Comparison of Protective Effects of Ethylestrenol, Norbolethone, and Spironolactone against Lethality from Acute Doses of Parathion and Paraoxon in Female Rats

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Abstract □ Protection against the toxicity of parathion (increased LD_{50}) was provided by preadministered ethylestrenol and, to a lesser extent, by norbolethone and spironolactone. Ethylestrenol and norbolethone also offered protection against paraoxon toxicity. With ethylestrenol and spironolactone, the protection against parathion lethality was greater than that against paraoxon lethality.

Keyphrases □ Ethylestrenol—effect on toxicity of parathion and paraoxon, compared to norbolethone and spironolactone, rats □ Norbolethone—effect on toxicity of parathion and paraoxon, compared to ethylestrenol and spironolactone, rats □ Spironolactone—effect on toxicity of parathion and paraoxon, compared to ethylestrenol and norbolethone, rats □ Parathion—toxicity in rats, effect of ethylestrenol, norbolethone, and spironolactone □ Paraoxon—toxicity in rats, effect of ethylestrenol, norbolethone, and spironolactone

Several steroids, including ethylestrenol, norbolethone, and spironolactone, offer protection against a variety of toxins when the steroid is given for a period of time prior to challenge with the toxic agent (1). These "catatoxic" steroids induce drug-metabolizing enzymes, and this action has been proposed as the probable mechanism of their protective effect (2). Their effect on paraoxon toxicity has not been reported, and earlier experiments (1) with parathion did not allow an evaluation of the relative protective efficiency of the three agents mentioned here.

In this study, the effects of prior administration of these steroids on the LD_{50}'s of paraoxon and parathion in female rats were determined.

EXPERIMENTAL

Two hundred and twenty-two female, drug-naive, albino Holtzman rats, ~105 g, were used. They were given 10 mg of ethylestrenol, norbolethone, or spironolactone suspended in 1 ml of water or 1 ml of water only, by gastric tube, twice daily for 3 days and once on the 4th day. This dose and dosage schedule had been used previously and shown to be effective in protecting against a variety of toxins (1).

The organophosphorus compounds were dissolved in dimethyl sulfoxide and administered intraperitoneally 2–4 hr after the dose of steroid on the 4th day. Concentrations were adjusted so that the appropriate doses were contained in 0.1 ml of dimethyl sulfoxide/100 g of body weight. The rats were observed for 24 hr, and acute LD_{50}'s and their 95% confidence intervals were computed from the data obtained by the method of Finney (3).

RESULTS AND DISCUSSION

The results (Table I) show that the steroids afforded protection against parathion in the following order of effectiveness: ethylestrenol > norbolethone = spironolactone > control. The protection to paraoxon lethality afforded by ethylestrenol was greater than that afforded by norbolethone. Spironolactone did not protect against paraoxon toxicity. Ethylestrenol and norbolethone afforded more protection to parathion-poisoned rats than to those poisoned with paraoxon. Although ethylestrenol and norbolethone are anabolic agents and spironolactone is a diuretic, no differences in weight gain were observed ($p > 0.05$, the Student t test for unpaired data) during the 4 days of treatment.

Table I—Effect of Pretreatment with Spironolactone, Norbolethone, or Ethylestrenol on the Lethality of Parathion and Paraoxon in Female Rats

Pretreatment	Poison	LD_{50}[a], mg/kg	95% Confidence Interval
Water sham	Parathion	1.40	1.27–1.58
	Paraoxon	0.43	0.35–0.52
Spironolactone	Parathion	2.80	2.52–3.05
	Paraoxon	0.48	0.21–0.71
Norbolethone	Parathion	2.86	2.59–3.10
	Paraoxon	0.88	0.68–0.98
Ethylestrenol	Parathion	5.90	3.70–7.68
	Paraoxon	1.06	1.03–1.12

[a] Each LD_{50} was calculated from observations of the lethality of the poison at at least three dosage levels with seven to nine rats in each group.

Pretreatment with catatoxic steroids has been previously shown to increase the metabolism rate of various compounds (2). In this study, the protection afforded by two of the steroids was greater against parathion lethality than against paraoxon lethality. This finding indicates that enzymes that inactivate both parathion and its active metabolite paraoxon are being induced if only an increased rate of metabolism is involved in the protective effects. It has been demonstrated previously that the enzymes metabolizing parathion and paraoxon can both be induced (4).

However, an increase in the LD_{50} does not necessarily indicate an effect on the rate of inactivation of the toxin. The toxicity of paraoxon can also be altered by an alteration in its binding to relatively nonvital tissues (5, 6). Other factors such as an altered rate of absorption from the peritoneal cavity or an alteration in the permeability of the blood–brain barrier also may be involved in the observed changes in lethality.

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Rapid GLC Determination of Fusaric Acid in Biological Fluids

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Abstract □ A simple, sensitive GLC assay was developed for fusaric acid, the active metabolite of bupicomide, to follow the disposition of this investigational antihypertensive agent in patients undergoing therapy. Fusaric acid is efficiently extracted from biological samples, derivatized by on-column methylation, and chromatographed using flame-ionization detection. An internal standard is utilized to quantitate results. The procedure is rapid and specific for fusaric acid, and has a lower limit of sensitivity of 0.1 μg/ml. The method is suitable for supporting pharmacokinetic studies of bupicomide following therapeutic doses in animals and humans.

Keyphrases □ Fusaric acid—active metabolite of bupicomide, GLC analysis, biological fluids □ GLC—analysis, fusaric acid (active metabolite of bupicomide), biological fluids □ Bupicomide—pharmacokinetic study by GLC analysis of fusaric acid metabolite, biological fluids

Fusaric acid (5-*n*-butylpicolinic acid) isolated from fungus culture filtrates was found to be a potent inhibitor of dopamine β-hydroxylase under both *in vitro* and *in vivo* (1) conditions and demonstrated significant hypotensive effects in animals (2–4) as well as in humans (5–6). Bupicomide[1], the amide derivative of fusaric acid, was reported to possess pharmacological activities similar to the parent compound (7).

Bupicomide is presently undergoing clinical trial as an antihypertensive agent. Studies using radiolabeled drug indicated that bupicomide underwent extensive metabolism rapidly when administered orally to animals (8). No unchanged drug was found by following the plasma time course of bupicomide. Only fusaric acid, a major metabolite, was identified.

Similar findings were reported in the same studies (8) after administration of a 15-mg/kg oral dose of bupicomide to humans. The biological half-life of fusaric acid was reported to be 8.7 hr in humans, utilizing a UV spectrophotometric procedure. However, for the analysis of fusaric acid in plasma, direct application of the UV assay to clinical studies involving therapeutic doses is limited by the lack of sensitivity and specificity.

The present work describes an analytical method in which fusaric acid is extracted from a biological sample along with an added internal standard into an organic solvent and subsequently is derivatized by on-column methylation for GLC determination. The procedure is applicable to blood and urine and is rapid enough to be performed routinely for clinical studies of patients on bupicomide therapy.

[1] Sch 10595.

EXPERIMENTAL

Reagents—All chemicals and solvents used were analytical reagent grade. The internal standard, *N*-*n*-butylphthalimide, was synthesized according to standard methods (9) by refluxing *n*-butylamine and phthalic anhydride in acetic acid. The crude product was recrystallized from alcohol–water before use, mp 32 ± 0.5° (10).

Procedure for Biological Samples—Blood samples were collected[2] and allowed to clot. The serum was separated by centrifugation and frozen until analysis. Spiked standards of fusaric acid in pooled serum were treated similarly. Urine samples also were collected, frozen, and stored until analysis.

A biological fluid, 0.5 ml, was transferred to a screw-capped[3] tube containing 2 μg of *N*-*n*-butylphthalimide, 0.5 ml of 0.1 *M* pH 4 citrate buffer that had been presaturated with sodium chloride, and 4.0 ml of methylene chloride. The mixture was mechanically shaken for 5 min and then centrifuged at 2200 rpm for 5 min to allow separation of the phases. The top layer was aspirated off, and the organic layer was decanted into a 5-ml vial[4] and evaporated to dryness under vacuum.

About 0.3 ml of acetone was used to wash the sides of the vial with intermittent vortex mixing. The acetone was removed under vacuum, and 10–15 μl of 0.1 *M* trimethylphenylammonium hydroxide[5] was added to the evaporated sample, followed by 1 min of vigorous vortex mixing. Then 1–2 μl of the mixture was injected into the gas chromatograph.

GLC—The assay was performed on a gas chromatograph[6] equipped with a flame-ionization detector. A 183-cm (6-ft) × 2-mm i.d. glass coiled column packed with 3% OV-1 on 100–120-mesh Gas Chrom Q[7] was used. GLC conditions were: column temperature, 150°; and detector and injector temperatures, 250°. The nitrogen carrier gas was maintained at 40 ml/min, while air and hydrogen flow rates were 250 and 30 ml/min, respectively. Methylation of the samples occurred in the injection port, with the derivatized product eluting as a sharp peak at ~6 min followed by the internal standard peak at ~7 min.

Injection could be resumed at ~12 min when the impurities had

[2] Vacutainers, no anticoagulant, Beckton, Dickinson and Co., Rutherford, N.J.
[3] Lined with Teflon (du Pont).
[4] Reacti-vial, Pierce Chemical Co., Rockford, Ill.
[5] Eastman Organic Chemicals, Rochester, N.Y.
[6] Varian model 2700 (Varian Aerograph, Walnut Creek, Calif.) equipped with a Hitachi Perkin-Elmer recorder, model 156.
[7] Applied Science Laboratories, State College, Pa.