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Relationship between brain and plasma carbaryl levels and cholinesterase inhibition☆,☆☆

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ABSTRACT

Carbaryl is a *N*-methylcarbamate pesticide and, like others in this class, is a reversible inhibitor of cholinesterase (ChE) enzymes. Although studied for many years, there is a surprising lack of information relating tissue levels of carbaryl with ChE activity in the same animals. The present studies were undertaken to describe the dose–response relationship about 40 min (approximate time of maximal ChE inhibition) after oral treatment in adult, post-natal day (PND) 17, and PND11 rats. Additionally, the time-course of plasma ChE activity and carbaryl levels in adult rats was determined after a 30 mg/kg dosage of carbaryl. The time-course study found that carbaryl levels could be detected in plasma 1 h after dosing, but rapidly decreased below the level of quantitation by the 2 h time point. In the dose–response studies, treatment-related increases in plasma and brain carbaryl levels were observed 40 min after dosing. Plasma levels of carbaryl increased linearly, while brain levels appeared to asymptote after 75 mg/kg carbaryl. Plasma and brain levels of carbaryl appeared to be linearly related with a slope close to 1 after various dosages (range: 1–75 mg/kg) of carbaryl at the 40 min time point. Finally, the dose-related relationship between tissue levels of carbaryl and ChE activity was described using a first order exponential decay function with an asymptote. The parameters of this function did not appear to differ between adult, PND17, or PND11 rats. This indicates that age-related differences in brain ChE inhibition by carbaryl are unlikely to be the result of greater tissue levels of the pesticide in PND11 animals. These are the first studies to report the relationship between brain and plasma tissue levels of carbaryl and ChE activity on an individual animal basis. The results of these experiments will be useful to extend physiologically-based pharmacokinetic models for carbaryl and their application in risk assessment.

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1. Introduction

Carbaryl is a member of the *N*-methyl carbamate family of insecticides. This class of pesticides has been studied for many years, and the mechanism of toxicity is related to inhibition of acetylcholinesterase activity in peripheral and central nervous systems (Cranmer, 1986; Mount and Ohme, 1981; World Health Organization, 1986, 1994). Several recent studies from our laboratories have examined the relationship between inhibition of ChE activity by carbaryl on behavioral and physiological measures (McDaniel et al., 2007; Moser et al., 2010; Mwanza et al., 2008). These published studies from our laboratories related the ChE activity in brain tissue to measures such as motor activity and altered photic afterdischarge. The inhibition of ChE activity was used as a surrogate measure of tissue levels of carbaryl.

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The metabolism of carbaryl has also been studied in detail (Carpenter et al., 1961; Knaak et al., 1965; Knaak, 1971; Krishna and Casida, 1966; Leeling and Casida, 1966; Nong et al., 2008; Strother and Wheeler, 1980). There are several metabolites of carbaryl and their conjugates (Tang et al., 2002), many metabolites are similar between man and rats (Knaak et al., 1965; Strother, 1972), and one commonly measured metabolite is 1-naphthol. Several of the above studies have also measured ChE activity in various tissues (Cambon et al., 1978; Carpenter et al., 1961; Ehrich et al., 1992; Mount et al., 1981). Unfortunately, several of these studies have used high dosages of carbaryl (450–1200 mg/kg) (Carpenter et al., 1961; Mount et al., 1981), diluted the tissues for ChE determination (Cambon et al., 1978), or used long incubation times (Carpenter et al., 1961). These two latter procedures may increase reactivation of ChE activity, underestimating the actual levels of inhibition (Hunter and Padilla, 1999; Hunter et al., 1997; Winteringham and Fowler, 1966). Additionally, many of these previous studies report the group mean levels of carbaryl or ChE activity (Carpenter et al., 1961; Ehrich et al., 1992; Mount et al., 1981). This prevents the determination of the relationship between carbaryl levels and ChE activity on an individual animal basis. Recently, there has been work developing a physiologically-based pharmacokinetic (PBPK) model for carbaryl and/or other carbamate pesticides (Nong et al., 2008; Zhang et al., 2007 [abstract]). The model by Nong et al. (2008) also measured ChE activity, but did not directly model the relationship between the tissue levels of carbaryl and ChE inhibition. Additionally, plasma carbaryl was detected only following intravenous dosing in their study. However, Nong et al. (2008) did fit previously published (Fernandez et al., 1982) blood [^{14}C]carbaryl, total blood ^{14}C activity, and plasma ChE activity data, using the PBPK model to show the predicted fit to a second data set.

The present work was undertaken to determine the relationship between brain and/or plasma levels of carbaryl and ChE activity in these two tissues of individual rats following oral dosing. Previously, behavioral and physiological changes, and/or ChE activity, produced in these same animals have been reported (McDaniel et al., 2007; Moser et al., 2010; Mwanza et al., 2008; Padilla et al., 2007). This manuscript correlates the previously reported ChE activity with tissue levels of carbaryl. We also examined the relationship between brain and plasma levels of carbaryl. Finally, one of the previous reports (Moser et al., 2010) has indicated that young animals (PND 11) were more sensitive than adult animals to carbaryl-induced brain ChE inhibition. We quantified tissue levels of carbaryl in the same animals used in Moser et al. (2010), to determine if the increased inhibition of brain ChE activity in PND 11 animals could be explained by greater brain levels of carbaryl.

2. Materials and methods

2.1. Animals

The rats used in these studies were housed in an AAALAC International accredited facility, and all investigations were approved by the National Health and Environmental Effects Research Laboratory Animal Care and Use Committee of the United States Environmental Protection Agency that requires compliance with National Institute of Health guidelines. Male Long Evans rats (minimum of 90 days old), or timed-pregnant female rats, were purchased from Charles River Laboratories (Wilmington, MA or Raleigh, NC), and housed with a 12:12 h light:dark cycle (lights on at 06:00 a.m.). Housing conditions were maintained at 22 ± 2 °C with 40 ± 20% humidity, and 10–15 air changes per hour of 100% filtered fresh air. Animals were housed singly with heat-treated pine shavings or hardwood chip bedding (Beta-Chip) in transparent polycarbonate cages. Pregnant females were provided with Enviro-Dri to serve as nesting material. All animals were provided food (Purina Lab Chow #5001, or Purina Formulab Diet #5008 for pregnant females, St. Louis, MO) and filtered tap water *ad libitum*, and were allowed to acclimate to the facility for 5–6 days prior to beginning the experiments.

2.2. Carbaryl preparation

Carbaryl (1-naphthalenol methylcarbamate, CAS: 63-25-2, 99% pure) was purchased from Chem Service (West Chester, PA) and was suspended in corn oil with vortexing and/or stirring to maintain uniformity in all studies.

2.3. Study 1: Time-course in adult rats

A detailed description of the dosing procedure, tissue collection, and the ChE assay can be found in Padilla et al. (2007). Briefly, 35 animals were assigned to 6 groups. The rats were dosed by oral gavage (1 ml/kg) with 0 (n = 1 at each time) or 30 (n = 5 at each time, except n = 4 at 24 h) mg/kg of carbaryl, and sacrificed 0.5, 1, 2, 4, 6, or 24 h later. Only plasma samples were analyzed for carbaryl concentration by HPLC from this time-course study.

2.4. Study 2: Dose–response in adult rats

A detailed description of the dosing procedure, tissue collection, and the ChE assay can be found in McDaniel et al. (2007). In summary, rats were assigned to 6 groups, with brain and erythrocyte ChE measured in 5 rats/dose. Animals were dosed by oral gavage (1 ml/kg) with 0, 3, 7.5, 15, 30 or 50 mg/kg of carbaryl, and sacrificed after behavioral testing at 40 min after dosing. In this study, both brain and plasma tissues were analyzed by HPLC. During the extraction procedure, one brain sample from the 3 mg/kg group was contaminated and discarded, resulting in a n = 4 in this group.

2.5. Study 3: Dose–response in adult rats after physiological testing

A detailed description of the dosing procedure, tissue collection, and the ChE assay can be found in Mwanza et al. (2008). For this report, the data from the two cohorts was combined (as in Mwanza et al., 2008). Briefly, the animals were implanted with epidural electrodes and allowed to recover for 1 week prior to dosing and neurophysiological testing. The rats were treated with 0 (n = 22), 1 (n = 17), 3 (n = 17), 10 (n = 17), 30 (n = 17), 50 (n = 23), or 75 mg/kg (n = 12) carbaryl. During tissue collection (40 min after dosing), the plasma for one animal in the 1 mg/kg and one rat in the 50 mg/kg dosage groups were lost. The tissue level data from these animals were removed from this study, to allow accurate statistical assessment of tissue-related differences in carbaryl levels. Thus, the final group sizes were n = 16 for the 1 mg/kg and n = 22 for the 50 mg/kg treatments. The carbaryl levels in brain tissue from these animals were included when examining the relationship with brain ChE activity. Both brain and plasma tissues were analyzed by HPLC in this study.

2.6. Study 4: Dose–response in different ages of rats

A detailed description of the dosing procedure, tissue collection, and the ChE assay can be found in Moser et al. (2010). Briefly, within each litter, pups were assigned to a treatment group. Adult animals were obtained separately. For the postnatal day (PND) 11, there were 8 rats/dosage, for PND17 there were 10 rats/dosage, and for adults there were 6 rats/dosage. Animals were dosed by oral gavage with 0, 3, 7.5, 15, or 30 mg/kg carbaryl in a 2 ml/kg dosage volume. Rats were sacrificed 40–45 min after dosing. Both brain and plasma tissues were analyzed by HPLC.

2.7. Tissue collection, storage, and cholinesterase assay

Details of tissue collection, storage, and the ChE assay have been previously reported (McDaniel et al., 2007; Moser et al., 2010; Mwanza et al., 2008; Padilla et al., 2007). Briefly, animals were sacrificed by decapitation and trunk blood was collected into a heparinized tube and placed in ice. Brains were rapidly removed, sectioned sagittally, weighed (Study 3), frozen on dry ice, and stored at −80 °C. The blood was centrifuged for 10 min at 950–1000 × g. The supernatant plasma was removed from the erythrocyte pellet and frozen at −80 °C until analysis. Cholinesterase activity was quantified using a slightly modified radiometric assay (Johnson and Russell, 1975). These storage and assay conditions minimize reactivation of ChE caused by factors such as sample dilution, elevated temperatures, and prolonged incubation times (Hunter and Padilla, 1999; Hunter et al., 1997; Winteringham and Fowler, 1966).

2.8. Tissue extraction

The tissues were defrosted on ice, and the brains weighed (Studies 1, 2, and 4). In Studies 1, 2 and 3, the brain tissue or 500 μl of plasma was transferred to a polypropylene tube, spiked with 15,000 ng chlorpyrifos methyl (99%, Chem Serve, West Chester, PA) in 125 μl acetonitrile (HPLC Grade; Fisher Scientific, Fair Lawn, NJ) as an internal standard, and 5 ml of ethyl acetate was added (Chromosolv, ≥99.7% pure, Sigma–Aldrich, St. Louis, MO). Brain tissue was homogenized on ice using a Polytron (Model PT6100; Kinematica AG, Switzerland) using three bursts of 20 s duration with a setting of 8.8 × 1000 rpm. The homogenate was then centrifuged at 4 °C for 10 min at 1000 × g. Plasma was vortexed for 1 min and allowed to separate.