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**THE STUDY OF MALDANE ANTI-INFLAMMATORY ACTIVITY UNDER PATHOLOGICAL CONDITIONS OF DIFFERENT GENESIS**

N. G. Vakhnina

The National University of Pharmacy

 The article is dedicated to the study of anti-inflammatory activity of the new compound di-(2,4-dimethyl) malonic acid anilide agreed upon the name of "Malden".

 The study of maldane anti-inflammatory action and the search of selected effective dose were performed on the models of carrageenan, formalin and dextrin and histamine rats edema.

 It was found that Malden has maximum antiexudative action, in the dose of 7.3 mg/kg. Maldane anti-inflammatory activity does not depend on the genesis of pathology (logogen objects: carrageenan, formalin, dextran, histamine) and not inferior to the efficacy of the drug in comparison to voltaren dose of 8 mg/kg.

 Malden (di-(2,4-dimethyl) malonic acid anilide) is a promising substance for further pharmacological study and introduction into medical practice as a new modern domestic drug with anti-inflammatory action.

*Keywords:* the derivatives of malonic acid, anti-inflammatory activity, Malden, carrageenan edema, formalin edema, dextranase edema, histamine edema

 The vast majority of a modern person illnesses has an inflammatory reaction in its pathogenesis, which is a typical pathological process and is accompanied by a complex of structural, functional and metabolic disorders at the level of an organ as well as a cell and is often complicated by or associated with pain syndrome [5].

 It’s known that non-steroidal anti-inflammatory drugs (NSAIDs), which millions of patients recieve today, are effective in the pharmacotherapy of acute and chronic inflammation. According to WHO, the number of patients receiving treatment with NSAIDs is about 30 million, there are 40% of the elderly among them, and this figure is constantly increasing [2,6].

 A pharmacological profile of current NSAIDs combines analgesic, anti-inflammatory, antipyretic action, which causes the wide use of this class of drugs [7]. It is known that the NSAIDs also helps to improve the quality of life and patients survival, but also, it should be noted that existing NSAIDs have significant drawbacks, namely induce the development of gastropathy, liver disorder, kidneys disorder, etc. [8].

 The development and introduction into medical practice of fundamentally new NSAIDs, which along with high pharmacological activity don’t induce the development of adverse reactions is a topical issue of modern pharmacy [9].

 A promising compound in this aspect can be considered as di-(2,4-dimethyl) malonic acid anilide which was synthesized by the scientists of the National Pharmaceutical University under the supervision of Professor P. A. Bezugly.

 The aim of this work was to study the anti-inflammatory action of di-(2,4-dimethyl) malonic acid anilide, hereinafter "Malden", and conducting some research for the selection of an effective dose in terms of inflammation of various origins.

**Materials and methods**

 The research of maldane anti-inflammatory action was conducted on white nonlinear rats male weighing 180-220 g. The groups of 8 animals in each were formed. The animals were kept in standard conditions of vivarium of the Central research laboratory of NUPh according to the rules of GLP. We worked in accordance to the EC Council Directive on the protection of animals used for experimental and other scientific purposes. The experiments were carried out in accordance with the "Rules of work with experimental animals" (Strasbourg,18.03.86) [4].

 The research of anti-inflammatory effect was carried out in terms of reproduction of the inflammatory response of various Genesis in experimental models recommended by the HEC of Ukraine for pre-clinical studies, namely on the models of carrageenan, formalin, dextran and histamine foot edema in rats [1].

 The carrageenan acute edema was caused by subplanetary injection of 0.1 ml of 1 % carrageenan solution [1]. To clarify the mechanism of antiexudative Malden action the formalin edema was modeled by injecting 0.1 ml of 3% formalin solution in the foot[1], dextran inflammation – by injecting of 0.1 ml of 6% dextran solution and histamine edema – by injecting of 0.1 ml of 0.1% of histamine solution [1].

 The tested compound was administered intragastrically to experimental animals at doses of 3.6 mg/kg 7.3 mg/ kg 14.6 mg/kg, the last dose corresponds to 1/20 LD50. A well- known NSAIDs voltaren (diclofenac sodium) was chosen as a reference drug at a dose of 8 mg/kg, which is recommended for experimental studies on rats.

 The obtained experimental data were processed statistically using t - Student criterion [3].

**Results and discussion**

 Carrageenan is an induced inflammation characterized by a multistage mechanism of pathogenesis. In the first 30-90 min of carrageenan edema histamine and serotonin are mainly used in the pathogenesis. In the interval of 1.5-2.5 hours - the system of kinins is used, and at 3-4 hours of exudation development there is the activity of prostaglandins and leukotrienes. Thus, the model of carrageenan edema allows to explore the expression of anti-inflammatory action of a test substance in dynamics and to find out the mechanism of its implementation.

 The results of the antiexudative maldane action study under the conditions of carrageenan edema are shown in table 1.

 It was found that Malden in doses of 3.6 mg/kg 7.3 mg/kg, 14.6 mg/kg has significant anti-inflammatory effect. During the first hour of the inflammation an average antiexudative maldane activity in different doses is 23.9%, during 2nd hour – 32,2%, 3 hour – 42.7 percent with the maximum at the 4th hour of inflammation of 50.3% (table1).

 Analyzing the results, we can conclude that a new compound of Malden has anticyclogenesis action mechanism, inhibits the synthesis of prostaglandins and leukotrienes in the centre of inflammation.

Table 1. Antiexudative activity of maldane and voltaren in conditions of carrageenan edema (n = 40)

|  |  |  |
| --- | --- | --- |
| Research conditions | Dose mg, kg | Antiexudative activity, % |
| Hour of inflammation |
| 1hour | 2 hours | 3 hours | 4 hours |
| Maldane  | 3,6 | 20,5±0,32 | 28,7±0,43 \* | 34,6±0,84 \* # $ | 41,6±0,63\* # $ |
| Maldane  | 7,3 | 26,3±0,84 | 35,7±1,72 | 48,8±2,12 | 56,9±0,97 |
| Maldane  | 14,6 | 25,1±1,74 | 32,4±1,70 | 44,7±3,10 | 51,6±2,80 |
| Voltaren  | 8,0 | 24,0±2,10 | 33,0±2,70 | 46,2±2,20 | 55,0±2,30 |

Note. \* – statistically significant differences with the indicators of maldane group dose of 7.3 mg/kg, p<0.05;

# – statistically significant differences with the indicators of maldane group at a dose of 14.6 mg/kg, p<0.05;

$ – statistically significant differences with the indicators of voltaren group dose of 8 mg/kg, p<0.05.

 It should be noted that di-(2,4-dimethyl) malonic acid anilide at a dose of 3.6 mg/kg is statistically inferior significantly to the activity of the voltaren at the second, third and fourth hours of the inflammatory reaction.

 It was found that Malden is characterized by a marked dose-dependent antiexudative effect, during 3rd and 4th hours of carrageenan inflammation, Malden at a dose of 3.6 mg/kg shows significantly less anti-inflammatory effect than at doses of 7.3 mg/kg and 14.6 mg/kg.

 The next piece of work was the study of maldane activity in conditions of a formalin edema, the pathogenesis of which is characterized by a significant destruction of membrane proteins. The results of the antiexudative maldane action study on the model of formalin edema are shown in table 2.

Table 2. Antiexudative activity of maldane and voltaren in conditions of formalin edema (n = 40)

|  |  |  |
| --- | --- | --- |
| Research conditions | Dose mg,kg | Antiexudative activity, % |
| Hour of inflammation |
| 1hour | 2 hours | 3 hours | 4 hours |
| Maldane  | 3,6 | 9,1±1,54 | 30,6±1,61 | 37,9±1,87  | 38,5±1,47\* # $ |
| Maldane  | 7,3 | 12,2±1,75$ | 35,1±1,73 | 42,1±1,93 | 51,3±1,56 $ |
| Maldane  | 14,6 | 13,0±1,36$ | 32,4±1,48 | 37,8±1,52 | 48,6±1,90 $ |
| Voltaren  | 8,0 | 3,2±1,85 | 31,3±1,92 | 39,4±1,33 | 28,2±1,84 |

Note. \* – statistically significant differences with the indicators of maldane group dose of 7.3 mg/kg, p<0.05;

# – statistically significant differences with the indicators of maldane group at a dose of 14.6 mg/kg, p<0.05;

$ – statistically significant differences with the indicators of voltaren group dose of 8 mg/kg, p<0.05.

 Malden in the dose range of 3.6 – 14.6 mg/kg has significant anti-inflammatory effects in conditions of formalin edema. During the first hour of the experiment di-(2,4-dimethyl)-malonic acid anilide is more effective than the reference drug and Malden at a dose of 3.6 mg/kg exceeds voltaren activity in 2,8 times, in the dose of 7.3 mg/kg – 3.8 times (p<0.05) in a dose of 14.6 mg/kg – 4.1 times (p<0.05).

 Under the terms of formalin edema the anti-inflammatory action of dose-dependent was confirmed. The activity of a new compound increases when injecting the dose of 7.3 mg/kg, in comparison with the dose of 3.6 mg/kg (with a possible difference of the indicators during the 4th hour) and decreases a little at a dose of 14.6 mg/kg (tab. 2).

 It should also be noted that during the 4th hour of inflammation Maldane possibly exceeds voltaren activity in all the tested doses. Malden exceeds the activity of the reference drug according to the ability to inhibit the development of exudative reaction in 1,3 times at a dose of 3.6 mg/kg (p<0.05) at a dose of 7.3 mg/kg – 1.8 times (p<0.05) at a dose of 14.6 mg/kg – 1.7 times (p<0.05).

 The next stage of the research was conducted on the model of dextran edema. The results of the study are shown in table 3.

Table 3. Antiexudative activity of maldane and voltaren in conditions of dextran edema (n = 40)

|  |  |  |
| --- | --- | --- |
| Research conditions | Dose mg,kg | Antiexudative activity, % |
| Hour of inflammation |
| 1 год. | 2 год. | 3 год. | 4 год. |
| Maldane  | 3,6 | 17,6±1,41\* | 21,5±1,61\* # $ | 48,8±3,17 \*  | 51,6±4,09 |
| Maldane  | 7,3 | 24,5±1,44 | 36,6±2,13 | 65,1±2,91 | 64,4±3,15 |
| Maldane  | 14,6 | 21,4±1,74 | 32,0±1,70 | 51,6±3,10 | 58,7±2,82 |
| Voltaren  | 8,0 | 23,2±2,15 | 34,1±3,11 | 51,4±4,27 | 63,7±4,30 |

Note. \* – statistically significant differences with the indicators of maldane group dose of 7.3 mg/kg, p<0.05;

# – statistically significant differences with the indicators of maldane group at a dose of 14.6 mg/kg, p<0.05;

$ – statistically significant differences with the indicators of voltaren group dose of 8 mg/kg, p<0.05.

 It is known that the injection of dextran solution promotes the release of biogenic amines - histamine and serotonin. Maldane antiexudative activity in the dose range of 3.6 – 14.6 mg/kg, over the study period, ranges from 17.6% to 65.1 per cent. Statistically significant difference of indicators was found in conditions of Maldane injection at a dose of 3.6 mg/kg according to the dosage of 7.3 mg/kg and 14.6 mg/kg at the second hour of the experiment.

 In times of injecting a new compound at a dose of 3.6 mg/kg its anti-inflammatory activity is possibly lower than voltaren during the first, second and third hour of inflammation.

 The maximum antiphlogogenic effect of a compound tested was found while being administered at a dose of 7.3 mg/kg. It should be noted that di-(2,4-dimethyl)- malonic acid anilide shows a tendency to exceed the anti-inflammatory activity of the reference drug of voltaren. For example, during the third hour of the development of dextran exudative reaction, the new compound predominated the effect of the voltaren for 14%.

 To confirm the anti-inflammatory activity of maldane, its anti-inflammatory effect on the model of histamine edema was studied . The injection of histamine has a negative impact on humoral link of microcirculation regulation. Histamine, which is a quite powerful inflammation mediator, activates biochemical and pathophysiological mechanisms of exudative reaction development.

 The results of maldane antiexudative action study on the model of histamine edema are shown in table 4.

Table 4. Antiexudative activity of maldane and voltaren in conditions of histamine edema (n = 40)

|  |  |  |
| --- | --- | --- |
| Research conditions | Dose mg, kg | Antiexudative activity, % |
| 1 hour of inflammation | 2 hour of inflammation |
| Мaldane | 3,6 | 25,4±1,37 \* | 31,7±2,20 \* # |
| Maldane  | 7,3 | 31,8±1,33 | 44,5±1,78 |
| Maldane  | 14,6 | 28,9±1,92 | 37,4±1,84 |
| Voltaren  | 8,0 | 29,2±1,90 | 41,7±1,74 |

Note. \* – statistically significant differences with the indicators of maldane group dose of 7.3 mg/kg, p<0.05;

# – statistically significant differences with the indicators of voltaren group dose of 8 mg/kg, p<0.05.

 On the model of exudative reactions induced with histamine, Maldane at a dose of 3.6 mg/kg is possible inferior to the effectiveness of the compound at a dose of 7.3 mg/kg (tab. 4), and that was confirmed in the previous experiments of dose-dependent anti-inflammatory activity of di-(2,4-dimethyl) malonic acid anilide.

 The maximum antiexudative activity Maldane has at a dose of 7.3 mg/kg, which does not have a statistically significant difference with the indicators of the reference drug group of voltaren at a dose of 8 mg/kg.

**Conclusion**

 The new compound of di-(2,4-dimethyl) malonic acid anilide, agreed upon the name of "Malden" provides the maximum antiexudative activity at a dose of 7.3 mg/kg.

 Antiphlogogenic maldane action is at the level of a well- known reference drug of voltaren at a dose of 8 mg/kg.

 Anti-inflammatory activity of the studied compound does not depend on the Genesis of pathology and shows equally powerful effectiveness in conditions of experimental inflammation, induced by different pagegenie objects (carrageenan, formalin, dextran, histamine).

 According to the results of the research Malden can be considered a promising substance for further pharmacological study and the development of modern domestic drug with anti-inflammatory action.

**Literature**

1. Chaparro M. New molecules in the treatment of inflammatory bowel disease / M. Chaparro, J.P. Gisbert //Gastroenterol Hepatol. – 2015. – Vol. 26. – Р.210-215.
2. Miazina M.A. Metyrapone effect on gastroprotective action of corticotropin-releasing factor administered centrally against indomethacin-induced gastric injury/ M.A. Miazina, T.R. Bagaeva, L.P. Filaretova //Ross Fiziol Zh Im I M Sechenova. – 2014. – №100(12). – Р.1421-1430.
3. Mkontwana N. Oral analgesia for relieving post-caesarean pain/ N. Mkontwana, N Novikova // Cochrane Database Syst Rev. –2015. – Vol. 29(3) . – Р.450-456.
4. Seyed Mirzaei S.M. Non-Steroidal Anti-Inflammatory Drug Related Peptic Ulcer Disease in Patients Referred to Afzalipour Hospital / S.M. Seyed Mirzaei, M.J. Zahedi, S. Shafiei Pour //Middle East J Dig Dis. – 2015. – Vol.7(4). – Р. 241-244.
5. Small molecules with anti-inflammatory properties in clinical development / T. Hanke, D. Merk, D. Steinhilber et al. // Pharmacol Ther. – 2015. – Vol. 25. – Р. 163-168.
6. Доклінічні дослідження лікарських засобів: метод. рек. / за ред. О. В. Стефанова. – К. : Авіцена, 2001. – 528 с.
7. Пахомова И.Г. Нестероидные противовоспалительные средства: фокус на безопасность при выборе препарата/ И.Г.Пахомова, Е.Ю.Павлова // Consilium medicum. Приложение «Неврология/ревматология». – 2014. – № 1. – С.14-18.
8. Программа статистического анализа. Режим электронного доступа [www.analystsoft.com/ru](http://www.analystsoft.com/ru)
9. Рєзников О. Г. Загальні етичні принципи експериментів на тваринах / О. Г. Рєзников // Ендокринологія. – 2003. – Т. 8, № 1. – С. 142–145.