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SCIENCE DIRECT®
Toxicology 220 (2006) 37–45
TOXICOLOGY
www.elsevier.com/locate/toxicol

Carbaryl inhibits basal and FSH-induced progesterone biosynthesis of primary human granulosa-lutein cells

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Received 20 October 2005; received in revised form 23 November 2005; accepted 23 November 2005
Available online 18 January 2006

Abstract

Carbaryl is known to impede female reproductive function, however, the mechanisms through which the adverse effects are mediated are not clearly elucidated. In order to get insight into the mechanisms, this study was conducted to raise fresh concerns about the potential effects of carbaryl on steroidogenesis by primary human granulosa-lutein cells (hGLCs) and explore the possible nature of this action. hGLCs were co-incubated with various concentrations of carbaryl at 0, 1, 5, 25, 125 μmol/L for 24 h to examine effects of this carbamate pesticide on progesterone accumulation. We observed that the carbaryl inhibited basal and FSH-induced progesterone production in a dose-dependent manner. We also investigated the effects of carbaryl on 22(*R*)-hydroxycholesterol (22*R*-HC)-stimulated progesterone yield, basal and FSH-stimulated StAR gene expression and cyclic adenosine monophosphate (cAMP) production, as well as forskolin (non-specific activator of adenylyl cyclase)-induced progesterone and cAMP production of hGLCs. We found that the decreased progesterone biosynthesis was accompanied with a reduced cAMP abundance on both basal and FSH-induced condition. Furthermore, our results demonstrated that the 22*R*-HC could remove the carbaryl-induced restraint of progesterone biosynthesis, suggesting that carbaryl caused a disruption of cholesterol transport across mitochondrial membranes, which was further confirmed by the observation that carbaryl inhibited the gene expression of steroidogenic acute regulatory protein (StAR). In addition, the inhibitory effects of carbaryl on progesterone and cAMP production were completely reversed by addition of forskolin to the cell culture, which indicated a repaired site on the upstream components of adenylate cyclase or adenylate cyclase per se by carbaryl in the cAMP-mediated signal pathway. All the effects mentioned above were not due to a detrimental action of carbaryl on cell viability by MTS assay. In conclusion, carbaryl may inhibit steroidogenesis, at least in part, by obstructing the delivery of cholesterol over mitochondrial membranes and attenuating cAMP generation.

Keywords: Carbaryl; Human granulosa-lutein cells (hGLCs); Steroidogenesis; Cyclic adenosine monophosphate (cAMP); Steroidogenic acute regulatory protein (StAR)

1. Introduction

Since increasing numbers of insecticides are used, there are serious concerns regarding the potential risks of exposure to these agents. Carbaryl (1-naphthyl-*N*-methyl carbamate), one of the most important broad-spectrum

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doi:10.1016/j.tox.2005.11.023

carbamate insecticides, has been applied for about 30 years on different crops, animals, and ornamental plants. The general population may be exposed constantly to carbaryl over many decades during pest control operation, contaminated food, and water consumption; workers are mostly through occupational exposure (CDC Report, 2003). Accumulated evidence has revealed that carbaryl has adverse effects on reproductive system in a wide variety of species. Pant et al. (1996) discovered the decline in spermatozoa and sperm motility and increase in sperm abnormalities of carbaryl administrated rats. Impaired fertility and decreased little size and increased mortality in offspring have been observed in rats exposed to carbaryl in their diet over three generations (Collins et al., 1971). Furthermore, Baranski (1993) reported that carbaryl could increase spontaneous abortion rate in wives of exposed workers. Studies in our lab also revealed the potential genotoxic effects such as DNA fragmentation and CA on human spermatozoa among the carbaryl-exposed workers (Xia et al., 2005). Exposure to carbaryl has been noted to disrupt female reproductive functions in fish, inducing a reduction in the number and size of oocytes, deformity in different stages of oocytes and an increase in the degree of follicular atresia (Kulshrestha and Arora, 1984), as well as causing the homeostatic unbalance of the reproductive regulatory system (Ghosh et al., 1990).

The mechanisms by which pesticides do harm to reproduction in all species are complex and may involve hormonal disruption because hormonal balance plays a critical role in female reproductive function. Studies have revealed that the steroid hormones play an important role in mammary gland development, establishment and maintenance of pregnancy and modulation of the uterine function (Devoto et al., 2000; McGee and Hsueh, 2000). To our knowledge, the effects of carbaryl on ovarian steroidogenesis have not been elucidated up-to-date. In this study, we performed our study on the model of primary human granulosa-lutein cells (hGLCs) that have the capacity to secrete large quantities of steroid hormones to evaluate the possible toxic effects of carbaryl on steroidogenesis. The hGLCs model has been used to assay the toxic potential of xenobiotics on reproduction (Badeaux et al., 1997). Thus, steroidogenesis of hGLCs in response to carbaryl may provide percipience to the potential effects of this pesticide on female reproductive function.

In the ovary, the interference with steroidogenesis may occur at a number of sites in the biosynthetic pathway (Piasek and Laskey, 1994) and is regulated through multiple signaling pathways (Stocco et al., 2005). The first, rate-limiting step for the synthesis of steroid hormones is medicated at the level of cholesterol import from the mitochondrial outer to inner membrane, which is mainly dependent upon the steroidogenic acute regulatory protein (StAR) (Wiltbank et al., 1993; Clark et al., 1994; Stocco, 2001). Changes in the level of StAR gene expression most likely account for progesterone production (Devoto et al., 2002). Walsh et al. (2000a) also suggested that StAR protein might be particularly susceptible to modulation by xenobiotic. Thus, it is possible that the effects of pesticides on steroid hormone levels might be acted on StAR. While steroidogenesis is controlled by multiple signaling events, research over three decades has established that cyclic adenosine monophosphate (cAMP)-dependent signaling is the major one. It is evidenced that ovarian steroid hormones production is controlled by the action of gonadotropins on steroidogenic cell surface receptors and the sequential activation of stimulatory G proteins (G_s proteins), adenylyl cyclase-directed generation of cAMP, and cAMP-dependent protein kinases (PKAs) and other protein kinases (Leung and Steele, 1992; Richards, 1994; Wood and Strauss, 2002). Based on the central role of the cAMP-dependent cascade in stereoidgenesis, it is logical to presume that carbaryl impacts its toxic effects on steroid hormones biosynthesis by altering the cAMP formation. This hypothesis is supported by the recent findings that the interference of steroidogenesis by other pesticides was associated with the changes of cAMP production (Ronco et al., 2001; He et al., 2004).

Carbaryl could impose its toxicity on female reproduction, however, the mechanisms behind the effects are not clear. In the present study, we hypothesized that it was associated with steroidogenesis disruption in ovary. The aim of this study was to investigate whether or not carbaryl has effects on steroidogenesis, concentrating on its effects on progesterone biosynthesis by hGLCs. Another purpose was to elucidate the possible cellular mechanisms involved in the effects.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle medium (DMEM), recombinant human follicle-stimulating hormone (FSH), fetal calf serum (FCS), bovine serum albumin (BSA), Percoll, 22(*R*)-hydroxycholesterol (22*R*-HC), forskolin, 3-isobutyl-1-methyl xanthine (IBMX), and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA). Antibiotic (10,000 U/mL penicillin G sodium, 10,000 U/mL streptomycin) and TriPure reagent were obtained from Roche (Nutley, NJ, USA), and M-MLV reverse transcriptase, dNTP mix, ribonuclease inhibitor and Taq polymerase were from Promega