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Degradation of biologically active substances by vapor-phase hydrogen peroxide

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Abstract We have examined the potential of vapor-phase hydrogen peroxide (VPHP) for degradation of 21 structurally different pharmaceutical substances. Our results show that VPHP can be used to degrade pharmaceuticals, but it was not universally applicable to all the drugs tested. Structural analysis revealed a significant correlation of the molecular structure of a compound with its susceptibility to VPHP. Tertiary amino groups seem to be the initiation centers, although the overall susceptibility of a substance depends on other factors also. Several tested substances underwent significant structural changes, suggesting a possible decrease in their biological activity. As far as we are aware, this is one of the first reports of application of VPHP to the decontamination of hazardous chemicals.

Keywords Vapor-phase hydrogen peroxide · Pharmaceutical drugs · Decontamination · Degradation · Bioactive compounds

Introduction

During the last three decades there has been massive development of the chemical and pharmaceutical industries, the products of which (carcinogenic pesticides, pharmaceutical substances, etc.) very often have a negative effect on the environment [1]. Among the most problematic types of chemical are pharmaceutical compounds, because they usually have high biological activity and their presence in

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the environment can have deleterious effects on fauna and flora [2]. Despite advances in water treatment and environmental regulations, many pharmaceutical agents escape into surface waters from a variety of sources, for example hospitals, households, or industrial production plants [2–5].

There has recently been rapid development of advanced oxidation processes (AOPs) for decontamination of the aqueous environment. These processes enable highly efficient tertiary wastewater treatment or direct treatment of effluent waters from plants or hospitals [6]. A different way of reducing the amount of chemicals in effluents is direct decontamination on site. The chemicals are thus inactivated before they can enter the sewers and become dispersed in wastewater [4]. Numerous vapor-phase methods have been developed to achieve such decontamination. They are mainly used for cleaning contaminated surfaces and for inactivation of residual contaminants in waste of various kinds (e.g. packaging, biological liquids) before release into wastewater or disposal at dumps [7, 8]. A frequently used method is decontamination with vapor-phase hydrogen peroxide (VPHP) [9]. This method is used with increasing frequency for bio-decontamination and has been approved by the US Environmental Protection Agency (EPA) for disinfection of enclosed premises [10].

In recent years, VPHP has been used in new areas of application, including biological decontamination of clean rooms, laboratories, production machines, and different process equipment, for example freeze dryers and centrifuges [11–13]. The main benefits of VPHP, for example its bactericidal effect [14, 15], superior penetration, thus reaching inaccessible surfaces and spaces [16, 17], and oxidizing potential toward a variety of chemicals [18, 19], have been proved in a number of scientific studies. As a decontaminant, vapor-phase hydrogen peroxide is regarded as less toxic than other fumigating agents (i.e. chlorine dioxide, ethylene oxide, methyl bromide, and formaldehyde). Moreover, hydrogen peroxide is neutralized by catalytic breakdown into water and oxygen [20]. Although a concentrated VPHP atmosphere, a strong oxidizing agent, is perfectly suitable for degradation of chemical contaminants, very few scientific articles on this topic have yet been published [18–21]—VPHP research has focused solely on bio-decontamination.

In this paper, we seek to emphasize the potential of VPHP for degradation of a variety of chemical compounds. We have focused on the decontamination of surfaces contaminated with bioactive compounds, which were selected from therapeutically used pharmaceutical substances.

Experimental

Chemicals

Pharmaceutical compounds were obtained from Teva Pharmaceuticals, Czech Republic, and Aldrich, USA. A complete list of the compounds tested is given in Table 1. Acetone p.a., CH_2Cl_2 p.a. (Lach-Ner, Czech Republic), methanol p.a., ethanol p.a., $CHCl_3$ p.a. (Penta, Czech Republic) or distilled water (ICT Prague, Czech Republic) were used as solvents. For NMR analysis of the original substances

Table 1 The pharmaceutical substances tested, the solvents used for transfer on to the model surface and extraction from the surface, and the solvents used for NMR analysis

No.	Pharmaceutical substance	Solvent/solvent for NMR
1	Buprenorphine hydrochloride	Methanol p.a./DMSO-d_6
2	Butorphanol	Methanol p.a./DMSO-d_6
3	Amoxicillin	Distilled H_2O/D_2O
4	Gentamicin sulfate	Distilled H_2O/D_2O
5	Chloramphenicol	Acetone p.a./DMSO-d_6
6	Nystatin	Methanol p.a./pyridine-d_5
7	Carbamazepine	Methanol p.a./methanol-d_4
8	Pimaricin (Natamycin)	Methanol p.a./DMSO-d_6
9	Ketoprofen	Ethanol p.a./DMSO-d_6
10	Testosterone	CH_2Cl_2 p.a./DMSO-d_6
11	Cyclosporine	CH_2Cl_2 p.a./DMSO-d_6
12	Mycophenolate mofetil	$CHCl_3$ p.a./D_2O
13	Bromocriptine	Methanol p.a./DMSO-d_6
14	Dihydroergotamine tartrate	CH_2Cl_2 : methanol (9:1) p.a./DMSO-d_6
15	Ergotamine	Methanol p.a./DMF-d_7
16	Nicergoline	Methanol p.a./DMSO-d_6
17	Lisuride	Methanol p.a./DMSO-d_6
18	Pergolide	Methanol p.a./DMSO-d_6
19	Imatinib	Methanol p.a./DMSO-d_6
20	Methotrexate	Distilled H_2O/DMSO-d_6
21	Paclitaxel	CH_2Cl_2 p.a./$CDCl_3$

and their potential degradation products, a variety of deuterated solvents were used: $CDCl_3$, DMF-d_7, D_2O, DMSO-d_6, methanol-d_4, pyridine-d_5 (Armar Chemicals, Germany). Hydrogen peroxide vapor was generated by evaporating a 70 % (*w*/*w*) aqueous solution of H_2O_2 (Overlack, Czech Republic). The molecular sieve Calsit 5 was used as desiccant (VÚRUP, Slovakia).

Methodology

Decontamination experiments were conducted in a novel apparatus (a so-called "peroxibox"), specifically designed to model surface contamination in large rooms and closed premises. Details of the construction and function of this device can be found in our previous publications [17, 18].

A selected pharmaceutical substance was dissolved in an appropriate volatile solvent (Table 1) and an adequate volume of the resulting solution was transferred on to a microscope slide to model a contaminated surface. The slide was subsequently left in a fume hood for the solvent to evaporate. Contamination of the model surface by the pharmaceutical drug was approximately 1.07 mg cm^{-2}.

The contaminated slide was then transferred into a "peroxibox", where it was exposed to VPHP for 12 h under defined conditions: $c_{VPHP} = 1{,}200 \pm 100$ ppm; $T = 25 \pm 1$ °C; initial relative humidity (RH_i) = 40 %. No condensation of the decontamination agent components (H_2O_2 and H_2O) was observed on the equipment. After exposure, the sample was transferred to the aeration chamber of the "peroxibox", where the concentration of VPHP was reduced below 1 ppm by input of aseptic air. The sample was then extracted into a suitable solvent and analyzed by NMR spectroscopy. This procedure was applied to all the pharmaceutical substances tested. To validate the experimental results, three samples of each pharmaceutical substance were tested in the VPHP atmosphere and two samples were exposed to ambient conditions.

Analysis of the parent drug and identification of its main degradation products were performed by NMR. ^{1}H and ^{13}C NMR spectra were acquired by use of the spectrometers Varian Mercury 300 (299.89 MHz for ^{1}H and 75.55 MHz for ^{13}C; Varian, USA), Bruker Avance III 400 (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C; Bruker BioSpin, Germany), and Bruker Avance III 600 (600.23 MHz for ^{1}H, 150.94 MHz for ^{13}C and 60.82 MHz for ^{15}N; Bruker BioSpin) in different deuterated solvents (Table 1) at 303 K. The samples were not treated in any way before analysis. The residual signal of the solvent was used as the internal standard. Experimental data were processed and interpreted by use of VNMRJ 2.1B (Varian) and Topspin 2.1 (Bruker BioSpin). ^{1}H NMR spectra were zero filled to twofold data points and multiplied by a window function to increase the resolution. The ^{13}C spectra were post-processed by 1 Hz line broadening to increase the signal/noise ratio. Structure elucidation was based on 2D NMR experiments (COSY, TOCSY, HMQC, gHSQC, HMBC, gHMQC, NOESY, ROESY, gHSQC-TOCSY, band-selective gHMQC, 1D-TOCSY, DPFGNOE, 1D-ROESY, 1D TOCSY–TOCSY, 1D TOCSY–NOESY, and NOE-difference). Because the number of substances examined was relatively high, the NMR spectra obtained are not included in this work.

Results and discussion

The purpose of this work was to study the potential of VPHP for degradation of active pharmaceutical substances and thus provide global information on its decontamination capabilities. To evaluate the efficacy of the method against the wide variety of registered active pharmaceutical ingredients, 21 pharmaceutical substances from different pharmacological groups (Table 1) were selected. Among these were analgesics (1, 2), antibiotics (3–6), an antiepilepticum (7), an antifungal drug (8), an antirheumaticum (9), a steroid hormone (10), immunosuppressants (11, 12), ergot alkaloids (13–18), and anti-cancer drugs (19–21). Although these compounds had diverse structures (Fig. 1), some common features (i.e. functional groups) could be found in many of them.

The results from the VPHP degradation experiments are summarized in Table 2. Many of the pharmaceutical substances were resistant to the treatment with VPHP and remained intact after exposure for 12 h. However, some of the compounds were

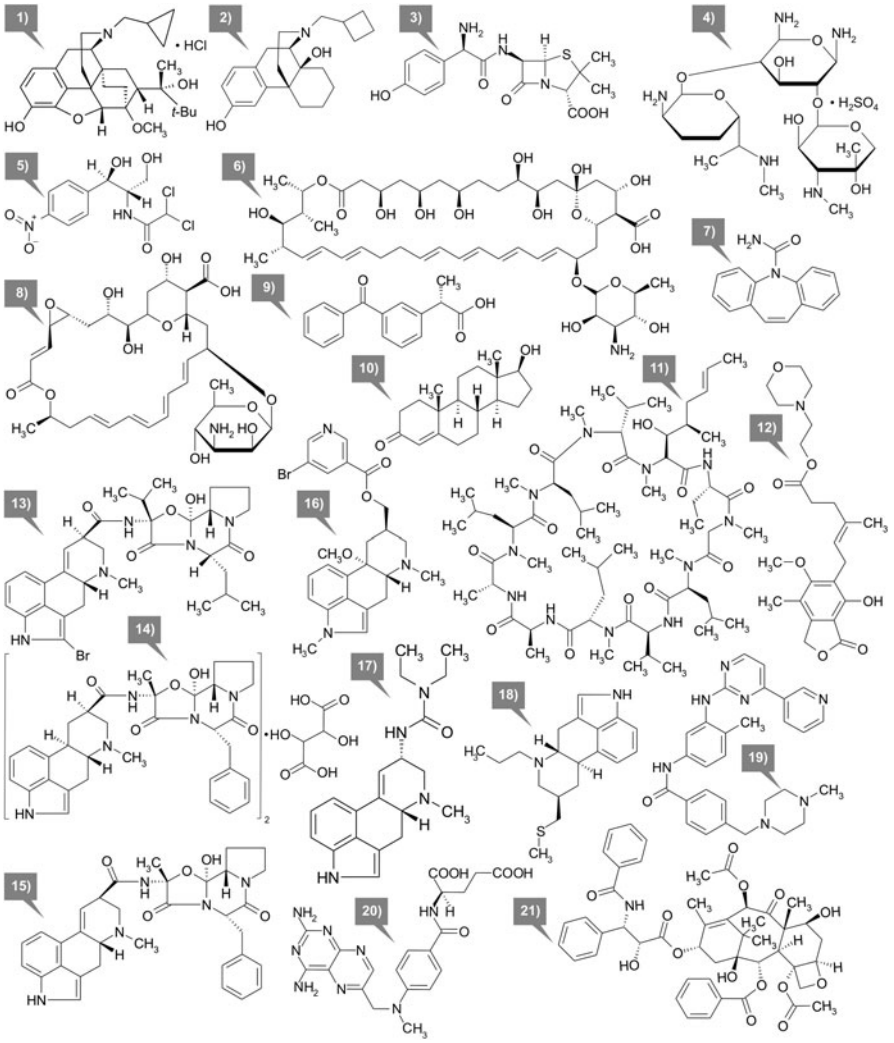

Fig. 1 Structures of the pharmaceutical substances tested

Table 2 Summary of the results from VPHP-induced degradation of the pharmaceutical substances tested

Degradation	The pharmaceutical substances
No	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 21
Yes	3, 14, 15, 16, 17, 18, 19, 20

highly sensitive to VPHP and underwent significant chemical changes. The sensitive substances were amoxicillin (3), ergot alkaloids (except bromocriptine, 13), and the anti-cancer drugs imatinib (19) and methotrexate (20).

It can be deduced from these results that substances sensitive to VPHP are those that contain a tertiary nitrogen atom in their structures (Fig. 1). The type of nitrogen-containing functional group is highly important, however. Whereas the amine undergoes degradation upon VPHP exposure the amide remains intact. It is obvious that overall molecular composition is very important, as is apparent for the ergot alkaloids. The alkaloids tested can be divided into ergines and ergopeptines. Structurally, ergines are formed with either an ergolene (Fig. 2a; 17) or a dihydroergolene (Fig. 2b; 16, 18) structure, and ergopeptines contain an additional peptide moiety connected via an amidic bond (13–15). Although these substances are similar in structure, VPHP-induced degradation proceeded in a different manner for both groups. The ergines nicergoline (16), lisuride (17), and pergolide (18) underwent complete degradation; only a mixture of lower aliphatic hydrocarbons was detected in the sample. From ergopeptines (14, 15), however, only the ergolene or dihydroergolene moiety degraded and the peptide structure (Fig. 2c) remained intact. The structure of bromocriptine (13) did not change at all. These observations unambiguously confirmed that the presence of an aliphatic tertiary nitrogen atom in the structure of a given substance facilitates its degradation by VPHP and this reaction center can be identified as the point of initiation of the degradation process.

It is also known that the tertiary nitrogen atom in the presence of hydrogen peroxide forms an *N*-oxide compound which can undergo Cope elimination, leading to the corresponding alkene and *N*-hydroxylamine. This reaction mainly occurs at high temperatures, although it can be expected at laboratory temperatures also [22]. In ergopeptines, the *N*-oxide moiety remains intact, because it contains only amide nitrogen. The resistance of bromocriptine (13) to VPHP can be attributed to the presence of a bromine atom in its structure, because it is well known that halogen atoms deactivate some structures because of their negative inductive effect. On the basis of these results, it can be concluded that VPHP enables highly effective degradation of ergot alkaloids, except the aforementioned bromocriptine (13).

However, results obtained for other structures also containing a tertiary aliphatic nitrogen were rather surprising. For instance, imatinib (19) contains a 1-methyl-piperazine moiety (Fig. 2d) bearing two tertiary aliphatic nitrogen atoms, which reacted to the corresponding di-*N*-oxides (Fig. 2e; the presence of oxygen atoms on both nitrogens was deduced from a change in their chemical shifts by about 50 ppm, as indirectly observed in $^{1}H–^{15}N$ HMBC spectra) and the final degradation product (Fig. 2f) did not react any further. It cannot be predicted with certainty whether such

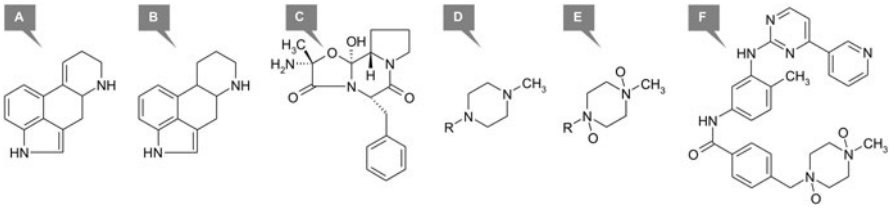

Fig. 2 Selected structural moieties or products of VPHP-induced degradation of some pharmaceutical substances

a small structural change can lead to any loss of biological activity of imatinib (19). The degradation outcome of morphinane compounds, for example buprenorphine (1) and butorphanol (2) was also unexpected. Although facile degradation was expected, because of the presence of a tertiary amino nitrogen atom, the experiments confirmed that not even an *N*-oxide species was formed.

Methotrexate (20) underwent complete degradation on exposure to the VPHP atmosphere and a complex mixture of degradation products was obtained. The NMR spectra of the sample after degradation indicate presence of several components. They have some common features. It was clear the glutamic part of the molecule was not affected by VPHP. Therefore we expect that the structural changes occurred in the pyridine moiety. We can expect oxidation and/or condensation reactions. It is therefore highly probable that this substance loses its biological activity as a result of the action of VPHP.

Another substance tested that was degraded by VPHP was amoxicillin (3). In this case, only the sulfur atom was oxidized to higher oxidation products (sulfoxide → sulfone). Table 3 summarizes the degradation products of individual pharmaceutical substances which can potentially be degraded by the action of VPHP.

It can be concluded that decontamination by VPHP to remove pharmaceutical substances is possible and useful for many different compounds. However, the method is not universally effective and testing of applicability is required for every compound. The need to test and validate the decontamination method for a particular substance is common practice and applies to all currently used decontamination methods; it should, therefore, not be regarded as a disadvantage of this method. One VPHP-susceptible molecular fragment was identified as the aliphatic tertiary amino group, which is easily oxidized in the VPHP atmosphere to the corresponding *N*-oxide, thus becoming a starting point for further degradation processes, as shown for the ergot alkaloids. Nevertheless, the results showed that this assumption did not apply to all substances, and their different sensitivity to VPHP must be ascribed to their overall molecular constitution. It can be expected that degradation of the compounds in the VPHP atmosphere—either completely or partially (i.e. to the *N*-oxide)—probably changed their biological activity. In-vitro

Table 3 Summary of the results of VPHP-induced degradation of the pharmaceutical substances tested

No.	Pharmaceutical substance	Degradation products
3	Amoxicillin	Products of sulfur oxidation
14	Dihydroergotamine tartrate	A mixture of aliphatic hydrocarbons and the peptide moiety of the molecule (Fig. 2c)
15	Ergotamine	A mixture of aliphatic hydrocarbons and the peptide moiety of the molecule (Fig. 2c)
16	Nicergoline	A mixture of aliphatic hydrocarbons
17	Lisuride	A mixture of aliphatic hydrocarbons
18	Pergolide	A mixture of aliphatic hydrocarbons
19	Imatinib	Fig. 2f
20	Methotrexate	A mixture of products

testing of biological activity would confirm this assumption and provide definite proof of the suitability of VPHP for decontamination for the chemicals tested.

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