrul cu animalele de laborator.

1. Introduction

The present study tries to demonstrate the type of pharmacodynamic interaction between a substance with anti-anginous properties (trimetazidine), and a non-steroidal anti-inflammatory drug ketoprofen in mice. The ketoprofen (KETO) is a non-steroidal anti-inflammatory highly used in inflammatory conditions, with an action mechanism based on its capacity to inhibit the cyclooxygenase, thus reducing the synthesis of the metabolites of arachidonic acid. In surgery and in human and veterinary practice, ketoprofen is used frequently in the post-operative care.

In the last years, a series of studies demonstrated the analgesic activity of compound with different main activity, like anti-anginous, anti-convulsant and so on. Thus, a series of compounds the block sodium or calcium channels demonstrated their analgesic action in various types of pain. For example, trimetazidine demonstrated both an anti-nociceptive and an anti-inflammatory action using various experimental models in rat (Abdel-Salam & El-Batran, 2005).

With these data in sight, we have proposed the demonstration of the antinociceptive action in inflammatory conditions of trimetazidine and ketoprofen together with the analysis of the combination between these two substances.

In experimental pharmacology, it is more and more acknowledged that the quantitative study of drug combinations, mainly the identification of the true synergism (potentiation), can be a useful step in identifying the action mechanisms (Raffa, Stone et al., 2000).

Materials and methods

Lab animals

The research in the present study were made using male Swiss mice (source Cantacuzino Institute Bucharest) weighing 18-22 g. the animals were placed in Plexiglas cages, type mini-Duna, with water dripper.

For accommodation, 10-15 animals were placed in each cage, following their behaviour for 15 days.

The habitation was realised in the Laboratory of Experimental Pharmaco-dynamics of the Department of Pharmac-odynamics and Clinical Pharmacy of the University „Grigore T. Popa" Iaşi, in a space with controlled temperature and humidity (21℃ ±2℃), and a 12/12 hours cycle light/dark (07.00 AM / 07.00 PM). The animals received water and standard fodder (source Băneasa Biobase), *ad libitum.*

The tests were made using groups of 6 animals beginning with 10.00 AM, and 3 hours before experiments access to water and food was discontinued.

All the experimental proceedings used in this study were made strictly according to the norms approved in the „Grigore T. Popa" University of Medicine and Pharmacy and international bioethics regulations concerning experiments performed using lab animals (Zimmermann, 1986).

Substances

For the tests were used: Ketoprofen (Sigma), Zymosan A (Sigma), Trimetazidine (Sigma), CMC-Na (Sigma) and normal saline (Zentiva).

The test of the constrictive abdominal response (method of Siegmund et al. 1957, technique of Koster et al. 1959) induced by Zymosan A, is made by administrating intra-peritoneal 40mg/kg a suspension of Zymosan A. The recording of the number of abdominal constrictive responses was made during 12 minutes after the administration of the irritant (Turner & Hebborn, 1965). Data interpretation was quantal, characterized by the presence or absence of the response, calculating a maximum possible effect: % (antinociception)

Inhibition = (no. of non-responders / total number of animal) x 100

Statistical data analysis

For the analysis of the dose-effect relationship, the working protocol imposes drawing regression lines and their analysis, and for measuring the relationship between the two variables (dose-effect) the correlation coefficient Pearson „R" was used.

For the evaluation of the antinociceptive potential, the value of the ED50 (Effective

Dose 50%) will be calculated from the maximum possible effects measured.

For the quantification of the interactions we used the method of the additive composite line, method that allows the quantitative evaluation of the pharmacodynamics interactions within the fixed - ration binary combinations (Tallarida, 2001; Tallarida, 2002).

It is based on the analysis of the regression lines (the regression line of the combination versus the composite additive line) for the activity level of 50%.

The interaction index (γ) indicated the strength of the synergism.

In the statistical analysis, for all tests of statistical significance, it has been considered that a p value <0.05 meant a significant statistical difference between the groups taken in study (ANOVA Test, t - Test).

Results and discussion

The substances taken in study were administered orally, in successive doses, in geometrical progression. For the ketoprofen we administered orally successive doses comprised between 0.31-5.00 mg/kg.

Through the administration of the above-mentioned dose sequence we obtained a maximum possible effect of 100% beginning with a dose of 1.25 mg/kg. The data obtained allowed for calculating an ED50 for ketoprofen (table 1, fig. 1).

For the trimetazidine were administered orally, in geometric progression, successive doses between 1.9-30.00 mg/kg. For the dose sequence taken in study no antinociceptive action was demonstrated. The testing in inflammatory conditions was made 60 minutes after the administration of trimetazidine. (Table 1).

Table 1

The ketoprofen ED50 value

Parameter	Ketoprofen (KETO)	Trimetazidine (TMD)
ED50 (SME)[1] mg/kg/p.o	0.606 (0.100) Y = 6.022 + 4.701*X R = 0.904 TLC: 0.363 - 0.959	-

[1]*SME, standard mean error*

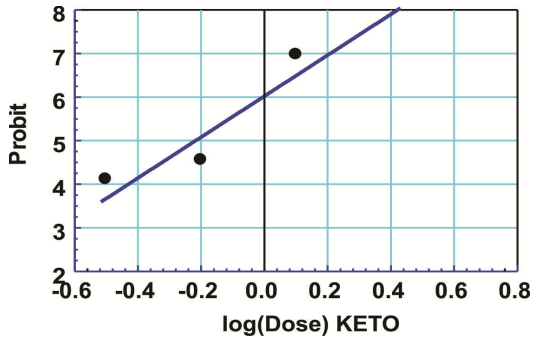

Fig. 1. Analysis of the regression line for ketoprofen

The methodology for the study of fixed-ration combinations requires a protocol for the combinations where only one of the compounds demonstrated the action followed in the given experimental conditions. Thus, dose pairs in various ratios are used following the EMP% for each ratio (Table 2, figure 2).

From table 2 we can observe that for a KETO/TMD ratio of 1:2 an EMP of 66.33% was obtained, which allows for the establishing of the Zadd value. To demonstrate this value is necessary for establishing the dose pairs in fixed proportions, for the evaluation of the type of pharmacodynamic interaction between those two substances.

Following the administration of the dose pairs in the ration KETO/TMD - 0.333/0.667 an antinociceptive effect of 55.50% was obtained for the combination.

The interaction between ketoprofen and trimetazidine was proven to be synergistic. The synergism is proven by the aspect of the regression lines, the left shift of the regression line for the association compared to the additive composite line (Fig. 2).

The statistical parameters of the regression analysis demonstrated the synergism between those two substances (F_c = 14.08, F_t = 5.79 T_c= 4.92, T_t = 3.84, P< 0.05).

Using the test of the constrictive abdominal response is made for evaluating both central and peripheral analgesia. The administration of an irritant suspension of Zymosan A gives the model its specificity for the pathogenesis of inflammatory pain [(Pettipher, Hibbs et al., 1997) (Vale, Marques et al., 2003).

A series of authors have shown that the administration of intraperitoneal Zymosan A in mouse induces an inflammatory response, characterized by abdominal constrictive response, leukocyte infiltration an eicosanoid biosynthesis [Doherty) cit (Pettipher, Hibbs et al., 1997)]. Also, it has been demonstrated the activation of the alternate pathway of the complement, the generation and release of PAF, free radicals of oxygen and lysosomal enzymes (Rao, Currie et al., 1994).

Table 2

EMP% values for each ratio

Ratio	Ratio dose pairs	Total dose mg/kg/p.o	(EMP) %	ED50 (SME)[1]		Statistical parameters
				Zadd (SME)	Zmix (SME)	
KETO/TMD	1/3	-	33.33	-	-	F_C = 14.08 F_t = 5.79 T_c= 4.92 T_t = 3.84 P< 0.05
	1/2	-	66.33			
	1/1	-	50.00			
	1/0.5	-	16.66			
	1/0.25	-	0.00			
KETO / TMD (0.333/0.667)	-	0.113	16.66	1.818 (0.326) Y = 6.02 + 4.70*X R = 0.959	0.458 (0.101)[1] Y = 6.19 + 3.52*X R = 0.991 γ = 0.251	
	-	0.225	33.33			
	-	0.45	66.66			
	-	0.90	100.00			

[1]*SME, standard mean error*

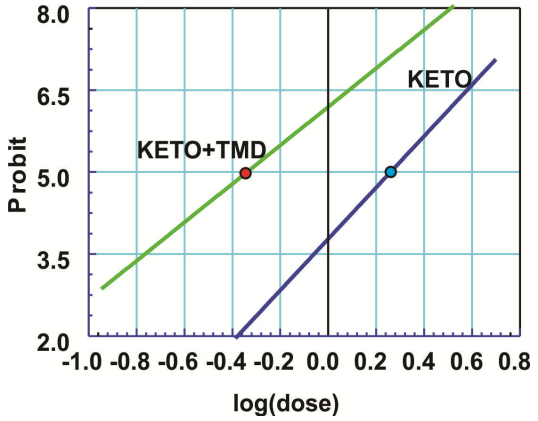

Fig. 2 Analysis of the additive composite line for the combination KETO - TMD

Recent studies show that the inflammatory agents do not directly stimulate the release of hyper nociceptive primary mediators, but that their release is preceded by a cytokine cascade (Cunha, Poole et al., 1999) (Ribeiro, Vale et al., 2000). In the mouse, the abdominal constrictive response to Zymosan is mediated through TNF-alpha, IL1-beta and IL8, which act simultaneously and synergistically (Ribeiro cit (Cunha, Verri et al., 2005).

The ketoprofen is a non-steroidal anti-inflammatory agent, used in this study as a racemic mixture. Unlike other NSAID's, KETO relies on a peripheral action mechanism and on a central one, but which have not been completely identified (Pinardi, Sierralta et al., 2001). The action mechanisms are based on the inhibition of the cyclooxygenase and of the nitric oxide synthase. Recently, it has been demonstrated at the central level the interaction of KETO with the serotoninergic system, which might explain its high efficacy compared with other NSAID's (Diaz-Reval, Ventura-Martinez et al., 2004). The spinal implications might be explained by its intervention on the 5HT3 receptor, while the supraspinal effects could be ascertained to the effects on the 5-HT1, 5-HT-2, 5-HT7 receptors. (Díaz-Reval, Ventura-Martínez et al., 2002). Other studies show that it inhibits the lypoxygenase, the generation of leukotrienes, stabilises the lysosomal membranes, has an anti-bradikinin action and is a scavenger for hydroxyl radicals (Qiu, Liu et al., 2007) .

The trimetazidine is a non-selective calcium blocker and an anti-ischemic agent that intervenes in the oxidative stress (Cristea, 2005). In mouse, the TMD reduces the oxidative stress induced by lypopolysaccharide E. TMD blocks the β-oxidation by inhibiting the 2-AcetylCoA thyolase, reduces calcium accumulation and superoxide production. Also, trimetazidine administration on models of cardiac ischemia demonstrated a tendency of decrease in the levels of IL6, C3, C5 IL8, TNFα and leukotrienes, which are responsible both for the omnipresent oxidative lesions both in reperfusion and acute inflammation and for

the activation of the mechanisms of pain perception in the inflammation (Martins, Siqueira Filho et al., 2012).

Conclusions

The synergism between those two substances might be explained through the complex action mechanism of ketoprofen, to which adds the antioxidant effect of TMD together with the reduction of the calcium influx in the primary afferent neurons due to the inhibition of the currents through the AMPA / kainite receptors, which might prevent neuronal depolarization and triggering, thus reducing the pain perception (Jain, Bharal et al., 2010).

These results suggest a potential advantage in the combined administration of KETO with TMD, the desired effects being obtained at significantly lower dosages.

Bearing in mind the antioxidant effect of TMD and the interferences of this effect with the reduction of the gastric protection by the NSAID's, TMD co-administration might also be beneficial concerning this aspect, which justifies further investigations in this direction too.

References

Abdel-Salam, O.M. & El-Batran, S. (2005) Pharmacological investigation of trimetazidine in models of inflammation, pain and gastric injury in rodents. Pharmacology, 75(3), 122-132.

Cristea, A. (2005). Tratat de farmacologie. Editura Medicala, Bucuresti.

Cunha, F.Q., Poole, S., Lorenzetti, B.B., Veiga, F.H. & Ferreira, S.H. (1999). Cytokine-mediated inflammatory hyperalgesia limited by interleukin-4. Br J Pharmacol, 126(1), 45-50.

Cunha, T.M., Verri, W.A., Jr., Silva, J.S., Poole, S., Cunha, F.Q. & Ferreira, S.H. (2005). A cascade of cytokines mediates mechanical inflammatory hypernociception in mice. Proc Natl Acad Sci U S A, 102(5), 1755-1760.

Diaz-Reval, M.I., Ventura-Martinez, R., Deciga-Campos, M., Terron, J.A., Cabre, F. & Lopez-Munoz, F.J. (2004). Evidence for a central mechanism of action of S-(+)-ketoprofen. Eur J Pharmacol, 483(2-3), 241-248.

Díaz-Reval, M.I., Ventura-Martínez, R., Déciga-Campos, M., Terrón, J.A., Cabré, F. & López-Muñoz, F.J. (2002). Involvement of serotonin mechanisms in the antinociceptive effect of S(+)-ketoprofen. Drug Development Research, 57(4), 187-192.

Jain, S., Bharal, N., Mediratta, P.K. & Sharma, K.K. (2010). Trimetazidine exerts protection against increasing current electroshock seizure test in mice. Seizure, 19(5), 300-302.

Martins, G.F., Siqueira Filho, A.G., Santos, J.B., Assuncao, C.R., Vieira, F.B., Valencia, A., Carvalho, K.G. & Jessen, B. (2012). Trimetazidine and inflammatory response in coronary artery bypass grafting. Arq Bras Cardiol, 99(2), 688-696.

Pettipher, E.R., Hibbs, T.A., Smith, M.A. & Griffiths, R.J. (1997) Analgesic activity of 2-amino-4-methylpyridine, a novel NO synthase inhibitor. Inflamm Res, 46 Suppl 2, S135-136.

Pinardi, G., Sierralta, F. & Miranda, H.F. (2001) Interaction between the antinociceptive effect of ketoprofen and adrenergic modulatory systems. Inflammation, 25(4), 233-239.

Qiu, H.X., Liu, J., Kong, H., Liu, Y. & Mei, X.G. (2007). Isobolographic analysis of the antinociceptive interactions between ketoprofen and paracetamol. Eur J Pharmacol, 557(2-3), 141-146.

Raffa, R.B., Stone, D.J., Jr. & Tallarida, R.J. (2000). Discovery of "self-synergistic" spinal/supraspinal antinociception produced by acetaminophen (paracetamol). J Pharmacol Exp Ther, 295(1), 291-294.

Rao, T.S., Currie, J.L., Shaffer, A.F. & Isakson, P.C. (1994). In vivo characterization of zymosan-induced mouse peritoneal inflammation. J Pharmacol Exp Ther, 269(3), 917-925.

Ribeiro, R.A., Vale, M.L., Thomazzi, S.M., Paschoalato, A.B., Poole, S., Ferreira, S.H. & Cunha, F.Q. (2000). Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol, 387(1), 111-118.

Tallarida, R.J. (2001). Drug synergism: its detection and applications. J Pharmacol Exp Ther, 298(3), 865-872.

Tallarida, R.J. (2002). The interaction index: a measure of drug synergism. Pain, 98(1-2), 163-168.

Turner, R.A. & Hebborn, P. (1965). Screening methods in pharmacology. Academic Press, New York,.

Vale, M.L., Marques, J.B., Moreira, C.A., Rocha, F.A., Ferreira, S.H., Poole, S., Cunha, F.Q. & Ribeiro, R.A. (2003) Antinociceptive effects of interleukin-4, -10, and -13 on the writhing response in mice and zymosan-induced knee joint incapacitation in rats. J Pharmacol Exp Ther, 304(1), 102-108.

Zimmermann, M. (1986). Ethical considerations in relation to pain in animal experimentation. Acta Physiol Scand Suppl, 554, 221-233.