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Is there any association between imidapril hydrochloride stability profile under dry air conditions and cancer initiation?

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ABSTRACT

Stability study for imidapril hydrochloride (IMD) was performed under stress conditions of increased temperature ($T = 373$ K) and decreased relative air humidity (RH = 0%) in order to obtain and identify its degradation product. The degradation sample stored for 15 days under the above environmental conditions was analyzed by LC–MS technique and it was found that the only degradation impurity formed in the course of the investigated drug degradation was IMD diketopiperazine derivative (DKP) which was produced by dehydration and intramolecular cyclization. The kinetics of its formation was analyzed by a revalidated RP-HPLC method and the kinetic model of this reaction was established. It was concluded that the DKP formation follows Prout–Tompkins kinetics with the rate constant $k \pm \Delta k = 2.034 \pm 0.157 \times 10^{-6}$ [s^{-1}]. The obtained degradation impurity was further assessed with respect to its mutagenic potential using commercial Ames MPF 98/100 microplate format mutagenicity assay kit equipped with *Salmonella typhimurium* strains TA 98 and TA 100. Both strains were exposed to six concentrations (in a range of 0.16–5.0 mg/mL) of DKP in the presence and absence of metabolic activation system. No mutagenic effect was observed confirming that the presence of DKP in IMD final dosage form has no impact on cancer initiation.

1. Introduction

The renin-angiotensin system (RAS) has recently gained an interest as a potential anticancer target mainly because of its participation in fundamental cell processes, such as: regulation of angiogenesis, induction of inflammatory responses, cell cycle control or moderation of cell motility. Thus, angiotensin-converting enzyme inhibitors (ACE-I), which attenuate the activity of main RAS effector, have become the subject of consideration as novel chemotherapeutic agents. Unfortunately, the available preclinical and clinical data provide conflicting evidence on their impact on cancer development and some studies even emphasize the positive correlation between patient exposure to various ACE-Is and the neoplastic disease incidence, however the exact mechanism responsible for this effect has not been established (George et al., 2010; Regulska et al., 2013b). This obviously rises concerns about safety of the ACE-I use and therefore it seems evident that individual class members should be thoroughly examined on both, pharmacodynamic and chemical level in order to conclusively verify their possible, molecule-dependent capability of carcinogenesis stimulation.

One of the factors that determines pharmacological properties of a drug product is its specific stability profile and this stems from the fact that not only an active substance but also its impurities, including degradants, can exert some biological actions that consequently influence the overall effect associated with drug administration. For this reason products of degradation have gained our interest as potential moderators of ACE-I-related increased cancer incidence after their long-term use. In fact, the occurrence of moisture- and heat-dependent degradation demonstrated for various ACE-Is (Stanisz, 2003a,b, 2004; Regulska and Stanisz, 2013; Regulska et al., 2013a) implies that small doses of their degradation impurities are likely to be introduced into organism when administered after improper storage or at the end of tablets' expiration date. Nonetheless their cancer-related properties still remain unexplored. Hence, we decided to investigate one of the possible modes of cancer induction after exposure to degradants formed in the course of ACE-I decay.

Generally, the most common mechanism by which drug or drug-related chemicals can stimulate cancer development is the induction of genome mutations. Drug mutagenicity is, thus, defined as the capability of any of its ingredients to cause changes in human DNA, RNA or proteins leading to the increased frequency of

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abnormalities in nucleotide sequence, as well as to cell death and organ damage, finally resulting in the elevated risk of carcinogenesis (Rao et al., 2004). Therefore, the assessment of drug mutagenic properties constitutes a justified prophylactic approach toward reduction of the potential lifetime cancer risk associated with patient exposure to mutagenic and carcinogenic chemicals. As the capacity to induce mutagenic effects is attributed not only to active substances themselves but also to any other ingredient, including impurities present in the final dosage form, the occurrence of drug degradation which cannot be controlled and frequently depends on patient habits constitutes a significant concern (Stanisz and Regulska, 2013). Thus, the identification of drug degradation impurities and the assessment of their mutagenic potential seems reasonable and should be performed for any pharmaceutical, with special impact on the drugs that are intended for long-term administration.

Unfortunately, the literature data on the mutagenicity of various ACE-I is highly limited. In fact, there are only two reports which assess the mutagenic potential of quinapril (McGuire et al., 1996) and trandolapril (Aruga et al., 1993) and they evidence that these drugs are non-mutagens at clinically-available doses, however the clastogenic effect has been obtained in the *in vitro* chromosome aberration assay for quinapril at its cytotoxic concentrations with metabolic activation. Also one paper is available for enalapril in which a positive result for a drug–nitrate interaction product (1.12 mg/mL) in the reverse mutation test on *Salmonella typhimurium* TA 1535 strain has been shown (Ozhan and Alpertunga, 2003). The preclinical data on drug mutagenic properties are also included in the drug reference documents such as summary of product characteristics. Unfortunately, all of these information regard only pure substance, not its degradation products. For this reason we decided to analyze the stability of one member of the ACE-I class, imidapril hydrochloride (IMD), and to preliminarily evaluate the mutagenicity of its degradation product in order to verify safety of its use with respect to cancer development. In fact, in our previous studies we have shown that IMD belongs to unstable drugs and its degradation under high humidity conditions occurs according to autocatalytic kinetic model with the formation of two degradation impurities, *i.e.*: a diacidic IMD derivative defined as imidaprilat formed by hydrolysis and diketopiperazine derivative (DKP) formed by intramolecular cyclization (Regulska and Stanisz, 2013). Imidaprilat is an active metabolite of IMD responsible for its pharmacological effect and given its close structural similarity to IMD, it is not considered to be a mutagen as indicated by the summary of product characteristics of the commercial dosage form. The mutagenicity of DKP derivatives, however, is a subject of controversy. In fact, they represent the group of compounds that could potentially exert mutagenic actions themselves (Witiak et al., 1977) or after exposition to nitrite in the acidic environment of gastric juice (Olney et al., 1996; Shephard et al., 1991, 1993), which was confirmed in *S. typhimurium* TA100 and TA98 strains (Shephard et al., 1991). Yet there are also studies indicating no mutagenic effect of DKP derivatives (Butchko et al., 2002; Rencüzoğullari et al., 2004). Also piperazine itself has been proven to be non-mutagenic (Arriaga Alba et al., 1989; Braun et al., 1977; Haworth et al., 1983; Stewart and Farber, 1973). These conflicting evidence support the need to determine the safety of the presence of DKP derivative in the IMD drug product.

It is also worth mentioning that our previous studies on IMD stability showed that this drug under dry air environment produces only one degradation product which has not been identified (Regulska et al., 2013a) yet the lack of moisture indicates that in this case the reaction of hydrolysis is less favorable and thus we hypothesized that under these conditions only DKP is produced. In order to confirm our assumptions we conducted the appropriate stability assay, according to ICH recommendations available in the guideline *Q1A(R2)*, *Stability Testing of New Drug substances and Products*, and then we evaluated the mutagenic potential of the formed degradation impurity. The mutagenicity assay for pure IMD and for its one degradation product – diacidic derivative, imidaprilat, was not a subject of this study since the relevant data confirming their non-mutagenic character are available in the summary of product characteristics of the commercial pharmaceutical dosage form. The adopted analytical approach involved the performance of stress testing under the conditions of dry air and increased temperature, and then the conduction of the bacterial reverse mutations test (Ames test) for the IMD degradation product obtained under RH = 0%. With this aim we utilized a commercial Ames MPF 98/100 microplate format mutagenicity assay kit which, according to ICH guideline *CMPH/ICH/174/95*, belongs to the standard test battery for genotoxicity testing of pharmaceuticals and its performance is mandatory for any drug prior its approval for use in humans. Additionally, the very extensive database available for this assay justifies its inclusion in any initial genotoxicity testing for mutagenic hazard. Ames MPF 98/100 microplate format mutagenicity assay also offers several advantages over the standard Ames procedure since it requires less test substance, reagents, plasticware and hands-on time, while the colorimetric readout allows for automation of substantial parts of the assay (Kamber et al., 2009).

2. Materials and methods

2.1. Chemicals for kinetic studies

Imidapril hydrochloride was kindly supplied by Jeleniogorskie Zaklady Farmaceutyczne "JELFA" (Jelenia Gora, Poland). Analytical grade potassium phosphate monobasic and *ortho*-phosphoric acid were purchased from POCh S.A. (Gliwice, Poland) and HPLC grade methanol and acetonitrile were obtained from Merck KGaA (Darmstadt, Germany). Bidistilled water was used.

2.2. Chemicals for bacterial reverse mutations test

A commercial Ames MPF 98/100 microplate format mutagenicity assay kit (Xenometrix, Switzerland) with *S. typhimurium* strains TA 98 (containing frameshifts mutation *hisD3052*, *rfa uvrB*, *pKM101*) and TA 100 (containing base-pair substitution mutation *hisG46*, *rfa uvrB*, *pKM101*) was used. It contained 2-nitrofluorene, 4-nitroquinolone-N-oxide, 2-aminoanthracene as positive controls, phenobarbital/β-naphtoflavone-induced rat liver fraction S9 as the activation system, sterile ampicillin (50 mg/mL), growth medium, exposure medium (histidine-rich) and indicator medium (lacking histidine). For preparing the S9 mix a ready-to-use kit from Xenometrix containing phosphate buffer pH 7.4, $MgCl_2$, KCl, G-6-P and NADP was employed. The tested samples were dissolved in sterile DMSO (Sigma–Aldrich).

2.3. Apparatus

2.3.1. High-pressure liquid chromatographic method

Shimadzu liquid chromatograph consisting of Rheodyne 7125, 100 μL fixed loop injector, UV–Vis SPO-6AV detector, LC-6A pump and C-RGA chromatopac integrator was used and the following operating conditions were applied: mobile phase – acetonitrile–methanol–aqueous phosphate buffer, pH 2.0; 0.035 mol/L (60:10:30, v/v/v) and stationary phase – LiChrospher 100 RP-18 (size 5 μm) 250 mm × 4 mm I.D. column. The chromatographic separation was performed isocratically at ambient temperature at the flow rate of 1.2 mL/min with the detector wavelength set at 218 nm (Stanisz et al., 2011). The injection volume of