Food and Chemical Toxicology 51 (2013) 330–336

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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

Genotoxicity evaluation of Mequindox in different short-term tests

Awais Ihsan [a,b,c,1], Xu Wang [a,b,1], Hong-Gong Tu [a,b], Wei Zhang [a,b], Meng-Hong Dai [a,b], De-Peng Peng [a,b], Yu-Lian Wang [a,b], Ling-Li Huang [a,b], Dong-Mei Chen [a,b], Shazia Mannan [c], Yan-Fei Tao [a,b], Zhen-Li Liu [a,b], Zong-Hui Yuan [a,b,*]

[a] National Reference Laboratory of Veterinary Drug Residues, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[b] MOA Key Laboratory for the Detection of Veterinary Drug Residues in Foods, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[c] Department of Bioscience, COMSATS Institute of Information Technology, Sahiwal, Pakistan

ARTICLE INFO

Article history:
Received 12 June 2012
Accepted 2 October 2012
Available online 12 October 2012

Keywords:
Carbadox
Genotoxicity
Mequindox
Quinoxaline-1
4-Dioxides

ABSTRACT

Quinoxaline-1,4-dioxides (QdNOs) are the potent heterocyclic N-oxides with interesting biological properties such as antibacterial, anticandida, antitubercular, anticancer and antiprotozoal activities. Here, we tested and compared the mequindox (MEQ) for mutagenic abilities in a battery of different short term tests according to OECD guidelines. When compared with the controls, a strong mutagenicity of MEQ and carbadox (CBX) was observed with an approximate concentration-effect relationship in Salmonella reverse mutation test, chromosome aberration test, unscheduled DNA synthesis assay and HGPRT gene mutation test, in the absence and presence of S_9-mix. In *in vivo* micronucleus test, CBX produced significant increase in the proportion of micronucleus formation than MEQ in mice bone marrow cells. From these results, we can conclude that MEQ had a strong genotoxic potential to mammalian cells *in vitro* as well as *in vivo* and its mutagenicity is slightly higher than CBX. Our results, for the 1st time, discuss the genotoxic potential of MEQ. These results not only confirm the earlier findings about CBX but also extend the knowledge and awareness about the genotoxic risk of QdNO derivatives.

1. Introduction

Genotoxic studies are designed to evaluate the mutagenic potential of industrial chemicals, candidate drugs and pharmaceutical agents. Both *in vivo* and *in vitro* testing methods are widely utilized for identifying the genetic damage directly or indirectly, by various mechanisms (Eisenbrand et al., 2002). The elucidation of the genotoxic potential plays a critical role in cancer risk categorization for carcinogens (Dearfield et al., 2002). Quinoxaline-1,4-dioxides (QdNOs) are the potent heterocyclic N-oxides with interesting biological properties such as antibacterial, antifungal, anticancer and antiprotozoal activities (Wang et al., 2012). Mequindox (MEQ) is a potent synthetic heterocyclic N-oxides belonging to QdNOs (Fig. 1). It is rather new and structurally related to quindoxin, carbadox (CBX), olaquindox (OLA), quinocetone (QCT) and cyadox (CYA). In China, MEQ is used in the feed of poultry, swine and livestock for growth promotion, improved feed efficiency, increased rate of weight gain, and control of *Brachyspira hyodysenteriae, Salmonella and E. coli* infections (Huang et al., 2010a; Li et al., 2012). It is also used to cure yellow and white scours of young piglets, and avian colibacillosis (Ihsan et al., 2010).

It was necessary to pay much more attention to the genotoxic potential of QdNOs, especially the drugs used in food producing animal. CBX and OLA have been continuously tested in various *in vivo* and *in vitro* genotoxicity assays, and both of these agents marked highest genetic risk (WHO, 1991a,b). Irrespective of metabolic activation, CBX and OLA were consistently identified as frame-shift mutagen in TA98 and base-pair substitution mutagen in TA100 strains of *Salmonella typhimurium* (Voogd et al., 1980; Beutin et al., 1981; Yoshimura et al., 1981; Nunoshiba and Nishioka, 1989; WHO, 1991b). Both of these agents were also found to induce SOS activity with DNA breaks in *Escherichia coli* (Von der Hude et al., 1988; Nunoshiba and Nishioka, 1989). The genotoxicities followed in prokaryotic cells were also confirmed in eukaryotic tests. Previous studies had also corroborated that CBX and OLA were effected the chromosomal material, caused

Abbreviations: 6-TG, 6-thioguanine; ATCC, American type culture collection; BaP, benzo-[*a*]-pyrene; CA, chromosomal aberration; CBX, carbadox; CCTCC, China Center for Type Culture Collection; CP, cyclophosphamide; CYA, cyadox; DMEM, Dulbecco's modified Eagle's medium; EMS, ethylmethanesulfonate; FCS, fetal calf serum; FCS, fetal calf serum; HAT, hypoxanthine aminopterin thymidine; MEQ, mequindox; MMC, mitomycin C; OLA, olaquindox; Quinocetone, Qct; QdNOs, quinoxaline 1,4-dioxides; ROS, reactive oxygen species; SD, standard deviation.

* Corresponding author at: National Reference Laboratory of Veterinary Drug Residues, Huazhong Agricultural University, Wuhan, Hubei 430070, China. Tel.: +86 27 87287186; fax: +86 27 87672232.

E-mail addresses: awais.dr@gmail.com (A. Ihsan), yuan5802@mail.hzau.edu.cn (Z.-H. Yuan).

[1] These authors contribute equally to this work.

0278-6915/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2012.10.003

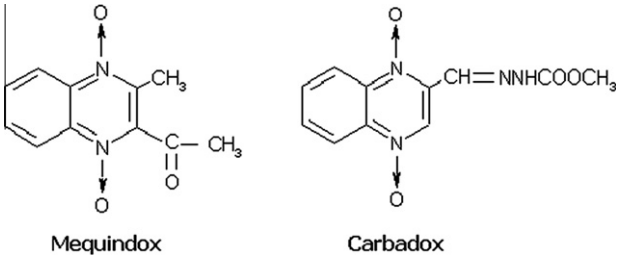

Fig. 1. Chemical structure of MEQ and CBX.

gene mutation in hamster V79 cells, SV40-based shuttle vector pSP189, African green kidney cell, and human lymphocytes (Oud et al., 1979; Scheutwinkel-Reich and von der Hude, 1984; Markovic et al., 2000a; Chen et al., 2008, 2009). In bone marrow micronucleus assay, CBX and OLA caused micronuclei formation in mice and rats when administered intraperitoneally or orally (Cihak and Srb, 1983; Cihak and Vontorkova, 1985; Markovic et al., 2000b: Carta et al., 2005). Besides, OLA led to increase the frequency of dominant lethal mutations and abnormal spermatozoa in mice with 200 and 500 mg/kg feed (Sram et al., 1986a,b). Because of undesirable characteristics, Commission of the European community and Health Canada restricted the use of CBX and OLA in food-producing animals (EU, 1998; Wu et al., 2007; Boison et al., 2009). Previously, we have been found that MEQ led to testicular and adrenal toxicities by involving oxidative DNA damage, dysregulation of hormonal secretions and apoptosis of different endocrinal cells in rats (Huang et al., 2010a; Ihsan et al., 2011; Wang et al., 2011). However, compared with the continuously tested of genotoxicity tests of CBX, no studies are available on the genotoxic potential of MEQ though it was widely used in food animal production. Thus, the aim of the present work was to test and compare the genotoxic potential of MEQ with CBX, under similar conditions.

Herein, MEQ was studied in a panel of tests, namely in *Salmonella* reverse gene mutation test with 6 *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535 and TA1537, induction of chromosomal aberrations and HGPRT gene mutation assay in metabolically competent cultures V79 cell, unscheduled DNA synthesis assay in human lymphocytes, and micronuclei formation (MN) in mice bone marrow cells.

2. Materials and methods

2.1. Chemical reagents

Mequindox (MEQ, $C_{11}H_{10}N_2O_3$, CAS: 13297-17-1, ⩾98%) and Carbadox (CBX, $C_{11}H_{10}N_4O_4$, CAS: 6804-07-5, ⩾98%) were purchased from Zhongmu Pharmaceutical Limited Company (Wuxue, PR China). Hypoxanthine aminopterin thymidine (HAT), trypsin, Dulbecco's modified Eagle's medium (DMEM) and RPMI1640 medium were provided by Gibco (NY, USA). Mitomycin C (MMC), benzo-[a]-pyrene, ethylmethane-sulfonate, 9-aminoacridine, 1,8-dihydroxy-anthraquinone, 2-aminofloracene and 6-thioguanine (6-TG) were procured from Sigma (St. Louis, MO, USA). Carboxymethylcellulose sodium (CMC), histidine and sodium azide were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Cyclophosphamide (CP) was supplied by Alfa Aesar (USA). Fetal calf serum (FCS) was bought from Hangzhou Sijiqing Biological Materials Limited Company. Tritiated thymidine (^{3}H-TdR) was supplied by Beijing Institute of Atomic Energy of CAS (Beijing, China). S_9 metabolic activation mixture (S_9-mix) was prepared from the Aroclor 1254-induced rat liver homogenate, according to the method of Mortelmans and Zeiger (2000). S_9-mix was used in a final concentration of 1% (1.85 mg protein/ml). All other chemicals were of analytical grade or complied with the standards need for cell culture.

2.2. Cell culture

S. typhimurium TA97, TA98, TA100, and TA102 were obtained from China Center for Type Culture Collection (CCTCC, Wuhan, PR China), while TA1535 and TA1537 were purchased from American type culture collection (ATCC). Chinese hamster lung fibroblasts (V79) were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Science (Shanghai, PR China). The cells were cultivated under standard conditions in DMEM supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% FCS at 37 °C in a humidified atmosphere at 5% CO_2 (Swierenga et al., 1991).

2.3. Salmonella reverse mutation test

Salmonella reverse mutation test was performed by the plate incorporation procedure (OECD, 1997a). Initial concentration levels were determined by a preliminary test using TA100 for both MEQ and CBX. The appropriate concentrations of these compounds were dissolved in dimethyl sulfoxide and stock solution of each drug was prepared. For the final assay, five different amounts (1.0–50 μg/plate) were tested in the presence or absence of the S_9-mix with the appropriate vehicle controls, positive and negative controls. Without S_9-mix, the positive control was dexon (50 μg/plate) (Ke et al., 2005; Li et al., 2006; Xia et al., 2004), except in TA100 where sodium azide (1.5 μg/plate) and TA1537 where 9-aminocridine (30 μg/plate) was used as positive control. 2-Aminofloracene (10 μg/plate) was used as positive control for all the strains with S_9-mix, except in TA102, where 1,8-dihydroxy-anthraquinone (50 μg/plate) was used as positive control with S_9-mix. An agent was considered positive only when the number of His$^+$ revertants was at least double the spontaneous yield (MI ⩾ 2; mutagenic index (MI): number of His$^+$ induced colonies in the sample/number of spontaneous in negative control). Triplicate plates were incubated in the dark at 37 °C for 48 h. The results are expressed as the Mean ± standard deviation (SD).

Table 1
Induction of His$^+$ revertants/plate in six strains of *Salmonella typhimurium* by MEQ and CBX without metabolic activation; $-S_9$-mix.

Substance	Dose (μg/plate)	TA 97		TA 98		TA 100		TA 102		TA 1535		TA 1537	
		Rev/plate[a]	MI index[b]	Rev/plate	MI index	Rev/plate	MI index	Rev/plate	MI index	Rev/plate	MI index	Rev/plate	MI index
Solvent[c]		152.3 ± 10.6		41.7 ± 4.7		164.3 ± 13.5		265.0 ± 16.5		22.0 ± 4.4		17.7 ± 6.0	
PC[d]		2548.0 ± 109.3	16.73	1071 ± 76.5	25.68	2825.3 ± 148.0	17.2	2146.7 ± 111.5	8.1	431.3 ± 37.5	19.6	290.3 ± 41.2	16.4
MEQ	1	336.4 ± 15.4*	2.21	47.4 ± 17.0	1.14	361.5 ± 10.2*	2.2	224.4 ± 19.8	0.85	55.1 ± 8.2*	2.5	22.2 ± 7.2	1.25
	2.6	652.0 ± 30.1*	4.28	79.0 ± 19.1	1.89	477.1 ± 9.4*	2.9	367.7 ± 25.3	1.39	65.4 ± 7.9*	2.97	37.9 ± 9.2*	2.14
	6.9	1521.0 ± 97.5*	9.99	119.7 ± 15.3*	2.87	814.0 ± 17.9*	4.95	615.5 ± 21.1*	2.32	77.9 ± 10.5*	3.54	47.5 ± 5.9*	2.68
	18.2	2145.3 ± 119.2*	14.09	221.8 ± 17.3*	5.32	1212.3 ± 19.6*	7.38	1183.0 ± 60.5*	4.46	84.4 ± 3.9*	3.84	78.7 ± 6.6*	4.45
	50	2410.3 ± 78.4*	15.83	314.7 ± 17.9*	7.55	1258.0 ± 157.3*	7.66	1591.0 ± 125.7*	6.0	114.7 ± 15.9*	5.21	119.2 ± 15.6*	6.73
CBX	1	379.3 ± 15.5*	2.49	42.3 ± 5.5	1.01	403.3 ± 20.3*	2.45	313.0 ± 9.5	1.18	53.0 ± 4.6*	2.41	30.3 ± 10.7	1.71
	2.6	587.7 ± 45.7*	3.86	81.3 ± 6.1	1.95	632.3 ± 24.0*	3.85	422.3 ± 34.2	1.59	63.7 ± 4.5*	2.9	46.0 ± 9.6*	2.6
	6.9	1237.7 ± 70.2*	8.13	164.3 ± 7.8*	3.94	945.0 ± 19.7*	5.75	621.7 ± 16.6*	2.35	76.7 ± 6.4*	3.49	67.7 ± 9.5*	3.82
	18.2	2281.0 ± 129.0*	14.98	228.7 ± 16.7*	5.48	1328.7 ± 35.9*	8.09	1241.3 ± 77.5*	4.68	89.0 ± 4.4*	4.05	93.7 ± 9.7*	5.29
	50	2288.7 ± 135.9*	15.03	335.3 ± 25.0*	8.04	1168.7 ± 72.1*	7.11	1774.3 ± 106.6*	6.7	126.3 ± 8.6*	5.74	121.7 ± 15.0*	6.88

[a] Number of His$^+$ revertants/plate: mean of three independent experiments (Mean ± SD).
[b] Mutagenic index (MI): number of His$^+$ induced in the sample/number of spontaneous His$^+$ in the negative control.
[c] Solvent: dimethylsulfoxide.
[d] Positive controls: (dexon at 50 μg/plate versus TA 97, TA98, TA102 and TA 1535; sodium azide at 1.5 μg/plate versus TA 100; 9-aminoacridine at 30 μg/plate versus TA 1537).
* MI ⩾ 2: number of His$^+$ induced was double in the sample/number of spontaneous in negative control.