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INDUCTION OF ACUTE PHASE REACTION AND SUPPRESSION OF THE IMMUNE SYSTEM IN THE PARAOXON-INTOXICATED RATS

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Widespread use of organophosphates, especially in agriculture, led us to study their toxic effects. Our investigations were focused on paraoxon, an oxygenated analog of a phosphothionate pesticide. This points to an analogy between the response to organophosphate intoxication and the acute phase reaction to injury. The capacity of paraoxon to elicit the acute phase response was assessed by studying two major processes characteristic of acute inflammation, the expression of acute phase proteins (APP) and the immunosuppressive activity of serum. After an LD_{50} paraoxon administration to rats, the serum APP levels increased with time reaching a maximal level at the 24 h time point. The several-fold increases of AGP, MG, Hp and TST concentrations in the circulation of intoxicated rats, as well as a significant immunosuppressive activity of examined animal serum, pointed to the role of APP, especially AGP and MG, as immune

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modulators. These processes are analog to those observed during the acute phase response to injury and aimed at reestablishing homeostasis.

Key words: paraoxon, acute phase proteins, immunosuppression

INTRODUCTION

Organophosphates have a widespread industrial use in the manufacture of flame retardants, plasticizers, lubricants and insecticides. Therefore, the risk of human exposure to their toxic effects is subject of numerous scientific researches. It is well known that organophosphates, besides acting as a potent cholinesterase inhibitors with extremely toxic effects, also exert effects on noncholinergic pathways which elicit changes similar to the reaction to stress (CLEMENT, 1985). Acute mammalian toxicity of organophosphorus insecticides has been generally ascribed to their ability to induce the generalized stress reaction (KUSHNER, 1982) that results in a number of metabolic and systemic changes that are aimed to protect the organism from toxic agents and reestablished homeostasis. The acute phase reaction (APR) is characterized by increased transcription rates of acute phase protein (APP) genes, corresponding mRNA and protein synthesis in the liver. Our studies were focused on paraoxon (O,O-diethyl O-p-nitrophenyl phosphate), the oxygen analog of a phosphothionate pesticide that is produced primarily through cytochrome P-450-dependent oxidative desulfuration of the parent pesticide in the liver (KULKARNI and HODGSON, 1980). The aim of our investigation was to examine whether intoxication with this organophosphate and exerts effects that are characteristic of the APR to tissue injury. The capacity of paraoxon to elicit the APR was assessed by studying two major processes that are characteristic for inflammation, the expression of APPs and immunosuppressive activity of the serum. The results presented here showed that changes in the plasma APP levels as well as immunosuppressive activity of the serum during the toxic response to paraoxon were similar to those observed during the APR.

MATERIAL AND METHODS

Ten week old male Wistar rats weighing between 200-300 g were used. The animals were exposed to LD_{50} of paraoxon (0.75 mg/kg) that was administrated intraperitoneally.

The concentration of plasma APPs was determined by crossed immuno-electrophoresis (GANROT, 1972) with polyspecific antiserum obtained after injection of turpentine-administered rat serum to rabbits. The concentration of α_1-acid glycoprotein (AGP), α_2-macroglobulin (MG), haptoglobin (Hp) and thyostatin (TST) were established by quantification of the areas under the immuno-precipitation peaks formed after immunoelectrophoresis, and expressed as a percent change relative to the control samples (100%).

The immune suppressive effect of paraoxon-intoxicated rat serum was estimated in a system of cultured thymocytes, and expressed as the extent to which their presence affected the concanavalin A (Con A)-stimulated proliferation

of normal rat thymocytes (JACOBSSON and BLOMGREN, 1974), and calculated according to the formula:

$$\text{ConA-index} = \frac{\text{cpm of ConA-stimulated thymocytes + 5\% test serum}}{\text{cpm of ConA-stimulated thymocytes + 5\% FCS}}$$

When the effect of AGP and/or MG on the proliferation of thymocytes was assayed, electrophoretically pure AGP and MG were isolated as described by CHARLWOOD *et al.* (1976) and OKUBO *et al.* (1981), respectively and added to thymocytes cultures in different concentrations: concentrations of AGP and MG in serum of untreated rats, 3- and 5-fold higher concentrations of AGP and 5-, 10- and 20-fold higher concentrations of MG than of untreated animal, respectively. ^{3}H-thymidine uptake was determined and the suppressive effect of AGP calculated as a percentage of the value registered in its absence. When the effect of AGP was studied in the presence of 5% rat serum, 5% fetal calf serum (FCS) was used as a control. The same procedure was used when purified AGP was replaced with purified MG in a culture of thymocytes *in vitro*.

RESULTS AND DISCUSSION

A summary of changes in the serum concentration of individual APPs at 4, 12, 24, 48 and 56 h after the administration of LD_{50} paraoxon is shown in Figure 1. The plasma concentrations of AGP, MG, Hp and TST increased with time reaching a maximal level at 24 h. At this time point, concentrations of AGP and TST were eight- and six-fold higher respectively in the paraoxon-intoxicated rats than in none treated animals, whereas those of MG and Hp exceeded the control value two-fold. The time course of changes in the serum APP levels was similar to those observed during the APR to tissue injury (KOJ *et al.*, 1982). The observed increase of APP concentrations in the circulation could be the consequence of gene transcription, accumulation of corresponding mRNAs and protein synthesis in the liver of paraoxon-intoxicated rats (ŠEVALJEVIĆ et al., 1990). The hepatic APR is elicited through interactions of liver cells with certain released mediators (Fey and FULLER, 1987). Those which determine the level of individual APPs have been identified as cytokines, whereas glucocorticoids were recognized as cofactors that enable the full induction of APP genes (NORTHEMANN *et al.*, 1988).

Resemblance of the APR and the response to paraoxon intoxication were further searched by studying the relationship between APP concentrations and the immunosuppressive potency of the serum. Figure 2 shows that paraoxon intoxication led to a remarkable immunosuppressive effect in rats which could be compared with other organophosphates (CLEMENT, 1985, IVANOVIĆ-MATIĆ *et al.*, 1999). Namely, the ratio of the value for ConA-stimulated proliferation of normal thymocytes in the presence of control rat serum and fetal calf serum (ConA-index) was 0.784. When control serum was replaced by the serum taken 4 h after injection of LD_{50} paraoxon, a 3-fold decrease in the Con A-index was observed. At 24 h

when APP concentrations were significantly enhanced in sera of intoxicated rats (Figure 1) the Con A-stimulated proliferation of normal thymocytes was significantly inhibited and was 2.5-fold lower than that of the control value. Although at the 48 h time point the Con A-index remained below the control level, at later intervals the immunosuppressive potency of the serum decreased linearly with time and normalization of the Con A-index was observed.

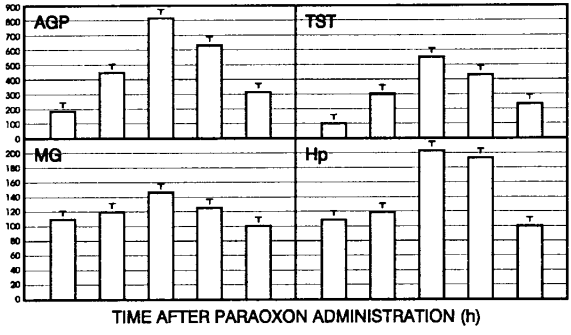

Fig. 1. - Time course of changes in the relative concentration of the major acute phase proteins in rats intoxicated with LD_{50} paraoxon.

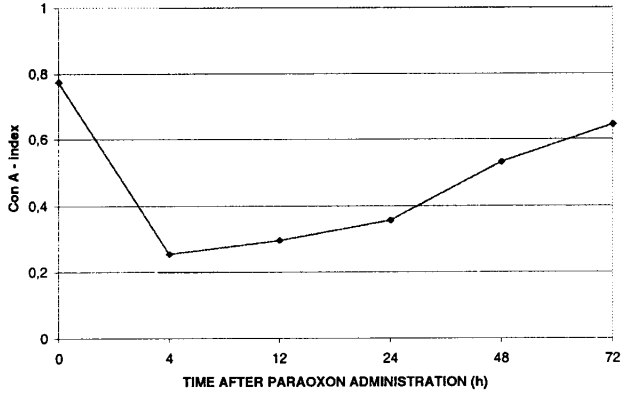

Fig. 2. - Effect of serum from control and LD_{50} paraoxon intoxicated rats on the Con A-induced proliferation of thymocytes from control rats.
Values are means ± SE calculated on the basis of triplicate determinations.

Direct evidence for the immunosuppressive activity of two major APPs, AGP and MG, was obtained in experiments when AGP and MG were isolated from rat serum, dissolved in culture medium and added to a standard thymocyte culture alone or mixed with control rat serum. The results in Table 1. showed that both APPs at the concentrations in the control serum (90 and 32 μg/ml for AGP and MG, respectively) caused significant inhibition of thymocyte proliferation (19% in AGP- and 17% in MG-culture, respectively). The percentage of suppression increased to 97 and 95% when AGP and/or MG concentrations were in 5-fold and

20-fold higher that in the control rat serum, respectively. Addition of the control rat serum to the culture medium caused 7 % inhibition of thymocyte proliferation. Its immunosuppressive activity increased with the amount of added AGP reaching the 80 % inhibition of thymocyte proliferation when 450 μg/ml of the examined protein were put in culture medium. A similar increase of the immunosuppression was observed when MG was added to the culture medium. The Con A-index of the control serum enriched either with AGP or MG was 30 % higher than that of the 24 h acute phase serum, which suggested that those two factors played a major role in controlling immunosuppressive activity during the APR. Moreover, on the basis of literature data significant increase of immunosuppressive activity of rat serum during the early phase of response to paraoxon intoxication (4 h) could be due to the immunosuppressive effect of glucocorticoids whose concentration increases during the early stage of the APR (ŠEVALJEVIĆ *et al.*, 1989). During later phases of the APR to paraoxon-poisoning, when the concentration of glucocorticoids rapidly decreases, the role of immunosuppression is taken over by APPs, especially AGP and MG, as delicate mediators of the immuno response, which prevent the overreaction of the immune system (MUNCK *et al.*, 1984) reestablishing the homeostasis after LD_{50} paraoxon-intoxication.

Table 1. - Effect of AGP and MG on the Con A-Stimulated Proliferation of Normal Rat Thymocytes

	AMOUNT ADDED TO CULTURE				UPTAKE OF ^{3}H-THYMIDINE		
THYMOCYTES (batch)	AGP (g/ml)	MG (g/ml)	FCS (%)	CONTROL SERUM (%)	(cpm/10^6 cells)	SUPPRESSION (%)	Con A-INDEX
1	-	-	-	-	160705–9773	0	
	90	-	-	-	129483–12157	19.4–9.26	
	270	-	-	-	57050–1822	64.5–1.39	
	450	-	-	-	5465–234	96.6–0.31	
2	-	-	5	-	259524–15332	0	
	-	-	-	5	239281–2672	7.8–1.42	0.927–0.017
	90	-	-	5	247822–11976	9.5–2.44	0.955–0.056
	270	-	-	5	158015–8541	39.1–3.30	0.609–0.040
	450	-	-	5	54161–4118	79.1–1.58	0.208–0.030
3	-	-	-	-	4309–34	0	
	-	32	-	-	3587–196	16.8–2.1	
	-	160	-	-	2727–155	36.7–3.6	
	-	320	-	-	2006–196	53.5–3.3	
	-	640	-	-	221–24	94.9–0.3	
4	-	-	5	-	66237–881	0	-
	-	-	-	5	50449–2699	23.8–1.3	0.762–0.041
	-	32	-	5	45105–267	31.9–0.2	0.681–0.004
	-	160	-	5	32731–915	50.6–1.4	0.494–0.014
	-	320	-	5	26783–1421	59.6–3.1	0.404–0.021
	-	640	-	5	17625–662	73.4–2.7	0.266–0.010

Values are means ± s.e. from two separate experiments.

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INDUKCIJA AKUTNO FAZNE REAKCIJE I SUPRESIJA IMUNOG SISTEMA KOD PACOVA TROVANIH PARAOKSONOM

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I z v o d

Široka upotreba organofosfatnih jedinjenja u industriji, a posebno u poljoprivredi, navela nas je da ispitujemo njihove toksične efekte. Istraživanja su bila fokusirana ka paraoksonu, kiseoničnom analogu fosfotionatnog pesticida, sa ciljem da se utvrdi analogija između odgovora organizma na intoksikaciju paraoksonom i akutno fazne reakcije na povredu. Sposobnost paraoksona da indukuje akutno fazni odgovor analizirana je ispitivanjem dva glavna procesa karakteristična za inflamaciju: ekspresije akutno faznih proteina i imunosupresivne aktivnosti seruma. Nakon ubrizgavanja LD_{50} paraoksona u pacove, nivo akutno faznih proteina u serumu životinja se povećava dostižući maksimalni nivo u 24 satu. Višestruko povećanje koncentracije α1- kiselog glikoproteina (AGP), α2-makroglobulina (MG), haptoglobina i tiostatina u cirkulaciji tretiranih pacova kao i značajna imunosupresivna aktivnost seruma tretiranih životinja ukazali su na ulogu ovih proteina, naročito AGP i MG, kao finih modulatora imunološkog odgovora. Ovi procesi su analogni procesima tokom akutno faznog odgovora organizma na povredu i odvijaju se u cilju uspostavljanja fiziološke ravnoteže.

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