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Effect of carbamate esters on neurite outgrowth in differentiating human SK-N-SH neuroblastoma cells

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Abstract

Carbamate esters are widely used as pesticides and can cause neurotoxicity in humans and animals; the exact mechanism is still unclear. In the present investigation, the effects of carbamates at sublethal concentration on neurite outgrowth and cytoskeleton as well as activities of acetylcholinesterase (AChE) and neuropathy target esterase (NTE) in differentiating human SK-N-SH neuroblastoma cells were studied. The results showed that 50 μM of either aldicarb or carbaryl significantly decreased neurite length in the retinoic acid-induced differentiation of the neuroblastoma cells, compared to cells treated with vehicle. Western blot analyses revealed that neither carbamate had significant effects on the levels of actin, or total neurofilament high molecular proteins (NF-H). However, increased NF-H phosphorylation was observed following carbamate treatment. These changes may represent a useful in vitro marker of carbamate neurotoxicity within a simple model of neuronal cell differentiation. Furthermore, activity of AChE, but not NTE, was significantly inhibited by aldicarb and carbaryl in differentiating cells, which suggested that cytoskeletal protein changes induced by carbamate esters in differentiating cells was associated with inhibition of AChE but not NTE.

Keywords: Carbamate; Neurite outgrowth; Neurofilaments; Acetylcholinesterase; Neuropathy target esterase; Neuroblastoma SK-N-SH cells

1. Introduction

Carbamates, which are all derived from the basic structure of carbamic acid, represent a broad variety of compounds. Many carbamates are potential neurotoxicants, particularly following occupational, accidental or intentional exposure. Acute toxic symptoms of carbamate poisoning are generally caused by inhibition of the enzyme acetylcholinesterase (AChE), which leads to accumulation of acetylcholine (ACh) [1]. However, studies on chronic exposure to carbamate insecticides and case reports of long-term exposure provide conflicting data, wherein some show no effects after chronic exposure [2], and others describe memory impairment [1,3,4], degenerative polyneuropathy [5], neurobehavioral effects [6] and other neurological disorders [3,7]. Overall, it appears that at least some carbamate esters may initiate neurological and behavioral changes at dose levels that produce few overt signs of acute toxicity or significant reduction in nervous tissue AChE activity [1]. However, the mechanisms involved in these changes remain to be elucidated.

Aldicarb is a commonly used and very potent carbamate pesticide. Aldicarb has appeared in some foods

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at toxic levels [8,9] and its toxicity and exposure hazards have been widely studied [2,10]. Carbaryl, another carbamate shown to cause acute neurotoxicity through inhibition of cholinesterase, is used extensively in agriculture and veterinary medicine. Additionally, subacute neurotoxicity and "delayed neurotoxicity"-like toxicity in man and animals after exposure to carbaryl have also been reported [7,11]. An extensive survey of carbaryl toxicology reports a variety of reversible neurobehavioral and neurotoxic effects in vertebrates, all associated with acute poisoning symptoms [12]. However, there is a paucity of data on the biochemical changes underlying neurodegenerative effects of aldicarb and carbaryl.

Potential models for studying morphological and biochemical damage from neurotoxic compounds include cultured cells with neuronal properties and stable cell lines. Differentiating mouse N2a neuroblastoma cells represent an appropriate in vitro cell system for conducting mechanistic studies. Studies in these cells with organophosphorus compounds (OPs) and carbaryl suggest these pesticides act through different cytotoxic mechanisms [13,14]. Human neuroblastoma SK-N-SH cells, the parental cells of SH-SY5Y, have been used to test the inhibitory effects of OPs on neural differentiation [15]. SK-N-SH cell is a useful in vitro cell model for studying potential mechanisms of neurotoxicity since they extend processes following retinoic acid treatment and may maintain many properties inherent to neuroblastoma cells [16]. In this investigation, aldicarb and carbaryl were chosen as representatives of carbamates because of their wide use and differing structures as well as the potential of causing different neurotoxicity in differentiating cells and were used at the same concentration as those OPs that was reported to have effect on cells differentiation and neurofilament heavy subunit protein (NF-H) at 50 μM [15,17]. As axon outgrowth proceeds, NF-H levels may increase and undergo post-translational events (e.g., phosphorylation) linked to axon stabilization in differentiating cells, additional phosphorylated cytoskeletal components were investigated and several relative target proteins were assayed in differentiating SK-N-SH cells for the possible target of different carbamates during this process.

2. Material and methods

2.1. Materials

The human neuroblastoma SK-N-SH cell line was purchased from the Cell Center of Chinese Academy of Medical Sciences (Beijing, China). Cell culture reagents were obtained from Gibco BRL (Grand Island, NY, USA). All-*trans* retinoic acid (ATRA), aldicarb [2-methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl)oxime], carbaryl (1-naphthalenol methylcarbamate) and paraoxon [O,O-dimethyl-O-(4-nitrophenylmethyl) phosphate] were purchased from Sigma (St. Louis, MO, USA). Mipafox and phenyl valerate (PV) were synthesized in our laboratory as described by USA patent no. 2678334 and by Johnson [18], respectively. Monoclonal anti-neurofilament 200 (phosphorylated and non-phosphorylated specific) antibody (clone N52), monoclonal anti-neurofilament 200 (phosphorylated specific) antibody (clone NE14), monoclonal anti-β-actin antibody (AC-15) and anti-mouse IgG (Fc specific) peroxidase conjugate were purchased from Sigma (St. Louis, MO, USA). Enhanced chemiluminescence (ECL) reagents were obtained from Pierce Biotechnology (Rockford, IL, USA).

2.2. Cell culture and maintenance

Cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 μg/ml penicillin and streptomycin. Incubations were carried out at 37 °C in a humidified atmosphere of 5% CO_2/95% air. The cells were maintained in the logarithmic phase of growth and subcultured at 3–4 days intervals.

2.3. Differentiation of SK-N-SH cells

Cells were plated at 1×10^4 cells/well in 24-well plates and cultured for 24 h before inducing differentiation. The cells were then induced to differentiate in DMEM medium containing 20 μM ATRA in the dark. Differentiating cells were treated with 50 μM of either aldicarb or carbaryl in 0.1% DMSO, with 0.1% DMSO treatment as vehicle control, the conditioned media was replaced every 48 h. After exposure of 7 days, cell cultures were washed in Tris-buffered saline (TBS; 50 mM Tris–HCl and 150 mM NaCl, pH 7.4) before fixation with ice-cold 90% (v/v) methanol in TBS at −20 °C for 20 min. Cells were stained using Coomassie Brilliant Blue for 2 min at room temperature, and then viewed using an inverted phase-contrast microscope (DMRBE, Leica). Cells were considered to be differentiated if they had at least one process longer than the cell body, which could be regarded as a neurite [19]. The length of the longest neurite was measured in at least 100 cells in randomly chosen fields with an inverted microscope. At least three independent experiments were conducted and the results are expressed as mean ± standard error (S.E.).