Environmental Toxicology and Pharmacology 40 (2015) 220–229

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

The role of multifunctional drug therapy against carbamate induced neuronal toxicity during acute and chronic phase in rats

Karan Singh Chahal [a], Atish Prakash [a,b,c,*], Abu Bakar Abdul Majeed [b,c]

[a] Department of Pharmacology, I.S.F. College of Pharmacy, Moga, Punjab, India
[b] Faculty of Pharmacy, Campus Puncak Alam, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia
[c] Brain Degeneration and Therapeutics Group, Brain and Neuroscience Communities of Research, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor Darul Ehsan, Malaysia

ARTICLE INFO

Article history:
Received 6 January 2015
Received in revised form 31 May 2015
Accepted 1 June 2015
Available online 4 June 2015

Keywords:
Carbofuran
Multifunctional drug therapy
Neurotoxicity
Mitochondrial dysfunction
Oxidative stress

ABSTRACT

The current study has been designed to examine the effect of multifunctional drug therapy on carbofuran induced acute (2.187 mg/kg, s.c.) and sub-acute (0.2187 mg/kg, s.c.) neurotoxicity in male wistar rats. Drug treatment which includes nimodipine (Ca^{2+} channel blocker), diazepam, ropinirole (dopamine agonist) and GSPE (antioxidant) was started 2 h after carbofuran administration. Morris water maze was employed for aiming spatial memory. Narrow beam walk and rotarod were employed for testing motor functions. Brain acetylcholinesterase activity, thiobarbituric acid reactive species, nitrite, reduced glutathione, catalase levels, and mitochondrial complexes were also estimated. Carbofuran treatment resulted in significant development of cognitive and motor functions manifested as impairment in learning and memory along with increased thiobarbituric acid reactive species, nitrite levels and decreased acetylcholinesterase activity, reduced glutathione, catalase levels, and mitochondrial complexes. The standard antidote therapy (atropine) was not able to provide neuroprotection but was able to provide symptomatic relief. The multifunctional drug therapy attenuated carbofuran induced cognitive and motor dysfunction, acetylcholinesterase activity and other biochemical parameters. The triple combination in sub-acute study may be avoided in future as two drug combinations provide adequate neuroprotection. Thus it can be concluded that standard antidotal therapy may not provide neuroprotection while the multifunctional drug therapy offers neuroprotection against carbofuran and may dramatically increase survival and life quality.

1. Introduction

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate; CF), a N-methylcarbamate ester (Fig. 1), is one of the most widely used insecticide, acaricide and nematicide and is less toxic than organophosphate group of pesticides (Azizullah et al., 2011). WHO assigned it in a toxic class of WHO 1b; highly hazardous in its WHO recommended classification of pesticides (Tennakoon et al., 2009). The lethal dose of CF in mammals is found to be 5–50 mg/kg (Eisler, 2007). As it is non-specific in action, it produced toxicity in both target (pests) and non-target (humans and other animals) species (Li et al., 2009). The pharmacological as well as toxicological effects of CF are due to inhibition of acetylcholinesterase (AChE) enzyme resulting in increased acetylcholine (ACh) concentration (Kaur and Sandhir, 2006) which is associated with overstimulation of cholinergic system (Darvesh et al., 2008). Apart from ACh, CF is also associated with modulating various other neurotransmitters like γ-aminobutyric acid, epinephrine, norepinephrine, and dopamine (Farage-Elawar and Blaker, 1992). In cortical neurons, it induces apoptosis and decreases expression of α-7 subunit of the nicotinic ACh receptor in the hippocampal neurons (Kim et al., 2004). The potential of CF to disturb prooxidant/antioxidant balance in brain has been well documented (Rai and Sharma, 2007) which is associated with decreased levels of high-energy phosphates (Gupta et al., 2007) and generation of oxidative stress and mitochondrial dysfunction which is thought to be its main neurotoxicity mechanism (Kamboj et al., 2006a, 2008). Apart from neurotoxicity, it has also been known to induce embryotoxicity and teratogenicity (Gupta et al., 1994), chromosome

* Corresponding author at: Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Campus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia. Tel.: +60 1116218215; fax: +60 332584602.
E-mail addresses: atishprakash@gmail.com, atishkumar@puncakalam.uitm.edu.my (A. Prakash).

http://dx.doi.org/10.1016/j.etap.2015.06.002
1382-6689/

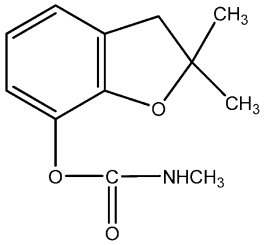

Fig. 1. Chemical structure of carbofuran.

aberrations, micronucleus formation and sperm abnormalities in mouse (Chauhan et al., 2000), endocrine disruption (Chatterjee et al., 2001) and delayed polyneuropathy (Yang et al., 2000).

Currently, carbamate poisoning management includes only one antidote i.e. Atropine (AT) (Rosman et al., 2009). AT, a competitive muscarinic anticholinergic agent, counteracts the peripheral effects of carbamates but due to its poor blood brain barrier penetration, it is not able to afford neuroprotection as a result of which secondary events takes place leading to death (Rosman et al., 2009). Diazepam is found to be neuroprotective in epilepsy by combining with other drugs (Trandafir et al., 2015; Sarnowska et al., 2009). It has been reported that, Ropinirole, a dopamine receptor agonist produced neuroprotective effect against animal models of Parkinson's disease (PD) (Park et al., 2013; Iida et al., 1999). Though the exact neuro-mechanism action of ropinirole against movement disorders is still not clear. However, It has been hypothesized that ropinirole acts by blocking the over activation of the glutamate receptors and maintains the normal physiological glutamate concentration in the synapse in addition to its own dopamine effect (Park et al., 2013; Bisht et al., 2014). Nimodipine is a 1,4-dihydropyridine calcium channel blocker (CCCB) used to treat many neurological disorders (Daschil and Humpel, 2014; Li et al., 2014; Lecht et al., 2012). Nimodipine passes the blood–brain-barrier more readily than other CCBs and blocks the calcium current through voltage-dependent channels. Therefore, it often produces neuroprotective action in neurological disorders (Sanz et al., 2012; Bailey et al., 2013). Grape seed proanthocyanidins have been demonstrated to exhibit neuroprotective effect glutamate-induced cell death through inhibition of calcium signals and nitric oxide formation in cultured rat hippocampal neurons (Ahn et al., 2011). Many beneficial effects of GSPE have been attributed to its anti-oxidant and free radical scavenging properties (Asha Devi et al., 2011; Yang et al., 2012). However, there are no reports of protective effects of these drugs against carbamate induced neurotoxicity alone or combination of other drugs.

Therefore, the present study is aimed to provide a new antidote combination of diazepam, ropinirole, grape seed proanthocyanidin extract (GSPE) and nimodipine, so as to afford neuroprotection against CF and thus eliminating the risk of short and long term neurotoxic effects of CF.

2. Materials and methods

2.1. Chemical

CF (FMC India, Mumbai) was reconstituted in corn oil and injected s.c. GSPE (100 mg/kg; Biogenix labs, Bangalore) (Farbood et al., 2009), Ropinirole (20 mg/kg; Sun Pharma, Ranipool, East Sikkim) (Fukuzaki et al., 2000) and Nimodipine (30 mg/kg; USV Limited, Mumbai) (Nascimento et al., 2005) were freshly prepared by suspending in 0.5% w/v CMC (carboxy methyl cellulose) in distilled water and were administered p.o. Corn oil was purchased from local market. AT (10 mg/kg) (Mehrania et al., 2008) and DZ (2 mg/kg) (Manna and Umathe, 2011) were dissolved in distilled water and injected i.p. Unless stated, all other chemicals and biochemical reagent of highest analytical grade were used in the study.

2.2. Animals

The experiments were carried out on 8–10 week old male Wistar rats (220–250 g) obtained from central animal house of ISF College of Pharmacy, Moga, Punjab (India). Animals were acclimatized to laboratory conditions and were fed standard pellet diet and water ad libitum. The animals were housed under standard laboratory conditions in polypropylene cages with husk bedding. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and care of the animals was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3. Calculation of LD_{50}

Mice were divided into 4 groups ($n = 3$). 4 doses were randomly selected for each group viz. 1, 2, 3, 4 mg/kg respectively. Behavioral, locomotary, toxic manifestations and mortality rate were observed for upto 14 days. 4 and 3 mg/kg dose resulted in death of all animals while 2 mg/kg dose resulted in the death of 2 animals and 1 mg/kg dose resulted in no death and produced no muscarinic side effects. LD_{50} was determined by Karber's method and was found to be 2.187 mg/kg.

2.3.1. Acute group

For inducing acute toxicity, LD_{50} dose of CF (2.187 mg/kg) was injected once. Each group consisted of 8 animals. Group 1: Vehicle control, Group 2: CF control, Group 3: CF + AT (10 mg/kg, i.p.) + DZ (2 mg/kg, i.p.), Group 4: CF + AT (10 mg/kg, i.p.) + DZ (2 mg/kg, i.p.) + GSPE (100 mg/kg, p.o.), Group 5: CF + AT (10 mg/kg, i.p.) + DZ (2 mg/kg, i.p.) + Nimodipine (30 mg/kg, p.o.),Group 6: CF + AT (10 mg/kg, i.p.) + DZ (2 mg/kg, i.p.) + GSPE (100 mg/kg, p.o.) + Nimodipine (30 mg/kg, p.o.).

For the normal control group, corn oil was injected s.c. All animals received AT and DZ after 30 min and test treatment after 2 h of CF exposure (O.D × 3 days). All animals were sacrificed on day 7.

2.3.2. Sub-acute group

For inducing sub-acute toxicity, 1/10th LD_{50} dose of CF (0.2187 mg/kg) was injected once per day for 28 days. Each group consisted of 8 animals. Group 1: Vehicle control, Group 2: CF control, Group 3: CF + GSPE (100 mg/kg, p.o.), Group 4: CF + Nimodipine (30 mg/kg, p.o.), Group 5: CF + Ropinirole (20 mg/kg, p.o.), Group 6: CF + Roipinirole (20 mg/kg, p.o.) + GSPE (100 mg/kg, p.o.), Group 7: CF + Ropinirole (20 mg/kg, p.o.) + Nimodipine (30 mg/kg, p.o.), Group 8: CF + Nimodipine (30 mg/kg, p.o.) + GSPE (100 mg/kg, p.o.), Group 9: CF + Roipinirole (20 mg/kg, p.o.) + Nimodipine (30 mg/kg, p.o.) + GSPE (100 mg/kg, p.o.).

For the normal control group, corn oil was injected s.c. once per day for 28 days. Drug treatment was started 2 h after administration of CF and continued on daily basis for 28 days. All animals were sacrificed on day 45.

2.4. Behavioral parameters

2.4.1. Rota-rod activity

Motor coordination task were evaluated by using rotarod apparatus on 0 and 7th day (acute) and 0, 15th, 30th, and 45th day (sub-acute) after CF injection. Before actual recording on rotarod apparatus, the animals were given a prior training session for acclimatization. The average results were recorded as fall of time (Kumar et al., 2010).