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PROTECTIVE EFFICACY OF NIMESULIDE IN PARAQUAT-POISONED MICE

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In male white mice (18-22 g) poisoned per os by paraquat (PQ) the protective efficacy of the non-steroidal antiinflammatory drug nimesulide (6 mg/kg ip) given either as a pretreatment (30 minutes before PQ) or treatment (immediately after PQ) was studied during 7 days after poisoning. The results showed that nimesulide given as a pretreatment protected poisoned animals significantly increasing their mean lethal time (LT-50) and mean lethal dose (LD-50) of PQ (7.54 vs 1.74 days, and 146.04 vs 98.39 mg/kg in protected and unprotected mice, respectively). Although the drug failed to offer any significant initial protection when given as a treatment, 10-15% of animals, poisoned with 100 mg/kg of PQ (absolutely lethal dose during 7 days after poisoning), survived the observed period. These results are encouraging and require further investigations of nimesulide as a potential drug assigned to the treatment of PQ poisoning.

Key words: paraquat, herbicide, poisoning, nimesulide, non-steroidal antiinflammatory drugs, mice

INTRODUCTION

Regardless of its high toxicity for humans and animals, paraquat (PQ), a bipyridyl herbicide, continues to be widely used in agriculture (Bismuth et al., 1990). Lethal outcomes from PQ poisoning occur due to either multiorgan system failure (early fatalities) or progressive pulmonary fibrosis (delayed deaths). Although systemic poisoning may occur by various means of exposure to PQ, ingestion is responsible for most severe and fatal cases (Bismuth et al., 1990; Honore et al., 1994; Bismuth and Hall, 1995).

PQ poisoning affects all body systems. Of its many effects, lung injury is the most deleterious. Type II alveolar cells are specific targets of PQ toxicity. After selective accumulation within these cells a redox reaction develops with auto-oxidation and formation of superoxide anion which is enhanced in the presence of alveolar O_2. Subsequently, a Haber-Weiss reaction leads to formation

of hydroxyl radicals and to lipid peroxidation of the alveolar epithelium. The end result is activation of the mononuclear macrophage system with fulminant obliterating pulmonary fibrosis (Honore et al., 1994; Bismuth and Hall, 1995). According to the described mechanism of toxicity the treatment of PQ poisoning consists of two general approaches. The first involves drugs and procedures for reducing the amount of PQ in the body by limiting absorption and promoting its excretion. The second one is based on drugs with antioxidant, antiinflammatory, immunomodulatory and antifibrotic actions (Bismuth et al., 1990; Honore et al., 1994; Bismuth and Hall, 1995).

Nimesulide is a new non-steroidal antiinflammatory drug (NSAID) with potent antiinflammatory, analgesic and antipyretic activities. It also inhibits neutrophil oxidative metabolism, thus preventing production of superoxide anion and hypochlorous acid, very potent oxidizing and damaging compounds (Bevilacqua et al., 1988; Dallegri et al., 1990; Magni, 1993).

Having in mind that agents with antioxidant and antiinflammatory actions have been used in the treatment of PQ poisoning the aim of this study was to evaluate the protective efficacy of nimesulide in PQ-poisoned mice.

MATERIAL AND METHODS

The experiments were performed on male white mice, weighing 18 to 22 g, divided into groups of 10-20 animals each. PQ in the form of the commercially available product, Galokson (Galenika Holding, Belgrade, FR Yugoslavia) containing 20% of active substance, was administered through an intragastric tube to animals that had starved for 24 hours before the poisoning. Nimesulide (Panacea Biotec, India) was given in a dose of 6 mg/kg ip either 30 minutes before or immediately after PQ. Control animals were given saline in a dose of 1 ml/kg ip. The protective efficacy of nimesulide was evaluated on the basis of survival of poisoned animals during 7 days after poisoning. The mean lethal dose (LD-50) of PQ and mean lethal time (LT-50) of poisoned animals were calculated and differences between groups were evaluated by the method of Litchfield and Wilcoxon (1949). A p value of <0.05 was used to establish statistical significance.

RESULTS

The results showed that nimesulide given as a pretreatment successfully protected poisoned mice, while given immediately after PQ it failed to offer any protection. Namely, when nimesulide was given 30 minutes before PQ it significantly increased LD-50 of the herbicide and lengthened LT-50 of

PQ-poisoned mice (100 mg/kg po) by 1.43 times and 4.32 times, respectively (Table 1).

Table 1. Influence of nimesulide (6 mg/kg ip) on mean lethl dose (LD-50) of paraquat (PQ) and mean lethal time (LT-50) in mice poisoned by PQ (100 mg/kg po)

Treatment	[1]LD-50 of PQ (mg/kg)	Potency ratio	LT-50 (days)	Potency ratio
Saline	98.39 (79.22-122.20)	1	1.74 (1.33-2.27)	1
Nimesulide (30 min before PQ)	146.04 (120.68-167.73)	1.48*	7.54 (3.63-15.66)	4.32*
Nimesulide (immed. after PQ)	110.96 (76.40-161.17)	1.13	1.01 (0.49-2.07)	0.58

[1]Based on the 24 hour-survival of poisoned mice
Potency ratio = LD-50 or LT-50 in protected (nimesulide-treated) mice/LD-50 or LT-50 in unprotected (saline-treated) mice; *p<0.05

Although nimesulide given immediately after PQ did not produce any significant initial protective effect, the 7 day-survival rate of mice, poisoned by an

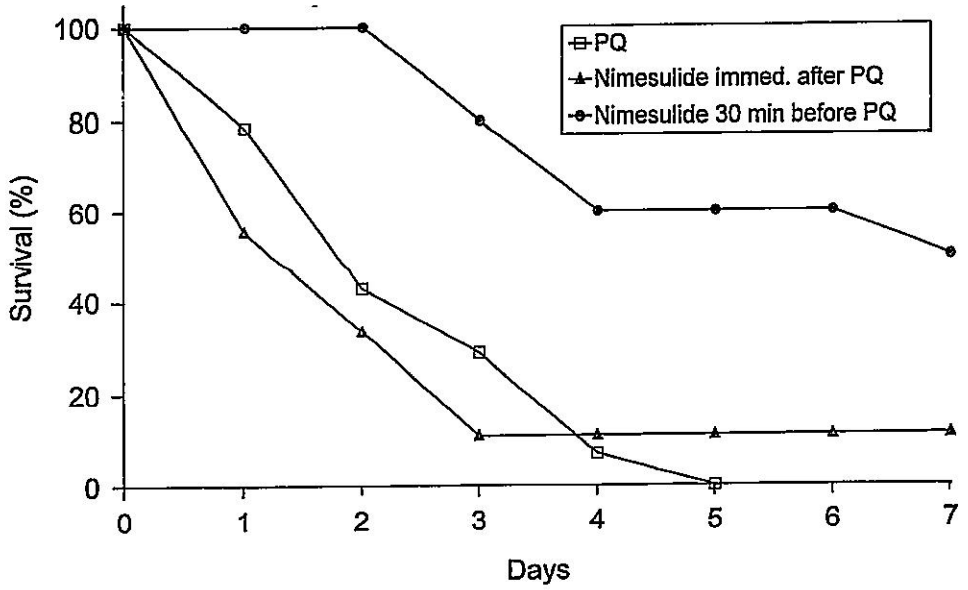

Figure 1. Survival of paraquat (PQ)-poisoned mice (100 mg/kg po) treated by nimesulide (6 mg/kg ip)

absolutely lethal dose of the herbicide for this period (100 mg/kg po), demonstrated that animals which survived a lethal outcome in the first 72 hours after poisoning, remained alive in the further course of the experiment (Figure 1).

DISCUSSION

When ingested, PQ can cause either rapid death from multisystem failure and cardiovascular shock or delayed death from progressive pulmonary fibrosis. The above-mentioned toxicity of PQ is mainly the consequence of its ability to form activated oxygen radicals which initiate lipid peroxidation of cell membranes. Besides agents and procedures which decrease gastrointestinal absorption of PQ (oral administration of Fulleržs earth, bentonite or activated charcoal) or promote its elimination (forced diuresis, hemofiltration, hemoperfusion, hemodialysis), a wide variety of treatments has been attempted in PQ-poisoning: hypooxygenation, superoxide dismutase (SOD), Vitamin E, vitamin C, selenium, sulfhydryl compounds (e.g. N-acetylcysteine), deferoxamine, clofibrat, riboflavin + niacin, methylene blue, zinc, xanthine oxidase inhibitors (e.g. allopurinol), surfactant synthesis inducer ambroxol, immunosuppresants (e.g. cyclophosphamide), corticosteroids, colchicine, lung radiotherapy, lung transplantation and nitric oxide inhalation (Bismuth and Hall, 1995). Unfortunately, most results of these treatments have been conflicting and none of them have been effective. Recently, it has been shown that delayed continuous nitric oxide inhalation along with complex therapy including oral Fulleržs earth, forced diuresis, hemofiltration, N-acetylcysteine, methylprednisolone, peroxidation cyclophosphamide, vitamin E and colchicine has resulted in a successsful outcome after PQ-poisoning (Eisenman et al., 1998). In any case, PQ-poisoning remains a serious and often fatal disorder and studies of new therapeutic procedures have been continued.

In the present study we demonstrated that nimesulide, a new potent NSAID, produced a significant protective effect in PQ-poisoned mice. Besides a strong inhibition of prostaglandin synthesis, this drug exerts also a strong antioxidant action. It inhibits superoxide anion production by human polymorphonuclear leucocytes (Bevilacqua et al., 1988) and this mechanism might be included in its action against PQ-toxicity. Namely, the end result in PQ-induced lipid peroxidation is activation of the macrophage system and further production of oxidizing compounds which have deleterious actions on tissues (Honore et al., 1994; Bismuth and Hall, 1995). Moreover, lipid peroxidation may cause an excessive release of arachidonic acid and thromboxane A_2 which produce an increase in bronchial resistance and pressure, and this may aggravate the lung-damaging effect of PQ. It has been shown that nimesulide strongly antagonises this effect of arachidonic acid being more effective than indomethacin, a known potent NSAID (Berti et al., 1990; 1991).

Our results demonstrated that nimesulide produced a significant protective effect only when given as a pretreatment. Thus, when the drug was given 30

minutes before a lethal dose of PQ the LT-50 of protected animals was 4.32 times longer than that of unprotected ones. These results confirm our previous findings that antioxidants, as protective agents in PQ poisoning, are more effective when given before the herbicide (Dobrić, 1994; 1997). The explanation is that the presence of an antioxidant in target tissue before exposure to lethal doses of PQ is of crucial importance for its protective action.

On the other hand, our results showed that nimesulide given immediately after PQ failed to produce any significant protective effect. However, the 7 day-survival rate of mice, poisoned by an absolutely lethal dose of the herbicide for this period (100 mg/kg po) and treated by nimesulide immediately after the herbicide, demonstrated that animals which had survived a lethal outcome in the first 72 hours after poisoning, remained alive in the further course. We suppose that nimesulide, with regard to its pharmacological action, might delay and prevent lung inflammation and fibrosis by suppressing PQ-induced release of neutrophil chemoattractants. Due to this in further experiments the drug should be given, not in a single dose as in this study, but in a multi-dose regimen, during several days after PQ poisoning, as well as in combination with other drugs used so far for this purpose.

In any case, our study demonstrated that the new NSAID, nimesulide, may offer protection against PQ poisoning which is an encouraging result, having in mind that this problem is not yet therapeutically solved.

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ZAŠTITNA EFIKASNOST NIMESULIDA U MIŠEVA TROVANIH PARAKVATOM

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SADRŽAJ

U mužjaka belih miševa (18-22 g) peroralno otrovanih parakvatom (PQ), tokom 7 dana posle trovanja ispitana je zaštitna efikasnost nesteroidnog antiinflamatornog leka nimesulida (6 mg/kg ip), datog preventivno (30 minuta pre PQ) ili terapijski (neposredno posle PQ). Rezultati su pokazali da nimesulid dat jedino kao pretretman uspešno štiti otrovane životinje značajno povećavajući njihovo srednje vreme preživljavanja (LT-50), kao i srednju smrtnu dozu (LD-50) PQ (7,54 naspram 1,74 dana i 146,04 naspram 98,39 mg/kg u štićenoj, u odnosu na neštićenu grupu). Premda lek, primenjen terapijski, nije pružio značajniju zaštitu, 10-15% životinja otrovanih sa 100 mg/kg PQ (apsolutno letalna doza tokom 7 dana), preživele su posmatrani period. Ovi rezultati ohrabruju i zahtevaju dalja ispitivanja nimesulida kao potencijalnog leka za terapiju trovanja PQ.