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Note

Quantitative determination of carbaryl and carbaryl metabolites by reversed-phase high-performance liquid chromatography

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Carbaryl (Sevin) is one of the most widely used industrial and domestic insecticides[1]. This anticholinesterase agent is effective against both insect and mammalian cholinesterases and excessive exposure to this compound has been associated with toxicity in both domestic animals and man[2,3]. A number of gas chromatographic methods are available for the detection of carbaryl in a variety of media; however, these methods involve extensive preliminary sample processing and are unable to determine carbaryl and its metabolites simultaneously[4]. Recently high-performance liquid chromatographic (HPLC) methods have been developed to measure carbaryl levels in biological fluids[5–7]; two of these methods can simultaneously determine both carbaryl and its hydrolysis product α-naphthol using fluorescence detection[6,7].

In this paper we describe two simple methods, one of which can simultaneously separate and quantitate carbaryl and α-naphthol and a second which simultaneously separates the 4- and 5-hydroxylated metabolites of carbaryl. The two methods differ only in the composition of the mobile phase used. These methods are suitable for use in pharmacokinetic studies.

EXPERIMENTAL

Reagents

Carbaryl, mesurol and betanol were supplied by the E.P.A. (Research Triangle Park, NC, U.S.A.), α-naphthol was obtained from Eastman Kodak (Rochester, NY, U.S.A.) and the hydroxylated metabolites of carbaryl were gifts from Dr. A. Strother (Linda Loma University, CA, U.S.A.). Tetrabutylammonium hydrogen sulphate (TBA) was obtained from Sigma (St. Louis, MO, U.S.A.). All solvents were of HPLC grade (Burdick and Jackson, Doraville, GA, U.S.A.).

Chromatography

The methods were developed on a Waters liquid chromatograph. The system consisted of a Model 6000A solvent delivery system, a Model 450 variable-wavelength UV absorbance detector which was set at 210 nm and a U6K universal liquid chromotograph injector. Analytical separations were carried out on a Biophase ODS 5-μm reversed-phase column (Bioanalytical systems, CA, U.S.A.). The mobile phase for the separation of carbaryl from α-naphthol consisted of water–acetonitrile (45:55)

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flowing at 1.5 ml/min. The mobile phase for the separation of 4- and 5-hydroxycarbaryl was water–acetonitrile (75:25), containing 3.5 m*M* TBA, at a flow-rate of 1.5 ml/min.

Extraction procedure

Extractions were carried out in 10-ml capacity glass culture tubes. Mesurol was used as the internal standard for the determination of carbaryl and α-naphthol and betanol was used as the internal standard for the hydroxylated metabolites. Samples of plasma or urine (0.5 to 1.0 ml) containing internal standard (500 ng) were extracted with 5 ml of hexane–ethyl acetate (3:1) by vortex mixing for 30 s. After centrifugation (100 *g* for 10 min) and separation, the organic phase was evaporated to dryness under a steady stream of nitrogen. Samples were reconstituted in acetonitrile (50 μl). An aliquot of 5–50 μl was injected onto the column.

Standard curves

Standard curves were prepared by adding known quantities of carbaryl, α-naphthol, 4-hydroxy- and 5-hydroxycarbaryl (0–200 μg) to a fixed amount of the appropriate internal standard (500 ng) in drug-free plasma or urine. Sample were analysed as described above and the peak height ratio of carbaryl or carbaryl metabolite to internal standard was plotted against the corresponding concentration. Peak height ratios of unknown samples were similarly determined and concentrations were calculated from the standard curve. The extraction efficiencies for carbaryl and carbaryl metabolites were calculated by comparison of the peak height of the extracted compound with that of directly injected stock solutions of equivalent concentration. Intra and inter-assay variations were determined from replicate samples spiked with carbaryl or metabolites (*ca.* 100 ng/ml).

Pharmacokinetic study

Carbaryl (10 mg/kg) was administered to a healthy male mogrel dog (15 kg) as an intra-portal bolus dose. Venous blood samples were taken pre-dose and at 5, 15, 30, 45, 60, 105, 120, 150, 180 and 240 min. Heparinized blood was centrifuged (1000 *g* for 15 min) and the separated plasma was removed and stored at −20°C until the time of analysis. The right and left ureters were cannulated and urine was collected over 4 h. Urine samples were treated with β-glucuronidase/arylsulfatase (200 units/ml) for 5 h at 50°C prior to extraction in order to hydrolyze carbaryl and metabolites excreted as conjugates.

RESULTS AND DISCUSSION

The extraction procedure resulted in simple sample preparation. The use of hexane–ethyl acetate as the extraction solvent was the most appropriate system evaluated, producing efficient extraction of carbaryl and metabolites without excessive co-extraction of endogenous substances. Fig. 1 shows chromatograms, obtained using the water–acetonitrile (45:55) mobile phase, from a plasma sample drawn prior to drug administration (Fig. 1A) and from a plasma sample obtained 1 h after administration of carbaryl to the dog (Fig. 1B). After drug administration, there were three distinct peaks with retention times of 5, 6 and 10 min corresponding to carbaryl

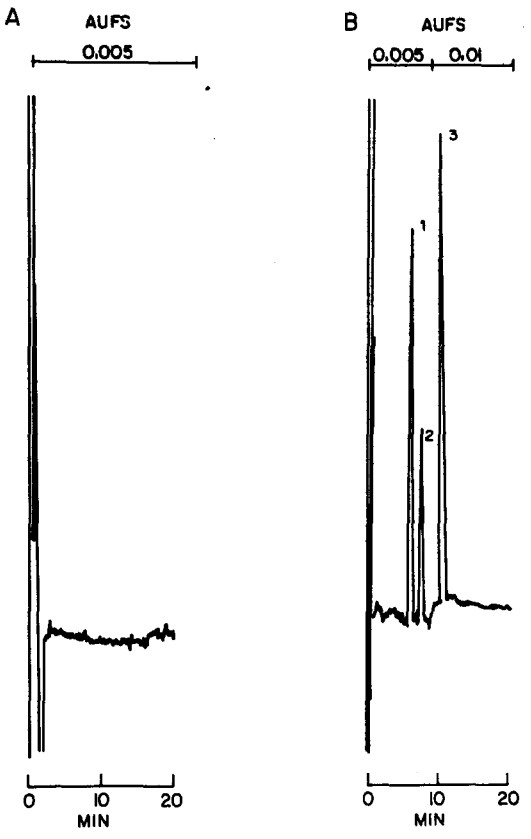

Fig. 1. Chromatograms from (A) a blank plasma extract and (B) an extract of dog plasma 1 h after the administration of carbaryl. Chromatographic separation of carbaryl (1), α-naphthol (2) and mesurol (3) was achieved using a mobile phase of water–acetonitrile (45:55) flowing at 1.5 ml/min.

(3.9 μg/ml), α-naphthol (2.2 μg/ml) and mesurol (internal standard), respectively. Using this mobile phase, there was no interference from endogenous compounds in either dog plasma or urine and all three compounds were completely resolved to baseline while maintaining excellent peak shape. Under the above conditions, the 4- and 5-hydroxylated metabolites were eluted with the solvent front.

Fig. 2 shows chromatograms from urine obtained before (Fig. 2A) and 2 h after carbaryl administration (Fig. 2B) using the water–acetonitrile (75:25) mobile phase containing 3.5 m*M* TBA. TBA was a necessary component of this mobile phase, producing a more lipid-soluble complex with the hydroxylated metabolites. At concentrations less than 3 m*M*, both metabolites were eluted with the solvent front and above 5 m*M*, both compounds exhibited excessively long retention times with a corresponding loss of peak shape and sensitivity. After drug administration, there were three distinct peaks with retention times of 5.2, 11 and 12.5 min, corresponding to the internal standard betanol, 5-hydroxycarbaryl (84 μg/ml) and 4-hydroxycarbaryl (93.2 μg/ml), respectively. There was no interference from endogenous

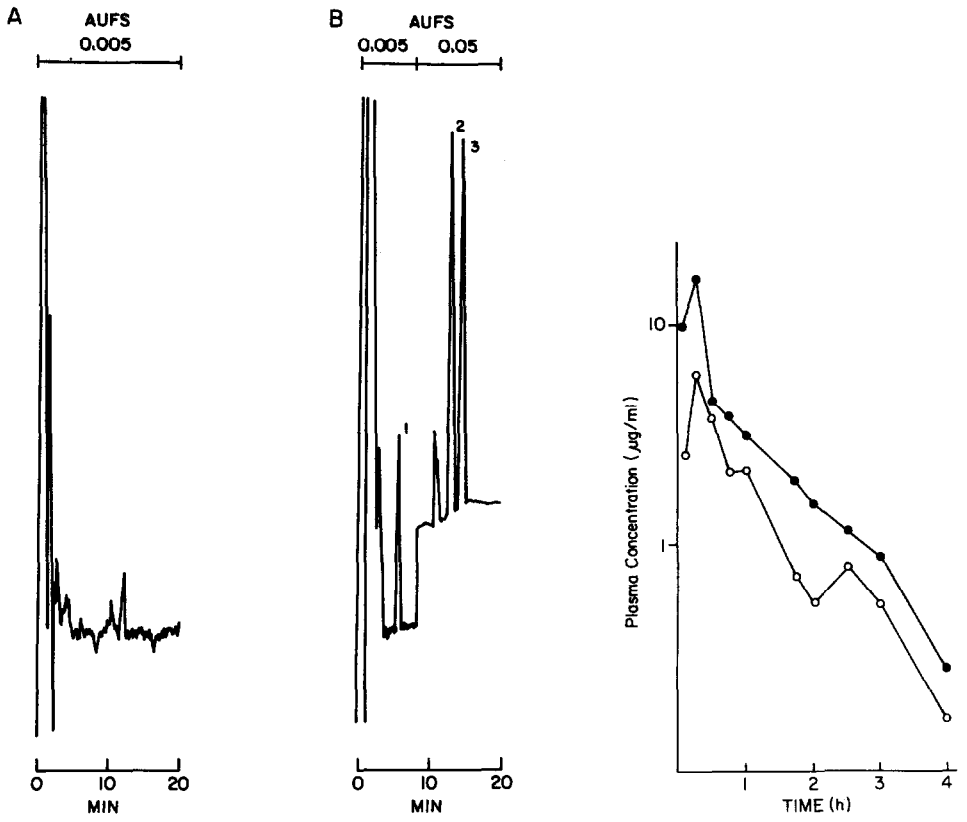

Fig. 2. Chromatograms from (A) a blank urine extract and (B) an extract of dog urine 2 h after administration of carbaryl. Samples had been hydrolyzed with β-glucuronidase/arylsulfatase. Chromatographic separation of betanol (1), 5-hydroxycarbaryl (2) and 4-hydroxycarbaryl (3) was achieved using a mobile phase of water–acetonitrile (75:25), containing (3.5 m*M*) TBA.

Fig. 3. Plasma concentration *versus* time profiles for carbaryl (●—●) and α-naphthol (○—○) after intraportal administration of carbaryl (10 mg/kg) to a dog.

compounds and all three compounds were completely resolved to baseline. The retention times of carbaryl and α-naphthol in this system were in excess of 30 min. In both mobile phases acetonitrile was preferred to methanol due to the improvement in peak shape, resulting from the reduction in non-specific hydrogen bonding of this solvent with carbaryl and its metabolites.

Calibration curves for all compounds were linear in the range 0–200 μg/ml (r = 0.997) and the analytical recoveries were 90, 60, 80 and 80% for carbaryl, α-naphthol, 4-hydroxy- and 5-hydroxycarbaryl, respectively.

The limit of detection, representing a peak four times baseline variation at the highest detector sensitivity (0.005 a.u.f.s.), was less than 5 ng/ml for carbaryl, 4-hydroxycarbaryl and 5-hydroxycarbaryl and 10 ng/ml for α-naphthol. The intra- and inter-assay coefficients of variation of spiked plasma samples at concentrations of approximately 100 ng/ml were 9.8% (n=5) and 9.1% (n=5) for carbaryl, 1.5%

($n = 5$) and 8.6% ($n = 5$) for α-naphthol, 4.5% ($n = 5$) and 5.6% ($n = 5$) for 4-hydroxycarbaryl, and 3.2% ($n = 5$) and 2.4% ($n = 5$) for 5-hydroxycarbaryl.

This assay was applied to a study of the disposition of carbaryl in the dog following an intra-portal bolus dose of 10 mg/kg. Fig. 3 shows the plasma concentration–time profiles for both carbaryl and α-naphthol. Plasma carbaryl concentration declined rapidly and monoexponentially with a half-life of 54 min. α-Naphthol was rapidly formed after carbaryl administration and was eliminated at a rate comparable to that of the parent compound. The hydroxylated metabolites of carbaryl were not detected in plasma. However, these compounds were excreted in significant quantities in the urine, mainly as their conjugated forms. The cumulative urinary excretion of carbaryl, α-naphthol, 4-hydroxycarbaryl and 5-hydroxycarbaryl in urine after deconjugation with β-glucuronidase/arylsulfatase accounted for only 3% of the dose with 0.007% as carbaryl, 0.06% as α-naphthol, 0.237% as 5-hydroxycarbaryl, and 2.5% as 4-hydroxycarbaryl. Thus, carbaryl, administered parenterally, is rapidly cleared from the dog. However, only a small proportion of drug is recovered from urine, implying either a substantial biliary excretion or excretion as additional unidentified metabolites.

The advantages of these assays over earlier assays are the simplicity of sample treatment without compromising selectivity and sensitivity, and the ability to quantitate both carbaryl and its known principal metabolites in biological fluids, enabling detailed pharmacokinetic studies to be carried out.

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