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APPLICATION NUMBER
20-838/S-015

Pharmacology Review(s)

MEMORANDUM

To: File, NDA 20-838/ S015

Through: Robert Temple, M.D., ODE I Office Director
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From: Jeri El-Hage, Ph.D., ODE I Associate Director for Pharmacology/Toxicology

Subject: NDA 20-838/ S015, Candesartan Cilexetil
Tertiary Review of Pharmacology/Toxicology Labeling Revisions

Date: July 25, 2002

Dr. Proakis completed a review of the preclinical sections of the labeling for ATACAND, dated April 29, 2002, in conjunction with the S015 labeling supplement. A re-review of the *in vitro* cytogenetics assays performed with Chinese hamster lung (CHL) cells was conducted using current ICH-specified evaluation criteria. The re-evaluation of the data resulted in a conclusion that Candesartan and it's O-deethyl metabolite tested positive for genotoxicity in this assay.

The revised labeling proposed on page 6 of Dr Proakis's review for the *Carcinogenesis, Mutagenesis , Impairment of Fertility* Section is acceptable as written.

Despite the positive findings in the CHL chromosomal aberration assay after re-evaluation of the results, based on a weight of evidence approach Candesartan and Candesartan cilexetil do not appear to demonstrate evidence of genotoxic potential.

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/s/

Jeri El Hage
7/26/02 01:33:55 PM
PHARMACOLOGIST

NDA # 20,838/SE4-015

REVIEW AND EVALUATION OF GENETIC TOXICOLOGY DATA

Anthony G. Proakis, Ph.D
4/29/02

SUBMISSION DATED: 9/27/01
CENTER RECEIPT DATE: 9/27/01
REVIEWER RECEIPT DATE: 10/02/01

PRODUCT: ATACAND® Tablets (Candesartan cilexetil, TCV-116)

SPONSOR: AstraZeneca
1800 Concord Pike
P.O. Box 8355
Wilmington, DE 19803-8355

PHARMACOLOGICAL CLASS: Angiotensin II Receptor Antagonist

INTRODUCTION

The sponsor submitted this supplemental NDA which provides revisions to some clinical sections of the currently approved labeling for ATACAND Tablets; new clinical studies conducted by the sponsor serve as the basis for the proposed labeling revisions. Review of the current ATACAND labeling for clarity and completeness of nonclinical information has revealed that results of a genotoxicity assay with candesartan may have been incorrectly described. Specifically, during the initial evaluation of study results from an *in vitro* chromosome aberration assay, despite the finding of a drug-related increase in chromosome aberrations, candesartan (the active metabolite of candesartan cilexetil) was judged negative for clastogenicity because the increase in chromosomal aberrations was associated with a high degree of drug-induced cytotoxicity (>50% reduction of mitotic index). The current ICH Guideline "Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals" (ICH S2A, April, 1996) describes the desired level of cytotoxicity for in vitro cytogenetics tests that, prior to this document, was poorly defined. Thus, it appears appropriate to re-evaluate the results of the in vitro Chinese Hamster Lung cytogenetics assay conducted with candesartan and one of its metabolites in order to include any non-clinical revisions to the labeling at this time.

GENOTOXICITY STUDIES

Chinese Hamster Lung In Vitro Cytogenetics Assay of Candesartan

Study Facility: [redacted]
Study No.: 1344/GE
Study Dates: 3/31/92-11/15/93

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Cell Culture: Chinese hamster lung (CHL) cells from a newborn female.

Procedure: Candesartan (Lot # A07807-03522) was dissolved in saline and added to CHL cell cultures in the presence and absence of metabolic activation (liver S-9 fraction obtained from Aroclor 1254-treated rats) at concentrations of 0 (vehicle), 0.625, 1.25, 2.5, 5 and 10 mM. Results from a preliminary study were used to select a range of non-cytotoxic concentrations of candesartan. Cells were incubated with drug, vehicle or positive controls (mitomycin-C, 3.75 and 7.5 x 10^{-3} mM and cyclophosphamide, 3.6 x 10^{-2} mM) for 24 or 48 hours in the absence of metabolic activation and for 6 hours in the presence and absence of metabolic activation. The metaphases (200 metaphases/dose group) of treated cells were analyzed for chromosomal aberrations (gaps, breaks, exchanges) at three successive doses set at two-fold intervals from 1.25 to 5 mM in the 24-hour treatment series, from 0.625 to 2.5 mM in the 48-hours treatment series and from 2.5 to 10 mM in the 6-hour treatment series. A cell having one or more chromosomal aberrations was recorded as an aberrant cell. The frequency of aberrant cells in each dosage group was compared for statistically significant difference from vehicle control by the Fisher's exact probability test.

Results: Incubation of candesartan with CHL cells for 6 hours with or without metabolic activation caused no adverse effect on cell viability as indicated by the mitotic indexes (Table 1). Exposure of the cell cultures for 24 and 48 hours reduced mitotic activity relative to saline controls. With 48 hours of exposure to candesartan concentrations equal to or greater than 1.25 mM and with 24 hours of exposure to candesartan concentrations equal to or greater than 2.5 mM, concentration-dependent reductions in mitotic indexes (relative to control) were observed. Six hours of exposure to candesartan, with or without metabolic activation, resulted in no significant increase in aberration frequency at any of the tested concentrations (up to 10 mM). Significant increases in the frequency of cells with chromosomal aberrations were detected with 24 hours of exposure to candesartan concentrations of 2.5 mM and 5.0 mM and with 48 hours of exposure to candesartan concentrations of 1.25 mM and 2.5 mM (Table 2). These candesartan concentrations that produced increases in chromosomal aberration frequencies correspond to concentrations that produced decreases in mitotic activity. Although the sponsor considers these positive clastogenic effects as cytotoxicity mediated and not related to a direct clastogenic effect of the drug, the degree of cytotoxicity was within levels considered (according to the ICH guideline) for detecting drug-induced genotoxicity. The increase in chromosomal aberration frequency by candesartan was dose-related at both the 24 hr and 48 hr exposure periods in the absence of metabolic activation. Assessment in the presence of metabolic activation at the 24 hr and 48 hr periods was not conducted. Thus, candesartan is judged to be positive in this assay.

Table 1. Candesartan Effects on Mitotic Index

Treatment (mM)	S-9	Mitotic Index (% of Control)		
		6-Hr	24-Hr	48-Hr
Saline	-	100	100	100
	+	100		
Candesartan				
0.625	-	99	111	103
	+	108		
1.25	-	87	120	79
	+	105		
2.5	-	97	89	48
	+	108		
5.0	-	97	43	23
	+	104		
10	-	117	17	12
	+	106		

Table 2. CHL In Vitro Cytogenetic Assay of Candesartan

Treatment (mM)	Exposure Period (hr)	S-9	Chromosomal Aberration Frequency (% Cells)[a]
Saline	6	-	0.5
	6	+	1.0
Candesartan			
2.5	6	-	0.5
5.0	6	-	0.5
10.0	6	-	1.5
2.5	6	+	0.0
5.0	6	+	1.5
10.0	6	+	0.5
Cyclophosphamide			
3.6×10^{-2}	6	-	0.0
	6	+	22.0*
Saline	24	-	0.0
	48	-	0.5
Candesartan[b]			
1.25	24	-	2.0
2.5	24	-	2.5*
5.0	24	-	7.5*
0.625	48	-	3.0
1.25	48	-	13.5*
2.5	48	-	25.5*
Mitomycin C			
7.5×10^{-3}	24	-	43.0*
3.75×10^{-3}	48	-	22.5*

[a] Based on 200 cells/dose group
[b] Due to severe reduction in mitotic activity, concentrations >5.0 mM with 24-Hr exposure and >2.5 mM with 48-exposure were not tested.
* Significantly greater than concurrent saline control ($p<0.05$)

Chinese Hamster Lung In Vitro Cytogenetics Assay of Candesartan Metabolite, M-II

Study Facility: [redacted]
Study No.: 1582/GE
Study Dates: 5/10/93-2/23/94

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Cell Culture: Chinese hamster lung (CHL) cells from newborn female.

Procedure: Candesartan O-de-ethyl metabolite, M-II, (Lot # M464-R0201) was dissolved in saline and added to CHL cell cultures in the presence and absence of metabolic activation (liver S-9 fraction obtained from Aroclor 1254-treated rats) at concentrations of 0 (vehicle), 0.375, 0.625, 0.75, 1.25, 1.5, 2.5, 3, 5 and 10 mM. Results from a preliminary study were used to select a range of non-cytotoxic concentrations of the M-II metabolite. Cells were incubated with drug, vehicle or positive controls (mitomycin-C, 3.75 and 7.5×10^{-3} mM and cyclophosphamide, 3.6×10^{-2} mM) for 24 or 48 hours in the absence of metabolic activation and for 6 hours in the

presence and absence of metabolic activation. The metaphases (200 metaphases/dose group) of treated cells were analyzed for chromosomal aberrations (gaps, breaks, exchanges) at successive doses set at two-fold intervals from 1.25 to 5 mM in the 24-hour treatment series, from 0.375 to 3 mM in the 48-hours treatment series and from 2.5 to 10 mM in the 6-hour treatment series. A cell having one or more chromosomal aberration was recorded as an aberrant cell. The frequency of aberrant cells in each dosage group was compared for statistically significant difference from vehicle control by the Fisher's exact probability test.

Results: The effects of candesartan metabolite, M-II, on mitotic activity are shown in Table 3. Incubation of M-II metabolite with CHL cells for 6 hours at concentration up to 5 mM with or without metabolic activation caused no adverse effect on cell viability as indicated by the mitotic indexes. With 6 hours of exposure, and only in the presence of metabolic activation, mitotic activity was reduced at an M-II concentration of 10 mM. With 48 hours exposure to M-II concentrations equal to or greater than 0.75 mM, and with 24 hours exposure to concentrations equal to or greater than 1.25 mM, concentration-dependent reductions in mitotic indexes (relative to control) were observed.

Table 3. Candesartan Metabolite, M-II, Effects on Mitotic Index

Treatment (mM)	S-9	Mitotic Index (% of Control)		
		6-Hr	24-Hr	48-Hr
Saline	-	100	100	100
	+	100		
M-II Metabolite				
0.375	-			98
	+			
0.625	-		108	
	+			
0.75	-			65
	+			
1.25	-	93	88	
	+	95		
1.5	-			69
	+			
2.5	-	82	66	
	+	94		
3.0	-			25
	+			
5.0	-	92	32	
	+	101		
10	-	115		
	+	78		

Six hours exposure to the M-II metabolite caused no significant increase in chromosomal aberration frequency at any concentration tested, with or without metabolic activation (Table 4). Significant increases in the frequency of cells with chromosomal aberrations were detected with 24 hours exposure to a concentration of 5.0 mM and with 48 hours exposure to concentrations of 0.75, 1.5 and 3.0 mM. These M-II metabolite concentrations that produced increases in chromosomal aberration frequencies correspond to concentrations that produced decreases in mitotic activity. Although the sponsor considers these positive clastogenic effects as cytotoxicity mediated and not related to a direct clastogenic effect of the drug, the degree of cytotoxicity was within levels considered (according to the ICH guideline) for detecting drug-induced genotoxicity. The increase in chromosomal aberration frequency by the candesartan metabolite

M-II was dose-related at 48 hr exposure period in the absence of metabolic activation. Assessment in the presence of metabolic activation at the 24 hr and 48 hr periods was not conducted. Thus, the M-II metabolite of candesartan is also judged to be positive in this assay.

Table 4. CHL In Vitro Cytogenetic Assay of Candesartan Metabolite, M-II

Treatment (mM)	Exposure Period (hr)	S-9	Chromosomal Aberration Frequency (% Cells)[a]
Saline	6	-	0.5
	6	+	1.0
M-II Metabolite			
2.5	6	-	0.5
5.0	6	-	1.0
10.0	6	-	1.5
2.5	6	+	0.5
5.0	6	+	0.5
10.0	6	+	1.0
Cyclophosphamide			
3.6×10^{-2}	6	-	0.5
	6	+	24.0*
Saline	24	-	0.5
	48	-	0.5
M-II Metabolite[b]			
1.25	24	-	1.5
2.5	24	-	2.5
5.0	24	-	10.0*
0.375	48	-	1.0
0.75	48	-	5.0*
1.5	48	-	4.5*
3.0	48	-	28.5*
Mitomycin C			
7.5×10^{-3}	24	-	35.0*
3.75×10^{-3}	48	-	29.0*

[a] Based on 200 cells/dose group
[b] Due to severe reduction in mitotic activity, concentrations >5.0 mM with 24-Hr exposure and >3.0 mM with 48-exposure were not tested.
* Significantly greater than concurrent saline control ($p<0.05$)

EVALUATION AND CONCLUSIONS

As indicated in the initial Pharmacology/Toxicology NDA review of candesartan cilexetil (NDA # 20,838, Review and Evaluation of Pharmacology and Toxicology Data, 2/18/98), candesartan and its M-II metabolite were judged to be negative in the in vitro Chinese Hamster Lung cytogenetics assay. This conclusion was based on the notion that excessive drug-induced cytotoxicity was undesirable in determining potential drug-induced chromosomal damage. Procedures for conducting and evaluating chromosome-breaking (clastogenic) agents that are found in reference texts (e.g. Principles and Methods in Toxicology; 2nd Ed., E. Wallace Hayes, ed. pp 428-29, 1989) advise that "doses are selected from a range of concentrations by bracketing the highest dose that shows no loss in growth potential with at least one higher and three lower doses". An acceptable level of inhibition of cell growth was not defined.

The current ICH guideline clarifies this previously ill-defined criterion. The guideline states that some genotoxic carcinogens are not detectable in *in vitro* genotoxicity assays unless the concentrations tested induce some degree of cytotoxicity and "the desired level of toxicity for *in vitro* cytogenetic test using cell lines should be greater than 50 percent reduction in cell number or culture confluency". Statistically significant increases in the frequency of chromosome aberrations were seen with candesartan at the 24 hr exposure at cytotoxicity levels of 11% to 57% and at the 48 hr exposure period at cytotoxicity levels of 21% to 52%. These levels of cytotoxicity would be suitable under the ICH guideline and, thus, candesartan would be judged positive in this assay. The candesartan metabolite M-II showed significant increases in chromosome aberration frequencies at suitable levels of cytotoxicity and, therefore, would also be judged positive in this assay.

LABELING RECOMMENDATION

Under the PRECAUTIONS, *Carcinogenesis, Mutagenesis, Impairment of Fertility* Section, the mutagenesis information in the current ATACAND product labeling reads:

> Candesartan cilexetil was not genotoxic in the microbial mutagenesis and mammalian cell mutagenesis assays and in the *in vivo* chromosomal aberration and rat unscheduled DNA synthesis assays. In addition, candesartan was not genotoxic in the microbial mutagenesis, mammalian cell mutagenesis, and *in vitro* and *in vivo* chromosomal aberration assays.

The paragraph above should be revised to read:

> Candesartan and it's O-deethyl metabolite tested positive for genotoxicity in the *in vitro* Chinese hamster lung (CHL) chromosomal aberration assay. Neither compound tested positive in the Ames microbial mutagenesis assay or the *in vitro* mouse lymphoma cell assay. Candesartan (but not it's O-deethyl metabolite) was also evaluated *in vivo* in the mouse micronucleus test and *in vitro* in the Chinese hamster ovary (CHO) gene mutation assay, in both cases with negative results. Candesartan cilexetil was evaluated in the Ames test, the *in vitro* mouse lymphoma cell and rat hepatocyte unscheduled DNA assays and the *in vivo* mouse micronucleus test, in each case with negative results. (Candesartan cilexetil was not evaluated in the CHL chromosomal aberration or CHO gene mutation assay.)

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HFD-110
HFD-110/Proj. Mgr.
HFD-110/AProakis
HFD-110/CResnick