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Mutagenic Assessment of Olmesartan Cilexetil by Bacterial Mutation Assay

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Hypertension is a serious health problem due to high frequency and concomitant other diseases including cardiovascular and renal dysfunction. Olmesartan cilexetil is a new antihypertensive drug associated with angiotensin II receptor antagonist. This study was conducted to evaluate the mutagenicity of olmesartan cilexetil by bacterial reverse mutation test using *Salmonella typhimurium* (TA100, TA1535, TA98, and TA1537) and *Escherichia coli* (WP2 uvrA). At the concentrations of 0, 62, 185, 556, 1667, and 5000 μg/plate, olmesartan cilexetil was negative in both *Salmonella typhimurium* and *Escherichia coli* regardless of presence or absence of metabolic activation system (S9 mix). These results demonstrate that olmesartan cilexetil does not induce bacterial reverse mutation.

***Key words*:** Hypertension, Olmesartan cilexetil, Angiotension II receptor antagonist, Mutagenicity

INTRODUCTION

Hypertension is a severe disease problem due to high frequency and concomitant increase in risk of cardiovascular and kidney diseases (1,2). Therefore, many antihypertensive drugs that possess mechanisms of blocking angiotensin converting enzyme (ACE) and angiotensin II (AII) receptors have been developed and shown in the market (3). ACE inhibitors regulate the activity of renin angiotensin system and have some adverse effects such as cough and angioedema (4-6), while AII receptor antagonists have been known to induce persistent dry cough and less frequent angioedema (7). Of these AII receptor antagonists, olmesartan is a nonpeptide AII antagonist that selectively and competitively inhibits the binding of angiotensin to type II receptors (7,8). Olmesartan cilexetil (1-(cyclohexyloxycarbonyloxy)ethyl 1-((2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl)-4-(2-hydroxypropane-2-yl)-2-propyl-1H-imidazole-5-carboxylate) was recently developed as a new AII receptor antagonist composed of different salt of cilexetil comparing to olmesartan medoxomil (Fig. 1). Thus, we tested genotoxicity of olmesartan cilextil to identify whether it could induce reverse mutation or not using *Salmonella typhimurium* (TA100, TA1535, TA98, and TA1537) and *Escherichia coli* (WP2 uvrA), according to OECD guideline (9).

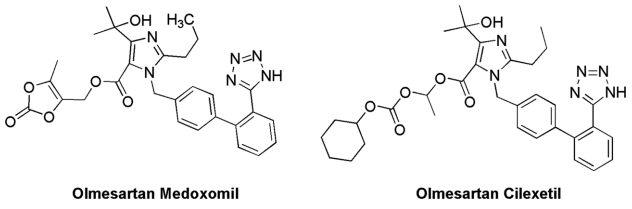

Fig. 1. Chemical structures of olmesartan medoxomil and olmesartan cilexetil.

MATERIALS AND METHODS

Materials. Olmesartan cilexetil was obtained from CTC Bio Inc. (Hwaseong, Korea). Nutrient broth No. 2 (Oxoid, London, UK) and bacto agar (Difco, Sparks, USA) were used as bacterial media. The following agents were purchased from commercial sources: magnesium sulfate heptahydrate, citric acid, and dipotassium hydrogen phosphate (Junsei, Tokyo, Japan); ammonium sodium hydrogen phosphate (YAKURI, Kyoto, Japan); sodium phosphate (mono and dibasic) and sodium azide (Bio basic, Ontario, Canada); L-histidine and D-biotin (Daejung, Siheung, Korea); L-tryp-

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tophan (TCI, Tokyo, Japan); 2-nitrofluorene, 2-aminoanthrancene, 9-aminoacridine hydrochloride monohydrate and dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St Louis, USA). For metabolic activation, S9 was purchased from Molecular Toxicology Inc. (Boone, USA) and kept at −80°C. Cofactor-I was purchased from Orient Yeast (Tokyo, Japan).

Bacterial strains and media. We used 5 strains including *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 and *Escherichia coli* WP2 uvrA. TA100, TA1535, and WP uvrA were used to identify the mutagenicity of base-pair substitution type, whereas TA98 and TA1537 were used as frame-shift type. The five strains were obtained from the Bio Toxtec (Cheongwon, Korea). Each strain was added in 2.5% Nutrient broth No. 2 and incubated for about 12 hr using shaking incubator (Jisco, Seoul, Korea). When bacteria counts were more than 1×10^9 cells/ml, they were used in the test.

The minimal glucose agar plate containing bacto agar, Vogel-Bonner (VB) salts and 20% glucose was divided by 25 ml and then used. Top agar was prepared to 0.6% NaCl and 0.5% bacto agar, and then 0.5 mM histidine-biotin and 0.5 mM tryptophan were respectively added to *Salmonella typhimurium* or *Escherichia coli* type agar.

Test ingredient and positive controls. Test samples, practically water-insoluble, were diluted in DMSO at different concentrations (0, 62, 185, 556, 1667, and 5000 μg/plate). The standard mutagens used as positive controls in experiments without S9 mix were sodium azide (1.5 μg/plate) for TA100 and TA1535, 4-nitroquinoline 1-oxide (1 μg/plate) for WP2 uvrA, 2-nitrofluorene (5 μg/plate) for TA98 and 9-aminoacridine (40 μg/plate) for TA1537. In addition, 2-aminoanthracene (2 μg/plate) for TA100 and TA98, and 2-aminoanthracene (10 μg/plate) for TA1535, TA1537 and WP2 uvrA were used in the experiments with metabolic activation. DMSO was used as the negative control.

Metabolic activation system (S9 mixture). The commercially available S9 fraction was stored at −80°C until use. When preparing the S9 mix, cofactor-I dissolved in distilled water was added to the thawed S9 fraction and then S9 mix was kept in ice cooling.

Experimental procedure. Using pre-incubation, we studied the effect of metabolic activation. In condition without metabolic activation, 0.1 ml of each concentration of test ingredient, negative control or positive control was added to 0.5 ml of 0.1 M phosphate buffer (pH 7.4) and 0.1 ml of each strain, and then incubated at 37°C for 20 min. After shaking incubation, 2 ml of top agar was added to the incubation mixture according to the strains, and then poured onto a plate containing minimal agar. The plates were incubated at 37°C for 48 hr and the revertant colonies were

Table 1. Bacterial reverse mutation assay of olmesartan cilexetil using *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and *Escherichia coli* (WP2 uvrA)

Dose (μg/plate)	Number of Colonies/plate (without S9 mix)				
	TA98	TA100	TA1535	TA1537	WP2 uvrA
0	39 ± 0	103 ± 4.2	8.5 ± 3.5	9.5 ± 2.1	154 ± 9.9
62	37.5 ± 3.5	118 ± 0	9 ± 0	9 ± 2.8	151 ± 14.1
185	33 ± 1.4	111 ± 2.8	10 ± 2.8	10 ± 4.2	153 ± 22.6
556	38.5 ± 6.4	115 ± 2.8	8 ± 0	6.5 ± 0.7	161.5 ± 6.4
1667	27.5 ± 4.9	104 ± 12.7	8 ± 5.7	7.5 ± 0.7	136 ± 4.2
5000	17 ± 2.8	85 ± 42	6.5 ± 2.1	2.5 ± 0.7	142 ± 7.1
Positive control[a]	232 ± 2.8*	763 ± 14.0*	570.5 ± 20.5*	67.5 ± 3.5*	1955 ± 202.2*
	Number of Colonies/plate (with S9 mix)				
	TA98	TA100	TA1535	TA1537	WP2 uvrA
0	39 ± 5.7	121 ± 0	11.5 ± 3.6	7.5 ± 2.1	131 ± 15.6
62	50 ± 4.2	140.5 ± 2.1	11 ± 1.4	9 ± 1.4	147 ± 8.5
185	41.5 ± 3.5	139.5 ± 12.0	8.5 ± 2.1	7 ± 1.4	160 ± 18.4
556	45 ± 8.5	131 ± 0	9.5 ± 0.7	9.5 ± 3.5	153.5 ± 17.7
1667	31.5 ± 49	112 ± 24	6 ± 0	11 ± 2.8	164.5 ± 47.4
5000	30 ± 1.4	100.5 ± 2.1	7 ± 0	7.5 ± 0.7	161.5 ± 6.4
Positive control[b]	473 ± 45.3*	1504 ± 142.8*	831.5 ± 3.5*	106 ± 2.8*	1211 ± 21.2*

[a]TA98: 2-nitrofluorene, 5 μg/plate; TA100: sodium azide, 1.5 μg/plate; TA1535: sodium azide, 1.5 μg/plate; TA1537: 9-aminoacridine, 40 μg/plate; WP2 uvrA: 4-nitroquinoline 1-oxide, 1 μg/plate.
[b]TA98: 2-aminoanthracene, 2 μg/plate; TA100: 2-aminoanthracene, 2 μg/plate; TA1535: 2-aminoanthracene, 10 μg/plate; TA1537: 2-aminoanthracene, 10 μg/plate; WP2 uvrA: 2-aminoanthracene, 10 μg/plate.
*More than two-fold increase in revertant colonies over negative control, 0 μg/plate.

counted manually. In the presence of metabolic activation, 0.5 ml of freshly prepared S9 mix instead of 0.1 M phosphate buffer (pH 7.4) was added to the incubation mixture. Other procedures were performed in the same way. All experiments were analyzed in duplicate.

RESULTS

The results are shown as the mean ± standard deviation of colony counts obtained from 2 plates per test group (Table 1). When the numbers of revertant colonies are more than two folds compared to the negative control and show dose-dependency and repeatability in at least one plate, regardless of the presence or absence of metabolic system, the results were considered positive.

In positive controls, revertant colonies were significantly increased in each strain compared with the negative control (Table 1). However, olmesartan cilexetil treatment (62, 185, 556, 1667, and 5000 μg/plate) did not show any significant change of revertant colonies compared with negative control in all strains (*Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 uvrA), regardless of the presence or absence of metabolic activation system (Table 1). Based on the above results, olmesartan cilexetil is considered as a substance that does not cause reverse mutation under current testing condition.

DISCUSSION

Olmesartan medoxomil as an antihypertensive drug selectively blocks the binding of AII to AT1 receptor, which mediates vasoconstrictive effect and is one of the key contributors to cardiovascular and renal diseases (10). Olmesartan medoxomil is rapidly converted to olmesartan, which is absorbed from the gastrointestinal tract into the body (11), thereafter, lowers ambulatory blood pressure and has low potential for interaction with other drugs (4,12). Olmesartan cilexetil was developed from olmesartan medoxomil by changing salt to cilexetil. Molecular weight of this drug is 16.71 g/mol and it has white to off-white amorphous powder. In current study, olmesartan cilexetil did not show positive outcome in bacterial mutation test. Genotoxicity studies for olmesartan and olmesartan medoxomil were performed and observed negative in revertant mutation test and *in vitro* Syrian hamster embryo cell transformation assay (13). However, both compounds showed a few positive responses in chromosomal aberrations using mammalian cell and in mouse lymphoma assay (13). Olmesartan medoxomil was negative *in vivo* micronucleus test using MutaMouse at oral doses of up to 2 g/kg and the weight-of-evidence shows that olmesartan medoxomil is not considered as a genotoxic agent at clinically relevant doses. In genotoxicity assays of other AII receptor antagonist drugs, candesartan and eprosartan induced chromosomal aberrations in mammalian cells and showed positive results in the mouse lymphoma assay (13). Therefore, further studies including chromosomal aberration and *in vivo* micronucleus assay should be performed in order to clarify the genotoxicity of olmesartan cilexetil.

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