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EFFECT OF AQUEOUS EXTRACTS OF *ACANTHOPANAX SENTICOSUS* ON PARATHION TOXICITY IN MICE[1]

Paul W Ferguson, Philip J Medon[2], Carl F Watson

(Division of Pharmacology, Toxicology and Nuclear Pharmacy, School of Pharmacy, Northeast Louisiana University, Monroe LA 71209, USA)

ABSTRACT Parathion toxicity in ♂ Swiss-Webster mice was enhanced by ip pretreatment with the aqueous extract (160 mg/kg/d × 4 d) of the root of *Acanthopanax senticosus* (*AS*). On d 5, *in vivo* RBC AchE was measured before and 30 min after ip parathion (5 mg/kg). *In vitro* parathion metabolism using the S-9 liver microsomal fraction and *in vitro* inhibition of RBC AchE by paraoxon were compared for control and *AS* pretreated mice. RBC AchE prior to parathion was similar to control values from mice not on a pretreatment schedule (280±20 nmol/min/ml RBC). RBC AchE of *AS* pretreated mice challenged with parathion (160±10 nmol/min/ml RBC) was significantly lower than non *AS* pretreated mice (250±10 nmol/min/ml RBC). *In vitro* RBC AchE activity after incubation with paraoxon (0.09 μ*M*) was similarly inhibited for control (55%) and *AS* pretreated mice (59%). No significant differences were observed in % of total analytes after a 30 min incubation with S-9 for parathion (66%, 62%), paraoxon (13%, 15%) and p-nitrophenol (21%, 21%) from control and *AS* pretreated mice, respectively.

KEY WORDS *Acanthopanax senticosus*; parathion; acetylcholinesterase

Reported effects of *Acanthopanax senticosus* (Rupr. et Maxim.) Harms (*AS*, "Siberian Ginseng") in experimental animals have included delayed growth of chemically-induced tumors[1], reduction of blood glucose in naive[2] and epinephrine-induced hyperglycemic[3] mice and decreased toxicity from such chemicals as alloxan and several cardiac glycosides.[3] Aqueous extracts of the root prolonged hexobarbital sleep times and inhibited microsomal (MFO) metabolism of hexobarbital *in vitro*.[4] These studies demonstrate the role of Siberian Ginseng extracts as "adaptogens".[5]

This study was designed to evaluate the effect of *AS* pretreatments on metabolism of the anticholinesterase insecticide, parathion *in vitro* and inhibition of AchE *in vitro* and *in vivo* by paraoxon, the active oxidative metabolite of parathion.

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2 To whom reprint requests should be addressed.

MATERIALS AND METHODS

Aqueous Extracts To every 100 g of powdered roots(obtained from the Svenski Ortmedicinski Institute, Ulricehamn, Sweden)1 L of water (60℃) was added. The mixture was stirred for 10 min, filtered twice and the filtrate frozen and lyophilized to obtain a dry powder weighing 1.6 g. Appropriate test solutions of this extract were made fresh daily with distilled water.[2]

AchE Inhibition Male Swiss Webster mice (31 ± SD 1 g) were pretreated ip with either *AS* (160 mg/kg/d × 4 d) or equal volumes of water. This dose has previously been shown to inhibit MFO.[4]

Red blood cell AchE inhibition was measured before and 30 min after 5 mg/kg ip parathion (98.9% Technical grade, Monsanto Co, St Louis MO) on d 5. RBC AchE activity was measured by a radiometric assay.

To ascertain possible effects of *AS* pretreatment on RBC AchE irrespective of metabolic factors the interaction of paraoxon with RBC AchE was studied *in vitro*. Ten μl RBC were collected from control and treated mice (4/group) and incubated with 0.09 μ*M* paraoxon (Sigma Chemical Co, St Louis MO). The incubation mixture contained 50 μl 0.1 *M* phosphate buffer (pH 7.4), 10 μl water, 10 μl paraoxon (0.25 μg/ml) and 20 μl [*acetyl*-^{3}H]Ach (50 mCi/mmol, New England Nuclear, Boston MA, USA). After 15 min the reaction was stopped with 100 μl of a mixture containing 1 *M* monochloroacetic acid, 0.5 *M* NaOH and 2 *M* NaCl. Four ml of a toluene based scintillation fluor were subsequently added. Free [^{3}H]acetate partitioning into the toluene fluor, indicative of RBC AchE hydrolytic activity, was measured by liquid scintillation counting using a Beckman Model LS-203 scintillation counter[6].

Parathion Metabolism In accord with the RBC AchE studies, Swiss-Webster mice were pretreated ip with either *AS* (160 mg/kg/d × 4 d) or water. On d 5 livers were removed to prepare a liver homogenate (25% in 1.15% cold KCl). The S-9 microsomal fraction was obtained by centrifugation (9000 × *g*, 15 min, 5℃). A 0.5 ml aliquot of the supernatant containing microsomes was added to a phosphate buffered (pH 7.4) mixture consisting of 0.65 μ*M* NADP, 50 μ*M* nicotinamide, 10 μ*M* glucose-6-phosphate and 25 μ*M* $MgCl_2$. After 30 min of incubation the mixture was analyzed for parathion, paraoxon and *p*-nitrophenol[7]. Total protein in the supernatant was measured[8].

Tab 1. Effects of *AS* pretreatments on *in vivo* and *in vitro* RBC AchE activity

Treatment	AchE(nmol/min/ml RBC)
in vivo (n = 5, $\bar{x} \pm$ SD)	
Vehicle only – no preRx	280 ± 40
Vehicle only – *AS* preRx	280 ± 110
Parathion – no preRx	250 ± 20
Parathion – *AS* preRx	160 ± 20
in vitro (n = 3, $\bar{x} \pm$ SD)	
Control – no preRx – no paraoxon	380 ± 10
Control – no preRx – paraoxon	170 ± 20
AS preRx – no paraoxon	450 ± 50
AS preRx – paraoxon	190 ± 20

Tab 2. Effects of *AS* pretreatment on *in vitro* parathion metabolism. The 3 chemicals analyzed totalled 100% of parathion originally added. For each experiment livers were pooled from 4 mice/group, triplicates/assay. $\bar{x} \pm$ SD

AS preRx	% of Total Analytes		
	Parathion	Paraoxon	*p*-Nitrophenol
–	66 ± 12	13 ± 9	21 ± 5
+	62 ± 7	15 ± 7	21 ± 3

Tab 3. Effects of *AS* pretreatment on *in vitro* formation of paraoxon and *p*-nitrophenol. Protein concentration of S-9 supernatant was 40 ± 7 mg protein/ml. For each experiment livers were pooled from 4 mice/group and tripliactes/assay. $\bar{x} \pm$ SD

AS preRx	Formation(pmol/min/mg protein)	
	Paraoxon	*p*-Nitrophenol
–	46 ± 21	75 ± 19
+	61 ± 24	80 ± 24

RESULTS

AchE Inhibition RBC AchE activity *in*

vivo prior to parathion challenge was similar for control mice pretreated with water and for control mice not on a pretreatment schedule (280 nmol/min/ml RBC). RBC AchE of *AS* pretreated mice challenged with parathion (160 nmol/min/ml RBC) was lower ($p<0.01$) than non-*AS* pretreated mice challenged with parathion (250 nmol/min/ml RBC)(Tab 1).

RBC AchE activity *in vitro* after incubation with paraoxon (Tab 1) was similarly inhibited for control (55%) and *AS* pretreated mice (59%). *AS* pretreatment in incubations without paraoxon resulted in higher ($p<0.05$) RBC AchE activity (450 nmol/min/ml RBC) than non-pretreated mice (380 nmol/min/ml RBC).

Parathion Metabolism *In Vitro* Comparison of incubations of parathion with the S-9 microsomal fraction (Tab 2) indicated no significant differences in total analysis for parathion (66%, 62%), paraoxon (13%, 15%) and *p*-nitrophenol (21%, 21%) from control and *AS* pretreated mice, respectively. Additionally, no significant differences in formation rate of paraoxon (46, 61 pmol/min/mg protein) or *p*-nitrophenol (75,80 pmol/min/mg protein) were observed for control and *AS* pretreated mice, respectively (Tab 3).

No significant differences were seen in liver weights or supernatant protein between control and *AS* pretreated mice.

DISCUSSION

Pretreatment with *AS* resulted in more significant *in vivo* inhibition (35%) of RBC AchE than non-pretreated mice when challenged with parathion. This was a surprising result in that adaptogens, like Ginseng, purportedly protect organisms from harmful biochemical stimuli.

To explain an enhanced *in vivo* parathion toxicity increased liver metabolite ratios of paraoxon/*p*-nitrophenol (oxidation/hydrolysis) were expected. This would result from either induction of phosphorothionate oxidases or inhibition of MFO hydrolases[9]. pretreatment with *AS* (160 mg/kg/d × 4 d) resulted in a slight but not significant increase in paraoxon formation (Tab 2,3).

Previous studies of *AS* effects on MFO metabolism are conflicting. Both enzyme induction[10] and enzyme inhibition[4] have been reported. No increase in liver weights after pretreatment were found in our study which suggests a lack of enzyme induction. This may support MFO inhibition as an effect of *AS* pretreatment. The chemical components of *AS* include a variety of "eleutherocides" which have been identified as sterols, lignans and sugar derivatives. One lignan, eleutheroside B_4, also called (−) sesamin, is known for its synergistic effect on the activity of pyrethrin insecticides through inhibition of MFO[3].

RBC AchE inhibition *in vitro* by paraoxon was enhanced slightly ($p<0.05$) over non-pretreated mice. Previous studies in rabbits indicated that *AS* had no significant effect on serum ChE[11]. It is interesting to note that non-paraoxon incubations in our study indicated greater RBC AchE activity for *AS* pretreated mice. Previous studies have indicated potential effects of Ginseng on protein synthesis[12] and this may, in part, account for the increased *in vitro* hydrolytic activity after *AS* pretreatment.

Although parathion activity was significantly enhanced *in vivo*, the *in vitro* assays for effects of *AS* on parathion metabolism and toxicity provided inconclusive information regarding the mechanism of action. The diversity of effects of *AS* on multiple organ systems indicates that isolated organ studies may not provide consistent results and that whole organ studies may not permit the required interplay of stress-related responses prior to an appearance of adaptogenic effects[2,5].

In these experiments we demonstrated an enhancement of parathion toxicity in lieu of an expected reduction. This study emphasizes the importance of investigating potential interactions between dietary products and environmental chemicals.

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刺五加水提取物对小鼠对硫磷毒性的影响

Paul W Ferguson, Philip J Medon[2], Carl F Watson (*Div Pharmacology, Toxicology and Nuclear Pharmacy, School of Pharmacy, Northeast Louisiana University, Monroe LA 71209, USA*)

提要 刺五加(*Acanthopanax senticosus, AS*)根水提取物(160 mg/kg/d×4 d)预先给小鼠ip增加对硫磷毒性，即降低红细胞AChE活力。若不用对硫磷攻击，则AChE活力相似于对照组。红细胞体外与对硫磷(0.09 μM)培养，抑制率相似，对照组55%，AS组59%，两组小鼠S-9肝微粒体与对酸磷试管内孵育，对照组与AS组代谢总分析没有明显差别，对硫磷(62,66%)，对氧磷(13,15%)，p-硝基酚(21,21%)。

关键词 刺五加(*Acanthopanax senticosus*)；对硫磷；乙酰胆碱酯酶

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