BUPRENNORPHINE – TRADITIONAL DRUG WITH A NEW POTENTIAL RISK OF ABUSE

IVANA KURCOVAa,b, HANA RAKOVCOVAa

a Toxicological Information Centre, Dept. of Occupational Medicine, General Teaching Hospital, 1st Faculty of Medicine of Charles University, Na Bojišti 1, 128 00 Prague 2, b Institute of Forensic Medicine and Toxicology, General Teaching Hospital, 1st Faculty of Medicine of Charles University, Na Bojišti 3, 128 00 Prague 2
kurcova.ivana@vfn.cz, ikurc@ff1.cuni.cz

Key words: buprenorphine, substitution therapy, abuse, respiration failure

Introduction

Buprenorphin (BUP) is a conventional analgetic, derived from an alkaloid opiate thebaine. As compared to other opiates, it is considered harmless, since it does not affect largely respiration or mind. Nevertheless, its introduction into therapy of drug addicts brought, together with predicted merits, also new risks. BUP was first semisynthetically prepared in 1968. Traditionally, it has been used as medicament suppressing severe pains and for premedication. At higher doses, it minimizes the need of drug addicts for additional doses of illegal intravenous opiates. Therefore, it was indicated as reportedly harmless peroral therapy in a degree comparable with antidote naltrexone.

Pharmacokinetics and pharmacodynamics

Biological accessibility during digestion is relatively low. Therefore, in addition to injections, sublingual tablets are manufactured for oral mucous absorption, which bypasses intestinal degradation and first past effect in liver. BUP is well distributed and as a lipophilic substance easy passes hematoencephalic barrier. A three-compartment model best characterizes its complex kinetics. Its plasma half life ranges between 1–7 hours; the terminal elimination process lasts 20–44 hours. Liver isoenzyme CYP 3A4 catalyzes the BUP conversion to an active metabolite – norbuprenorphine and bought are inactivated by glucuronization. BUP and metabolites are secreted into bile and eliminated by stool (70–80 %), partially by urine (10–30 %).

BUP is an opioid agonist-antagonist. It has a strong affinity to receptors μ and κ (100 times higher than morphine) with a slow reversible dissociation, which causes its long-term effect. It react less with δ receptors and, therefore, exhibits lower euphoric effects, which results in milder risk of addiction and moderate withdrawal symptom. Both experimental and clinical studies confirm the unique pharmacological property of BUP that, in contrast to selective agonists, as morphine and methadone, does not affect significantly the respiratory functions and shows so called “ceiling effect”. As a consequence, the severity of over dosage is reduced. Moreover, it acts with k receptors as a weak antagonist, and in this way can block the effect of other opiates in a degree comparable with antidote naltrexone.

Dosage and indications

BUP is 25 to 50-fold more effective analgesic than morphine and the ordinary doses are therefore low, up to 0.5 mg. The maximal overall dose is not indicated because BUP is considered as a medical preparation with wide-safe therapeutical profile. As analgesic it is registered in Czech Republic as Temgesic in the form of sublingual tablets of 0.2 mg or ampoules of 0.3 mg. For maintenance therapy (substitution) of opiate addicts, BUP is administrated at higher doses in a form of sublingual tablets Subutex of 0.4, 2.0 or 8.0 mg. The medicament has to be administrated only sublingually; once a day for adults and adolescents over 15 years. The dosage is individual in dependence on the addiction. The day dose should not exceed 32 mg.

Substitution therapy

Maintenance substitution is an official method for drug addiction therapy. It represents the first medical help for an addict, which should continue in further programs oriented on abstinence. It consists in administration of a substitute with similar effect as originally used drug but deliberately administrated in a different way than intravenously. It suppresses withdrawal symptoms and leads to a reduction of drug abuse. Consequently it leads to the prospective effect is “harm reduction”, i.e., minimizing the health and social impacts of dangerous behavior of addicts as, e.g., communication and social dystrophy and, above all, the criminality. Corporate benefit is the reduction of medical costs spent for the treatment and minimization of incidence of infectious diseases expanded by hazardous injection applications (hepatitis B and C, AIDS).

Side effects and toxicity

Side effects of buprenorphine are negligible and only rarely reported for the high-dosage substitution therapy. They include sedation, dizziness and nausea. As well, poisoning symptoms by BUP are similar, more marked but not serious. Toxic doses are individual and the lethal doses are even not determined. Only a single decease caused by a BUP massive per oral intoxication has been described so far.

Abuse and its risk

Abusers discovered that the sublingual tablets of Subutex could be misused. Crushed tablets applied intravenously elicited an effect comparable to heroine. BUP began to appear at the black market and became more and more popu-
lar drug. In contrast to heroine, it can be obtained legally, it is less expensive, and available in constant and reliable quality. However, in contrast with its reputation of a safe preparation, recently a number of cases were described, in which BUP abuse led to typical symptoms of serious intoxication found with conventional opiates, which leads to patient death caused by respiratory collapse. In Europe, particularly in France and northern states, more than 100 such cases were described till 2006 (ref.1). In Singapore counting 4.5 millions inhabitants, 21 such cases was reported during 15 months2. In most cases, coincident usage of psychotropic drugs, esp. benzodiazepines (BZD), was documented. It is surprising, that the lethal asphyxia occurred even in the cases with relative small over dosage or even at therapeutic dosages, e.g., combination of buprenorphine and diazepam.

Asphyxic deaths operation

Neither BUP nor BZD alone causes the lethal intoxications. However, it was shown that in an interaction, they show synergic pharmacodynamic effect1. BZDs elevate respiratory effort of upper airways and BUP disturbs normal response to this load in attenuation of respiratory centers in brain stem2. Apparently, this is the mechanism of alteration protective ceiling affect alteration. The combination of BUP with other sedatives as alcohol and tranquillizers can be similarly dangerous.

Situation in Czech Republic

The report3 of National Drug Monitoring Center of The Council of the Government for Drug Policy Coordination in 2006 estimates in our countries 32 000 troubled drug users, i.e., persons with long-term, regular or intravenous drug applications. One third of these (11 300) are the persons addicted to opiates. In the last year, sum of 949 were treated by replacement therapy; most of them by methadone and only 375 by buprenorphine. However, as judged from the amounts of distributed Subutex, the total number of BUP users is about 8 times higher (3100) than the number of the officially treated patients. In contrast to methadone, which is prepared from a registered substance and can be obtained only in specialized pharmacies. Every physician regardless of his professional specialization can prescribe it. Thus, the abusers can visit several physicians, can obtain several packages and sell some of them. Partial restriction was introduced in 2005 since when the blue-strip recipes has to be used for better check-up. Subutex illegal dealing is monitored mainly in Prague, and northern and southern Bohemia.

Deaths connected with opiate abuse

The introduction of the substitution therapy led to a decrease of deaths caused by heroine and other opiates’ overdose. In comparison to the years 2001–2005 in our republic, this number decreased more than to a half (from 56 to 24). Among these in 2005, three incidents with methadone were registered while no diagnosis of BUP appeared in official statistics. Nevertheless, in one lethal incident BUP was diagnosed in our laboratory. It is evident that not all the cases can be verified. The analysis is carried out only for the sake of autopsy injunction together with suspicion for Subutex consumption. That is, BUP is not detected in screening test for opiates in urine, nor in ordinary toxicology tests for medicaments. Moreover, in the forms of official Czech statistic, there is no separate column among opiodes, for BUP, as it is the case for methadone.

How to prevent the abuse risk

After introduction otherwise beneficial substitution therapy by BUP, in number of countries new risk arose: BUP abuse as an intravenous drug can have lethal effects. Can we prevent the growth of such incidents?

a) One of the prerequisite is an extension of laboratory diagnostics for Subutex abuse. Urine test for BUP should be included into routine screen testing and its presence should be examined in the lethal incidents caused by unknown substance.

b) Further education among physicians, e.g., seminars organized by The Scientific society JEP for addictive diseases.

c) To restrict illegal dealing with Subutex tablets, State Institute for Drug Control suggests to establish electronic registration of prescriptions for the registered patients. This would prevent multifold prescriptions of the medicament to the same person.

d) To prevent BUP overdose, new preparation Suboxon (combination of BUP and naloxone) is planned for the distribution. The opioide antagonist – naloxone, is present in the tablets in a form, which is not absorbed per os, while applied venously it blocks euphoria and provokes severe withdrawal. This prevents the abuse or overdose. In Czech Republic, it has to be introduced to the market at the end of 2007; in the meantime together with Subutex.

Conclusions

It is necessary to be aware that the abusers’ community is inventive. To hamper the negative impact of their activities, all possible measures restricting the abuse of other medical preparations have to be installed, at the best, a step in advance.

The study was supported by project MSM0021620807.

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THE EFFECT OF NiO NANOPARTICLES ON IMMUNE SYSTEM OF RATS

MIROSŁAWA KURICOVÁ, JANA TULINSKÁ, AURELIA LIŠKOVA, EVA NEUBAUEROVÁ, ZUZANA KOVÁČIKOVÁ, ELIZABETH TÁTRAI

"Laboratory of Immunotoxicology, Department of Immunology and Immunotoxicology, Research Base of Slovak Medical University, Liptovská Jaworina, Bratislava, Slovak Republic, b Laboratory of Biochemistry, Department of Bioactive Materials, RB SMU, Bratislava, Slovak Republic, c NIEH, Budapest, Hungary

E-mail: miroslava.kuricova@studs.uniba.sk

Key words: NiO nanoparticles, Immunotoxicity, Cellular immunity, Sprague-Dawley rats

Introduction

Nickel and its alloys are used in a wide variety of industrial applications. Therefore, there are assumptions of possible contamination and following impact on environment. Data on the influence of these materials on human health, especially on immune system are rare and more studies are needed to examine possible immunomodulatory effect.

Epidemiological studies have demonstrated increased mortality from lung carcinoma and nasal cavities in workers chronically exposed by inhalation to nickel-containing dusts in nickel refinery. The respiratory tract cancers in nickel refinery workers have been associated with inhalation exposures to nickel compounds with low aqueous solubility such as NiO. Our study has monitored the effect of nickel nanoparticles on selected cellular parameters of immune response in Sprague-Dawley rats.

Material and methods

Male Sprague-Dawley rats (ISASZEG, Hungary) with starting weight of 200–220 g were treated by intratracheal instillation with one single dose of 0.5 mg NiO nanoparticles (0.5 mg animal−1). After one and four weeks, the blood samples and spleen were aseptically removed. The following immune assays were performed: (i) Proliferative assay – splenocytes and peripheral blood were incubated in vitro with mitogens: 5 mg ml−1 concanavalin A (ConA), 25 mg ml−1 phytohemmaglutinin (PHA), 2.5 mg ml−1 pokeweed (PWM) and 100 mg ml−1 Salmonella typhimurium (STM). The proliferative activity of cells was determined by incorporation of [3H]-thymidine into DNA and measured using beta scintillation counter. (ii) Expression of adhesion molecule CD11b on monocytes, lymphocytes and granulocytes was quantified using monoclonal antibodies conjugated to fluorescein isothiocyanate and evaluated by flow cytometry. (iii) Differential white blood cells count examined percentage of each type of white blood cells. Analyses were performed on slides where the drop of blood was smeared and stained with contrast dye using microscope. Statistical analysis was done using SPSS. Differences between groups were analyzed using Student T-test or Mann-Whitney test.

Results and discussion

The DNA synthesis in spleen lymphocytes in vitro stimulated with PHA, PWM and STM as well as in non-stimulated cells derived from rats exposed to NiO nanoparticles was significantly suppressed in comparison with cultures derived from control rats one week after exposure (Table I). Similarly, proliferative activity of

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Statistical significance ** P<0.01, ***P<0.001
blood lymphocytes (in vitro stimulated with PHA and non-stimulated) taken from NiO exposed rats was significantly decreased in comparison with belonging control rats (fig. 1). Suppression of proliferation was significant in both groups – after shorter and longer exposition time. Our data correspond with study of Haley et al. who found that inhalation exposure of mice to nickel can result in varying effects on the immune system. Particularly, study showed decreased number of spleen antibody forming cells in mice exposed to NiO. These nickel-induced changes may contribute to significant immunodysfunction and effect depends on dose and physicochemical form of the nickel compound2.

In group of animals with shorter exposure we recorded significantly lower percentage of neutrophils in comparison with control group. The same effect was observed also on percentage of monocytes (fig. 2). Depletion of phagocytic cells might be due to cytotoxic effect of nickel oxide as was described by Takahashi et al.3.

Four weeks after exposure, the expression of adhesion molecule CD11b on monocytes and granulocytes of NiO exposed rats was significantly higher than in control animals (fig. 3). This enhanced expression of adhesion molecule might be associated with increased cell transfer into region of chronic inflammation. It has been recently shown that the beta(2)-integrin molecule (CD11b) is upregulated on circulating neutrophils in chronic obstructive pulmonary disease (COPD) subjects4. Nickel oxide can cause inflammation, hyperplasia, and fibrosis in the lungs of rats, and to a lesser extent in mice, exposed to nickel compounds. Published papers found histopathological changes in the lungs as the most sensitive parameters for nickel toxicity in exposed animals where chronic active inflammation, fibrosis, and alveolar macrophage hyperplasia were associated with nickel exposure5.

Conclusion

Our results indicate immunomodulatory effect of NiO nanoparticles in exposed rats. Immune changes were observed one week after exposure of animals and most of them persisted also one month after exposure to NiO.

We would like to express our gratitude to Viera Vačhálková, Helena Turazová and Edita Mrvíková for their excellent technical help. This work was supported by OTKA T46733 – Hungarian Research Fond and EU grant HEAR NAS Centre of Excellence in Environmental Health, QLAM-2001-00445.

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Key words: urban air, industrial sites, pollutants measurement, Ames test

Introduction

Genetic toxicology testing is capable of finding substances, or their mixtures, that can damage DNA. These processes could result in the initiation and progression of cancer.

Ostrava region is one of the most polluted regions in the Czech Republic. Air quality is frequently evaluated by comparison of contaminant’s concentration with its legislated limit. The real environmental air is a complex mixture of many together acting compounds. The interactions among all of them are complicated not only on the chemical but even more on the biological level.

The aim of study has been determining the mutagenic potentials of sampled air and levels of selected pollutants (during one year), based on which correlations, if any, between biological (mutagenic) effect of the real air and its components could be evaluated.

Materials and methods

Air sampling has been performed at three industrial sites of the city (coking plant, chemical works, iron and steel industry, vehicle emission) daily (for 24 hours) four times a month during one year.

Chemical analyses of some carcinogenic and mutagenic substances has been carried out: benzene, trichlorethene, styrene, toluene – gas chromatography with FID detector; arsenic, nickel, cadmium, chromium – X-ray spectrometry; eight polycyclic aromatic hydrocarbons (PAHs) including benzo/a/pyrene (B/a/P) – HPLC with fluorescence detection.

Concurrently every month pooled air sample from every site has been tested using the standard plate-incorporation Ames test.

Monthly means of individual pollutants (to find correlations with mutagenic potentials) and their annual means (to compare them with residential annual limits) have been calculated.

Statistical evaluation of the mutagenic potentials using Genetox Manager v. 2.1 program and the statistical evaluation of pollutant’s concentrations using ANOVA has been carried out. Associations between monitored factors have been analyzed by the correlation and regression analyses.

Results and discussion

Mutagenic activity of air

Mutagenic potentials (per volume of air) of monitored air samples have been found higher during winter period in comparison with summer period at all sites. Levels of indirectly acting substances (for example PAHs) have been observed higher than those of directly acting ones.

Pollutant levels

Annual values of B/a/P concentration exceeds the limit at all monitored sites, annual arsenic value is within the limit at one site while above it at two other sites and the benzene annual concentration exceeds the limit at one site only.

PAHs’ and metals’ monthly levels display season-related differences during the year in contrast with the monthly levels of volatile compounds.

Correlations between biological effect and presence of pollutants

The closed correlations have been noted at all sites between mutagenic potentials of the air and the presence of B/a/P and all measured PAHs. The weakly one has been found between the levels of air mutagenic activity and concentration of arsenic (only at one site, where the arsenic annual value has been within the limit). Furthermore, at two sites with benzene annual value under the limit, weak correlations between the biological effect and the presence of volatile compounds (trichlorethene, styrene, benzene) have been registered.

Conclusion

Our study confirmed that interactions among components of the complex mixture with a number of potentially genotoxic substances are complicated. Taking into account mutagenic potency of Ostravian air the major contribution seems to occur due to PAHs action.

REFERENCES

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COMPARISON OF ACUTE TOXICITY OF POTASSIUM PERMANGANATE TO JUVENILE AND EMBRYONIC STAGES OF Danio rerio

STANISLAVA MÁCOVÁ, VLADIMÍRA PIŠTĚKOVÁ, ZDEŇKA SVOBODOVÁ, IVETA BEDÁŇOVÁ, EVA VOSLÁŘOVÁ

Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-2, 612 42 Brno
smacova@vfu.cz

Key words: potassium permanganate, age, LC50, Danio rerio, fishes

Introduction

Zebrafish (Danio rerio) is one of the model organisms most commonly used in toxicity testing. It is a popular aquarium fish from the family Cyprinidae (Cyprinids) which originates from the Ganges river system in the Indian Peninsula. The main advantage of this species is easy availability, low price, and easy rearing. At suitable conditions, one female is able to produce 50–200 eggs per day. Eggs sized 0.8 mm² are non-adherent and fully transparent.

Potassium permanganate (KMnO₄) is a violet, crystalline substance with oxidative effects, well soluble in water. The compound is used as a disinfectant in immersion, short-term or long-term baths to purify water in fishery and as a traditional oxidant. The substance gained its significance and became widely used thanks to its versatile use and easy availability. Therapeutic baths are used worldwide in both fishery and aquarium fish breeding to treat and prevent bacterial skin and gill infections and ectoparasitic and fungal diseases of freshwater fish. The compound exerts its powerful oxidative effect on pathogens. It reacts as an oxidative agent when in contact with organic substances including pathogens that cause external diseases of fish. The effective concentration of the active ion (i.e. MnO₄⁻) used to control ectoparasitic, bacterial, and fungal diseases is usually 2 mg l⁻¹.

Generally, manganese-containing compounds do not pose any major risk to fish species. The only exception is potassium permanganate whose toxicity to fish is moderate to strong. The toxicity of permanganate was determined in a number of different fish species at various factors that affect the toxicity of this chemical in fish. The toxic effect of potassium permanganate is not exactly known. Some authors have confirmed that permanganate or its products such as MnO₂ may cause damage to gill tissue as they precipitate in gills at high pH. Acute toxicity of potassium permanganate was investigated in a number of fish species at different age. Generally, it is assumed that lower developmental stages of fish are more susceptible to toxic effects of chemicals. Some authors report that earlier developmental stages of fish have greater tolerance to toxic effects.

The main aim of this work is to compare acute toxicity of potassium permanganate in embryonic and juvenile developmental stages of the aquarium fish species Danio rerio.

Materials and methods

Acute toxicity tests were performed in the juvenile stages of zebrafish (Danio rerio) according to OECD 203 methodology (Fish acute toxicity test). A series of six tests was performed. The fish aged 2–3 months, weighed 0.3±0.1 g and being 30±5 mm long were used in the experiment. The tests were performed using a semistatic method, with solutions being changed after 48 hours. Each vessel contained 10 fish randomly selected from the stock population; 96-hr acute toxicity tests were then performed.

Embryo toxicity tests were performed in zebrafish embryos (D. rerio) in compliance with the OECD No. 212 methodology (Embryo toxicity tests). A series of six tests was performed. The tests used a series of six concentrations. 20 fertilized eggs placed in one Petri dish were tested for each concentration and in control. The bath was changed every 48 hrs.

Statistical significance of the difference between LC50 values in juvenile and embryonic stage of D. rerio was tested using the Mann-Whitney non-parametric test implemented in the Unistat 5.1 programme.

Results and discussion

Since potassium permanganate is frequently used in fishery, it is necessary to test the toxicity of this substance in order to ensure that suitable therapeutic concentrations

Fig. 1. Variation in LC50 KMnO₄ with the duration of the test for the embryonic and juvenile stages of D. rerio
will be used in the treatment of fish. This topic was addressed by a number of authors whose investigations particularly focused on freshwater species3,5.

Acute toxicity of KMnO4 expressed as 96h LC50 varied in a range of 2.09–5.76 mg l\(^{-1}\) for the embryonic stage of zebrafish (the mean value of 96h LC50 = 4.075±1.585 mg l\(^{-1}\)) and in a range of 1.09–1.53 mg l\(^{-1}\) for the juvenile developmental stage of zebrafish (the mean value of 96h LC50 = 1.233±0.174 mg l\(^{-1}\)). The value of LC50 KMNO4 for the embryonic stage of zebrafish was found to vary in a range of 2.14–4.07 mg l\(^{-1}\) (the mean value of LC50 = 3.021±0.735). The values of 96h LC50 KMNO4 reported for the Cyprinidae family equal to 40 mg l\(^{-1}\) in common carp *Cyprinus carpio*6. Slightly lower values of 96h LC50 potassium permanganate but still similar to those found in our tests were reported for *Morone saxatilis* (1.58 mg l\(^{-1}\))9, *Pimephales promelas* (2.13 mg l\(^{-1}\))11, *Lepomis macrochirus* (3 mg l\(^{-1}\))7, *Carassius auratus* (3.6 mg l\(^{-1}\))8. Higher tolerance of toxic effects of KMnO4 were observed in perch (96h LC50=6 mg l\(^{-1}\))13 and the fish species *Angilla rostrata* (96h LC50=21.6 mg l\(^{-1}\))12

It is generally assumed that the lower developmental stages of fish are more susceptible to the toxic effects of chemicals. A comparison of LC50 mean values for both developmental stages of zebrafish (*D. rerio*) showed that the LC50 in the embryonic stage of zebrafish was significantly higher (P<0.01) than that for the juvenile stage of zebrafish. This result indicates greater tolerance of potassium permanganate in the embryonic stage of zebrafish. It follows from the results of the tests that the resistance of zebrafish embryos (*D. rerio*) to the effects of potassium permanganate decreases with the increasing duration of the test. The exponential relation \(y=18.227e^{-0.954x}\) between the mean values of LC50 KMnO4 and the duration of the test was found (Graph 1). The highest mortality rate occurred in zebrafish at the juvenile developmental stage within the first 48 hrs of the 96-hr test. Similarly, the tolerance to the tested dose decreased with the increasing duration of the test in embryos, with the linear relation being found between the mean values of LC50 KMnO4 and the duration of the test, i.e. \(y = -0.155x+2.763\) \((r=0.978)\)

Greater tolerance in earlier developmental stages of fish was also confirmed by other authors who compared the resistance of embryonic, juvenile, and adult stages of various species of fish1,5,12 to different pollutants.

Acute toxicity tests in embryos revealed that permanganate at individual tested concentrations affects the hatching time in fish at individual tested concentrations, which is considered to be a side effect of permanganate.

**Conclusion**

Potassium permanganate has been used worldwide in fishery and aquaculture for treatment and prevention of skin and gill bacterial infectious, fungal infectious and external parasites in freshwater fish. The aim of the study was to compare acute toxicity of potassium permanganate to juvenile and embryonic stages of zebrafish (*Danio rerio*). The semistatic method according to OECD 203 was used in the acute toxicity tests with juvenile fish and OECD 212 methodology was used in the acute toxicity tests with embryonic stages of *D. rerio*. The LC50 KMnO4 values ranged from 1.09 to 1.20 mg l\(^{-1}\) in juvenile *D. rerio* fish while LC50 KMnO4 values ranged from 2.14 to 4.07 mg l\(^{-1}\) in embryonic stages of *D. rerio*. The study proved statistically significantly higher (P<0.01) sensitivity of juvenile fish to potassium permanganate as compared with embryonic stages.

The work was realized with the support of MSM Project No. 6215712402 Veterinary Aspects of Foodstuff Safety and Quality.

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SUPEROXIDE GENERATION AND MYELOPEROXIDASE RELEASE IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES TREATED WITH CARVEDILOL

TATIANA MAČÍČKOVÁ*, JANA PEČIVOVÁ†, RADOMÍR NOSÁĽ*, DAGMAR HOLOMAŇOVÁ‡

* Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, † National Transfusion Service, Ružinovská 6, 820 07 Bratislava, Slovak Republic

esfatama@savba.sk

Key words: carvedilol, superoxide generation, myeloperoxidase, human polymorphonuclear leukocytes

Introduction

Polymorphonuclear leukocytes (PMNL) are one of the main types of inflammatory cells. The production of reactive oxygen metabolites (ROM) including generation of superoxide anions (SO) by activated PMNL is critical for their successful bactericidal action, yet under pathological conditions they may contribute to host tissue injury. Activation of PMNL induces also release of enzymes from PMNL granules which play a crucial role in the destruction and phagocytosis of microorganisms, but also in potential tissue damage.

Carvedilol (CARV) is a lipophilic vasodilating anti-hypertensive drug that selectively blocks $\alpha_1$-receptors and non selectively antagonizes $\beta_1$- and $\beta_2$-adrenoceptors. Moreover, CARV was suggested to possess antioxidant properties and have a potential for myocardial and vascular protection. Due to its antioxidant effects, CARV has been suggested to provide greater benefit than $\beta$-blockers in the treatment of chronic heart failure. Dandona et al. described the inhibitory effect of CARV on SO anion release from activated PMNL in vitro. CARV was also found to inhibit luminol-enhanced chemiluminescence of ROM in blood cells in vitro and to decrease SO generation and myeloperoxidase (MPO) release from isolated human PMNL activated with FMLP.

The aim of the present study was to compare the effect of CARV on SO generation and MPO release by human PMNL stimulated with opsonized zymosan (OZ), a specific receptor activator, and with phorbol-12-myristate-13-acetate (PMA), a receptor bypassing stimulus.

Material and methods

Carvedilol (CARV) – (Slovakofarma, Slovak Republic) was dissolved in tartaric acid (100 µmol l$^{-1}$) and then diluted in PBS(-). Cytochrome c, Dextran T500 (Pharmacia Fine Chemicals), Lymphoprep (Nycomed Pharma AS), cytochalasin B, PMA (phorbol-12-myristate-13-acetate) and zymosan A (Sigma). All other chemicals used were of analytical grade.

Zymosan A from Saccharomyces cerevisiae was opsonized according to Lojek et al.

Polymorphonuclear leukocytes (PMNL) were isolated from blood of healthy volunteers into 3.8 % trisodium citrate. Erythrocytes were removed by dextran sedimentation and centrifugation on lymphoprep by the modified BOYUM’S method.

Superoxide dismutase inhibitable reduction of cytochrome c was used to measure superoxide (SO). Suspension of PMNL (10$^6$ cells/1.5 ml PBS with 1.8 mmol l$^{-1}$ CaCl$_2$ and 0.5 mmol l$^{-1}$ MgCl$_2$) was preincubated for 5 min at 37 °C with CARV (0.1, 1, 10 and 100 µmol l$^{-1}$) and then stimulated with PMA (1 µmol l$^{-1}$) for 15 min or with 0.5 mg l$^{-1}$ OZ for 60 min, at 37 °C. Controls were included for the effect of each stimulus and of CARV on cytochrome c reduction. After centrifugation at 4,200 g for 2 min at 4 °C, absorbance was measured at 550 nm using a spectrophotometer Hewlett Packard 8452 A.

For myeloperoxidase (MPO) determination, PMNL were preincubated with cytochalasin B (5 µg ml$^{-1}$) at room temperature for 5 min. Subsequently the suspension of PMNL (2.10$^6$ cells/ml PBS) was incubated with CARV (0.1, 1, 10 and 100 µmol l$^{-1}$) and then stimulated with PMA (1 µmol l$^{-1}$) for 15 min or with 0.5 mg l$^{-1}$ OZ for 60 min, at 37 °C. Controls for direct interaction of CARV and MPO were included. The activity of MPO was assayed in the supernatant after centrifugation at 983 g for 10 min at 4 °C by determining the oxidation of o-dianisidine in the presence of hydrogen peroxide in a Hewlett Packard 8452 A spectrophotometer at 463 nm (ref.11).

All data are expressed as the mean ± SEM. The data were analysed by one-way analysis of variance (ANOVA), and $P$ values below <0.05 were taken as significant.

Results

Unstimulated cells showed neither SO formation nor enzyme release after preincubation with CARV. Figure 1 shows the effect of CARV (0.1, 1, 10 and 100 µmol l$^{-1}$) on OZ activated SO generation and MPO release in isolated human PMNL. CARDV dose-dependently decreased SO generation and MPO release after OZ stimulation, however a significant decrease was recorded only with CARV concentration of 10 and 100 µmol l$^{-1}$ to 86.70±1.82 % and 90.01±2.27 %, respectively, and MPO release to 62.74±4.40 % and 63.44±3.70 %, respectively.

The effect of CARV on SO generation and MPO release in isolated PMNL stimulated with PMA is given in fig. 2. Its action is also dose-dependent. CARV in the concentration 10 and 100 µmol l$^{-1}$ significantly decreased SO generation to 70.21±4.45 % and 90.01±2.27 %, respectively, and MPO release to 62.04±4.84 % and 91.27±5.56 %, respectively.
Discussion

Activated PMNL produce ROM and liberate enzymes. Whereas the intracellular ROM and enzymes mediate destruction of phagocytosed material and regulate PMNL activity, the extracellular generation and release results mainly in inflammatory damage of surrounding tissues. ROM and granular enzymes are thought to be involved in the development of a number of pathological conditions and diseases, especially of the cardiovascular system. It has been suggested that CARV may provide greater benefit than traditional β-blocking drugs because of its antioxidant actions that synergize with its nonspecific β- and α-blocking effects.

The results presented in our study showed in vitro inhibitory effect of CARV on activated human PMNL. The two stimuli used are able to activate PMNL to evoke a respiratory burst and enzyme release via various mechanisms. The particulate receptor-operating stimulus OZ and the soluble stimulus PMA, which bypasses receptors and activates NADPH-oxidase via protein kinase C. In our experimental conditions, CARV decreased SO generation similarly as recorded for PMA-stimulated PMNL. CARV was shown to be a poor scavenger of SO, as proven an a cell-free system. In human PMNL, CARV interfered in vitro and ex vivo with ROM generation as well as with already generated ROM, suggestive of its both "preventive" and "therapeutic" effect.

MPO is the most abundant enzyme in PMNL and has been implicated directly or through its metabolites, hypochlorous acid and subsequently derived ROM in many life-endangering diseases. CARV inhibited MPO activity of the cell-free system in a concentration-dependent manner and CARV treatment reduced MPO levels at both the area-at-risk and the necrotic zone in the ischemic myocardium.

Our results showing that CARV decreased SO generation and MPO release both by membrane-operating stimulus (OZ) as well as membrane bypassing activator (PMA) lead to the conclusion that the inhibitory effect may be attributed to its non-specific action. Since CARV affected SO generation and MPO release only in higher concentrations, and that after both stimuli, each active in a different way, its effect seems to be of non-receptor type. Physicochemical properties of CARV and its antiplatelet activity support this conclusion. The inhibitory effect of CARV on MPO release from stimulated human PMNL indicates the possibility that CARV similarly to other lipophilic β-adrenoceptor/blocking drugs interferes with membrane structure, influencing predominantly phospholipid metabolism. Because CARV effectively participated in the decrease of SO generation in stimulated human PMNL and reduced MPO release, it can be concluded that the toxicity and damage to surrounding tissues caused by MPO, SO itself, and/or by subsequently derived metabolites, would also be diminished.

This work was supported by scientific grants VEGA 2/7019/27 and APVV 51-029602.

REFERENCE

P31

OXIDATION OF ELLIPTICINE BY HUMAN AND RAT CYTOCHROMES P450 CORRELATES WITH ITS BINDING TO DNA

EVA MARTÍNKOVÁa, PETR HODEKa, JIŘÍ HUDEČKA, EVA FREIb, MONIQUE DONTENWILLc, MARIE STIBOROVÁa

a Department of Biochemistry, Faculty of Science, Charles University, 128 40 Prague, Czech Republic, b Division of Molecular Toxicology, German Cancer Research Center, 69120 Heidelberg, Germany, c Department of Pharmacology and Physicochemistry, UMR 7175 CNRS, Université L Pasteur Strasbourg, Illkirch, France

stiborov@natur.cuni.cz; evamartinkova@seznam.cz

Key words: anticancer drug, ellipticine, metabolism, cytochrome P450, DNA binding

Introduction

Ellipticine (fig. 1), an alkaloid of Apocynaceae plants, exhibits significant antitumor and anti-HIV activities1–3. The prevalent mechanisms of its antitumor, mutagenic and cytotoxic activities are intercalation into DNA and inhibition of DNA topoisomerase II1–3. We demonstrated that ellipticine covalently binds to DNA after enzymatic activation with cytochrome P450 (CYP) or peroxidases2–7. Human and rat CYP1A and 3A are the predominant enzymes catalyzing oxidation of ellipticine either to metabolites that are excreted from organisms (7-hydroxy- and 9-hydroxyellipticine) or form DNA adducts (13-hydroxy- and 12-hydroxyellipticine, the latter formed also spontaneously from another ellipticine metabolite, ellipticine N2-oxide)2–6 (fig. 1 and 2). Human cyclooxygenase (COX)-2, ovine COX-1, bovine lactoperoxidases and human myeloperoxidase also generated ellipticine-DNA adducts (fig. 1)7. The same DNA adducts were also detected in V79 Chinese hamster lung fibroblasts transfected with human CYP3A4, 1A1 and 1A2 (ref.5), in human breast adenocarcinoma MCF-7 cells9, leukemia HL-60 and CCRF-CEM cells10 and in vivo in rats exposed to this anticancer drug7. On the basis of these data, ellipticine might be considered a drug, whose pharmacological efficiency and/or genotoxic side effects are dependent on its enzymatic activation in target tissues. Here, we compare the efficiency of human and rat CYP enzymes in Suspersomes™ (microsomes from Baculovirus transfected insect cells containing recombinantly expressed human CYPs and NADPH:CYP reductase with or without cytochrome b5) to oxidize ellipticine and to form DNA adducts. Moreover, we evaluated whether oxidation of ellipticine to metabolites generating DNA adducts correlated with formation of these adducts.

Material and methods

Incubation mixtures used to study ellipticine metabolism and procedures for separation of the metabolites were as described2. Incubation mixtures used for modifying DNA by ellipticine and procedures for analysis of ellipticine-derived DNA adducts by the 32P-postlabeling assay were as shown26.

Fig. 1. Metabolism of ellipticine by human CYPs and peroxidases showing the characterized metabolites found to form DNA adducts

Fig. 2. Ellipticine-DNA adduct levels in relation to concentration of rat CYP3A1 reconstituted with NADPH:CYP reductase; 50 pmol (A), 100 pmol (B) and 250 pmol CYP3A1 (C). Analyses were performed by the nuclease P1 version of the 32P-postlabeling assay
Results and discussion

Oxidation of ellipticine by rat and human CYP enzymes in Supersomes™

To characterize the capability of the in vitro CYP systems to oxidize ellipticine several rat and human CYPs in Supersomes™ were used (fig. 3). We focused on ellipticine metabolites responsible for covalent DNA adduct formation. As cytochrome b5 is known to influence the oxidation of several substrates by some CYPs (ref.7), this protein was either co-expressed in Supersomes™, either added into incubations. All CYPs in Supersomes™ oxidized ellipticine to up to five metabolites: 9-hydroxy-(M1), 12-hydroxy-(M2), 13-hydroxy-(M3), 7-hydroxyellipticine (M4) and ellipticine N2-oxide (M5). 13-Hydroxyellipticine, forming the major ellipticine-deoxyguanosine adduct in DNA, was generated predominantly by CYP3A of both humans and rats, followed by CYP1A1, 1A2, rat 2D1 and human CYP2D6 enzymes. Differences were found in efficiencies of CYPs of a 2A subfamily in both species. While rat CYP2A1 and 2A2 oxidized ellipticine to 13-hydroxyellipticine with efficiency similar to CYP2D1, human CYP2A6 was much less active (fig. 3). 12-Hydroxyellipticine was produced mainly by rat CYP2A1, followed by human CYP2C19 and rat CYP1A1. Human CYP2D6 was the most efficient enzyme generating ellipticine N2-oxide, followed by human CYP3A4 and rat CYP3A2.

Table I
Correlation coefficients between levels of ellipticine metabolites and those of ellipticine-DNA adduct formation

| Metabolite | Human CYPs | | Rat CYPs | |
|------------|------------|--|----------|--|----------|
|            | adduct 1   | r | P       | adduct 1 | r | P       |
| M1         | 0.309      | 0.356 | 0.412 | 0.206 | -0.199 | 0.493 |
| M2         | 0.203      | 0.573 | 0.370 | 0.325 | 0.498 |
| M3         | 0.832      | 0.001 | 0.644 | 0.032 | 0.759 | 0.003 |

Formation of ellipticine-DNA adducts by recombinant CYP enzymes in Supersomes™

Two major DNA adducts 1 and 2 (fig. 2), formed on deoxyguanosine from 13-hydroxy- and 12-hydroxy-ellipticine5, were generated from ellipticine by all CYPs. Among the CYPs tested, rat CYP2A were the most efficient in generating adduct 1, which corresponds to the highest levels of 13-hydroxyellipticine formed by these enzymes. Rat and human CYP3A, 2D and 1A also effectively form this adduct (fig. 3). Rat CYP2D1 and human 1A1 were up to four times less efficient than CYP2A, followed by rat CYP1A1, 3A and human 2C9, 2C19 and 2D6. Surprisingly, CYP2D6 did not generate such high amounts of adduct 2 as it was expected, because of its high efficiency to form ellipticine N2-oxide. In contrast to human CYP2D6, rat CYP2D1 was efficient in formation of the adduct 2. This adduct was also effectively produced by both, rat and human CYP1A1, and human CYP2C19 and 2D6 (fig. 3). Rat CYP2C6 and 2C11 also generated another DNA adduct (assigned as adduct A) (not shown), while nor other human CYP2C neither other CYP subfamilies generated this adduct. The levels of 13-hydroxyellipticine correlated with those of the DNA adduct 1 (Table I). In the case of adduct 2, two metabolites, 12-hydroxellipticine or ellipticine N2-oxide, are responsible for its production, thus influencing the correlation coefficients.
Conclusion

The results of this work demonstrate that oxidation of ellipticine correlates with the DNA adduct formation and confirm the results of our former studies, indicating that ellipticine activation to species forming DNA adducts by rat CYPs is similar to that by human enzymes. However, one exception was found; rat CYP2A were significantly more active to form ellipticine-DNA adducts than their human orthologues.

Supported by the grants 203/06/0329, 303/06/0928, MSM 0021620808 and 1M4635608802.

Abbreviations

COX cyclooxygenase
CYP cytochrome P450

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DANICA MIHALOVÁ, SILVESTER PONIŠT, JARMILA KUCHARSKÁ, DENISA KOMENDOVÁ, KATARÍNA BAUEROVÁ

a Institute of Experimental Pharmacology, Slovak Academy of Science, Bratislava, b Pharmacobiochemical Laboratory of Medical Faculty, Comenius University, Bratislava, Slovakia

exfadami@savba.sk

Key words: adjuvant arthritis, oxidative stress, total antioxidant status, glucomannan, Imunoglukan®

Introduction

Several clinical studies as well as preclinical animal model of rheumatoid arthritis have documented an imbalance in the body’s reduction/oxidation (redox) homeostasis to a more pro-oxidative environment, suggesting therapies that restore redox balance may have beneficial effects on the disease process. Different sources of oxygen species (ROS) and reactive nitrogen species (RNS) are produced locally at the site of the inflamed joint.

Adjuvant arthritis (AA), induced with intradermal administration of mycobacterial adjuvants, is a widely used model of evaluation of potential antirheumatics. ROS and RNS significantly present in this model contribute to systemic and local damage, assessed on the basis of selected clinical and biochemical parameters of AA.

In the present study, the oxido-redox balance was monitored in the AA model on systemic level by the total antioxidant status (TAS) in plasma.

The aim was: i) to compare TAS measured in plasma from the eye retroorbital sinus (RES) and from the heart; ii) to monitor TAS during the progress of the disease; iii) to compare the clinical parameter hind paw volume (HPV) with TAS.

Materials and methods

AA was induced in Lewis rats by a single intradermal injection of heat-killed Mycobacterium butyricum suspended in incomplete Freund’s adjuvant. The experiments included healthy intact animals as reference controls (CO), arthritic animals (AA) without any drug administration, and arthritic animals with administration of glucomannan (AA-GM) in the oral daily dose of 15 mg kg⁻¹ b.w. and Imunoglukan® (AA-IMG) in the oral daily dose of 2 mg kg⁻¹ b.w. The treatment involved administration of the substances tested from day 0, i.e. the day of immunization, to the experimental day 28. The volume of hind paws (HPV) was measured twice a week. In control, arthritic, and treated animals, TAS was measured in plasma samples collected from the RES on experimental days 14 (AA 14 D), 21 (AA 21 D), and 28 (AA 28 D). At the end of the experiment, on day 28, the rats were sacrificed in anesthesia, and plasma was collected from the heart. To determine the TAS of plasma samples, Randox total antioxidant status manual commercial kit was used. The data were expressed as arithmetic mean and SEM. The arthritis group (AA) was compared to healthy control animals (CO), for significance calculations Student’s t-test was used with: ns (not significant); * P<0.05 (significant); ** P<0.01 (extremely significant). The treated arthritis groups (AA-GM, AA-IMG) were compared to untreated arthritis (AA), for the significance calculations Student’s t-test was used with: ns (not significant); + P<0.05 (significant); ++ P<0.01 (very significant).
Results and discussion

Oxidative stress is a factor in many human diseases either as cause or effect. Any inflammatory condition inevitably leads to an increased oxidative burden since the release of ROS by macrophages is a part of body’s defense system. It is recommended to use a “battery” of measurements, as no single determination of antioxidant status is sufficient. Various assays for total antioxidant capacity (total radical trapping antioxidant potential, trolox equivalent antioxidant capacity, oxygen radical absorbance capacity, and FRA in plasma measure either radical scavenging or reducing capacity. They were devised, or at least have been used, to assess individual antioxidant status, as reflect in body fluids. In this experiment with AA we attempted to clarify the optimal condition for TAS measurements via a Randox kit, namely i) to compare TAS measured in plasma from the RES and from the heart; ii) to monitor TAS during the progress of the disease; iii) to compare the clinical parameter HPV with TAS. We found no differences between TAS data measured in plasma from the RES and from heart (fig. 1). The progress of AA was manifest by decreased TAS already on day 14 in comparison with day 0, and the decreasing trend continued to day 28. In AA animals, the plasmatic level of TAS was 0.49 mmol l⁻¹, which is half of that on day 0, showing an extremely significant drop (fig. 2). The clinical parameter HPV correlated negatively with the measured TAS data both in the arthritic group of animals and the arthritic groups treated with glucomannan and Immunoglukan®. In the latter groups, however, a significant increase in TAS was found. In the AA-IMG group, the HPV was significantly decreased compared to untreated animals, while in the AA-GM group the difference was not significant (fig. 3).

Conclusion

In our further experiments we intend to expand the parameters by measuring not only TAS but also malondialdehyde and its protein adducts. The present study highlighted the importance of TAS as rather helpful parameter for quantification of oxidative stress in AA and for the effect of pharmacological intervention by substances with antioxidant capacity.

This work was supported by the grants: VEGA 2/5051/25, VEGA 1/3442/06, APVV-51-017905, APVV-21-055205.

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P33

KINETICS OF 3-AMINOBENZANTHROLINE OXIDATION BY RAT HEPATIC MICROSOMES

JANA MIZEROVSKÁ a, HELENA DRAČÍNSKÁ a, MARKÉTA MARTINKOVÁ a, JIŘI HUDEČEK a, PETR HODEK a, HEINZ H. SCHMEISER b, VOLKER M. ARLT c, EVA FREI d, MARIE STIBOROVÁ e

a Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Praha 2, Czech Republic, b Section of Molecular Carcinogenesis, Institute of Cancer Research, Surrey SM2 5NG, United Kingdom, c Department of Molecular Toxicology, German Cancer Research Center, 69 120 Heidelberg, Germany, d Nucleosil 100-5 C 18 (Macherey-Nagel, Duren, Germany, stiborov@natur.cuni.cz, janna.mizerovska@seznam.cz

Key words: 3-aminobenzanthrone, 3-nitrobenzanthrone, oxidation, cytochrome P450, HPLC

Introduction

The aromatic nitroketone 3-nitrobenzanthrone (3-nitro-7H-benzde]anthracen-7-one; 3-NBA) is one of the most potent mutagens and a suspected human carcinogen that is found in diesel exhaust and ambient air pollution. We found that 3-NBA is activated by cytosolic and microsomal reductases by simple nitroreduction. Previous work indicated that N-hydroxy-3-aminobenzanthrone (N-OH-ABA) appears that it might be the critical intermediate in 3-NBA-derived DNA adduct formation, which can be further activated by N-acetytransferases (NATs) and sulfotransferases (SULTs). The main metabolite of 3-NBA, 3-aminobenzanthrone (3-ABA), was recently detected in the urine of smoking and nonsmoking salt mining workers occupationally exposed to diesel emissions demonstrating that exposure to 3-NBA can be significant and is detectable. In addition, 3-ABA was evaluated to be suitable for coloration of microporous polyethylene films, or an advantageous fluorescent ratiometer set at 254 nm, and peaks were integrated with a CHROMELLEON 6.01 integrator. The column used was a Nucleosil 100-5 C 18 (Macherey-Nagel, Duren, Germany, 25 cm x 4.6 mm, 5 μm) proceeded by a C-18 guard column. Chromatography was under isocratic conditions of 70 % methanol, with a flow rate of 0.6 ml min⁻¹.

Results and discussion

3-Aminobenzanthrone is oxidized by rat hepatic cytochromes P450 in micromotes to three metabolites. These metabolites were separated by HPLC as distinguish product peaks (fig. 1a). Using co-chromatography with synthetic standards (fig. 1b–d), two of them were identified to be oxidative metabolites of 3-ABA, N-hydroxy-3-ABA [fig. 1c, the retention times (r.t.) of 6.5 min] and 3-NBA (fig. 1d, r.t. of 25 min). The structure of another metabolite eluted with r.t. of 18 min, assigned as M18 (fig. 1a), remains to be characterized.

Because 3-ABA was found to be the most effectively activated by enzymes of a CYP1A subfamily to species forming DNA adducts, kinetics of oxidation of 3-ABA by micromotes isolated from livers of rats treated with β-NF, a known inducer of these CYP enzymes, was analyzed. Oxidation of 3-ABA by hepatic micromotes was dependent on the concentration of this compound (fig. 2) as well as on time of incubation (fig. 3) Time-dependence of 3-ABA oxi-
Chem. Listy 101, s73–s310 (2007)

12. mezioborová česko-slovenská toxikologická konference

Table I
Kinetic parameters for 3-ABA oxidation by β-NF microsomes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$, $\mu$M</td>
<td>51.0</td>
</tr>
<tr>
<td>$V_{max}$, nmol 3-ABA min$^{-1}$ mg$^{-1}$</td>
<td>14.2</td>
</tr>
<tr>
<td>$n$</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Conclusion

The study, showing for the first time identification of two metabolites formed from 3-ABA by CYP-mediated oxidation, suggest the participation of CYP1A enzymes in activation metabolites of 3-ABA forming DNA adducts.

Supported by the GACR (grant 303/05/2195) and the Czech Ministry of Education (grants MSM 0021620808 and 1M4635608802 – Centrum of targeted therapeutics).

Abbreviations

- CYP: cytochrome P450
- DMSO: dimethylsulfoxide
- HPLC: high performance liquid chromatography
- NADPH: nicotinamide adenine dinucleotide phosphate reduced
- NATs: N-O-acetyltransferases
- SULTs: sulfotransferases
- N-OH-ABA: N-hydroxy-3-aminobenzanthrone

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P34

CHRONIC EFFECT OF GARDOPRIM PLUS GOLD 500 SC (S-METOLACHLOR, TERBUTHYLAZINE) ON COMMON CARP (Cyprinus carpio)

HELENA MODRÁa, KAMILA KRUŽÍKOVÁa, RADKA DOBŠÍKOVÁa, ZDEŇKA SVOBODOVÁa, MARKÉTA LICKOVÁb

a University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Dept. of Veterinary Public Health and Toxicology, Palackého 1/3, 612 42 Brno, b Natonale Institute of Public Health, Šrobárova 48, 100 42 Prague 10
modrah@vfu.cz

Key words: bioaccumulation, terbuthylazine, fish, chronic effect

Introduction

Currently used pesticides should be generally less environmentally persistent, that means have shorter half-lives and lower bioaccumulation. For all that, pesticides constitute one of the most hazardous groups of contaminants to human health, fauna and environment in general. s-Metolachlor (chloracetanilides herbicides) and terbuthylazine (chlorotriazine pesticides) are used primarily for control of annual grasses and broadleaf weeds in corn. Monitoring pesticides in agricultural water displays content of terbuthylazine from 0.1 to 4.5 µg l⁻¹ (ref. 2).

Fish are suitable test model for monitoring the effects of contaminated water on the environment.

The aim of our study was to monitor the effect of preparative Gardoprim Plus Gold 500SC containing s-metolachlor (312.5 g l⁻¹) and terbuthylazine (187.5 g l⁻¹) on hematological parameters of common carp (Cyprinus carpio). Another goal was to determine the ability of bioaccumulation of terbuthylazine in the tested fish.

Materials and methods

Exposures

Juvenile common carp (initial weight 5.35±1.75 g) were accidently assigned to five concentrations of tested solutions and a control. Each concentration and control contained 23 fish in 100 l fiberglass aquaria. The test was carried out in a semistatic method, the bath water was exchanged every 48 h. Fish were maintained on a 12 h light: 12 h dark photoperiod. During the test the water temperature varied between 21 to 23 °C, water saturation with oxygen ranged from 60 to 70 % and pH was in range 7.7 to 8.6. The fish were left to adapt to these conditions for three weeks before the beginning the experiment. Then the fish were exposed to concentrations 0.01; 0.1; 1; 5 and 10 mg l⁻¹ of Gardoprim Plus Gold 500 SC for 21 days (which corresponds to concentration of terbuthylazine 1.88; 18.8; 188; 935; 1880 µg l⁻¹). The tested concentrations were selected according to LC₅₀(12.3 mg l⁻¹).

The fish were fed by the dose of 1.5 % of the mean weight of the fish. At the end of the test, blood was sampled by cardiac puncture and the dead fish were frozen (−80 °C).

Blood samples were used for determination of red blood count (RBCC), haematocrit (PCV), haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), leukocyte count (WBCc) and leu-kogramme.

Analysis of chemicals in water

Analysis of s-metolachlor was performed on the gas chromatograph Shimadzu GC 2010 (Shimadzu Handelsgesellschaft mbH) equipped with the autoinjector AOC 20i. The electron capture detector (ECD) was used for detection. Helium was the gas carrier. An Equity²⁵-5 Capillary Column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Supelco, Sigma-Aldrich r.o., CZ) was used. The SPE sorbent Strata C18-E (1000 mg/6 ml) were used for solid phase extraction. Analysis of terbuthylazine was performed on the gas chromatograph Thermo Quest Trace GC with Finnigan Polaris Q detector. The J&W DB-5MS (30 m × 0.25 mm; 0.25 µm) was used for analysis.

Analysis of terbuthylazine in fish homogenate

Analysis of terbuthylazine was performed on the gas chromatograph Thermo Quest Trace GC with Finnigan Polaris Q detector. The J&W DB-5MS (30 m × 0.25 mm; 0.25 µm) was used for analysis. Samples of fish were homogenized and 10 g homogenate was used for analysis. Samples were cleaned with the aid of gel permeation chromatography (GPC) and then analysed on the gas chromatograph with MS detection. The Tukey test was used to compare the values of hematological indices in blood plasma of the control and the experimental groups of fish.

Results and discussion

The Table I shows the effects of Gardoprim Plus Gold 500 SC on hematological indices of common carp. In the two highest concentrations (5 and 10 mg l⁻¹) the fish took the food badly, that is the reason why the statistical differences were noticed in weights between control and the lowest test concentration (0.01 mg l⁻¹). This influence was showed in the final observed length of the fish too. The statistical significant differences were not noticed between groups in haematocrit values and the amount of
erythrocytes. In the test group 1 mg l⁻¹ the number of erythrocytes were not estimated. On the other hand, the effect of Gardoprim Plus Gold 500 SC caused reduction of leukocytes in all the test concentrations. The statistical significant difference was noticed only between control and the highest concentration (10 mg l⁻¹). The reduction of the amount of haemoglobin was established in concentration 1.5 and 10 mg l⁻¹. Differential leukocyte count has lymphocytic characteristics with lower proportion of mature granulocytes (band and segmentes). The statistical significant differences were not noticed between groups in leukogramme.

Effect of experimental terbuthylazine exposure on the cells of Dicentrarchus labrax (L.) was observed³. Treated fish in concentration 3.55–7.08 mg l⁻¹ displayed cellular and ultrastructural alterations in all the examined organs.

The fig. 1 presents contents of terbuthylazine in fish (compound samples from each group). The concentrations of terbuthylazine in fish were 19–27 times higher than in the test solutions. The amount of residues of atrazine over the 48 hours exposition in the carp's liver was only 2.5 times higher than the atrazine concentration in water⁴. It implies that terbuthylazine has better ability to bioaccumulate in fish tissue in comparison with atrazine. Concentrations of terbuthylazine in wild fish have not been monitored yet, although it seems that fish would be a good indicator of pollution of water environment by this substance.

The bioaccumulation of terbutryn increased significantly with increasing water temperature. However, temperature seemed to have no effect on bioaccumulation of terbuthylazine⁵.

**Conclusions**

Gardoprim Plus Gold 500 SC, containing s-metolachlor (312.5 g l⁻¹) and terbuthylazine (187.5 g l⁻¹), is cumulated in tissue of common carp. Highest effect on change of haematological parameters was significant decrease of leukocyte count.

**This study was supported by a grant MSM 621571240.**

**REFERENCE**

THE EFFECT OF CARVEDILOL ON THE OXIDATIVE BURST OF RAT PHAGOCYTES

ANETA MORAVCOVÁa, ANTONÍN LOJEKb, MILAN ČÍZb, JANA PEČIVOVÁa, VIERA JANČINOVÁa, RADOMÍR NOSÁL b

a Institute of Biophysics AS CR, Královovalpolská 135, 612 65 Brno, Czech Republic, b Institute of Experimental Pharmacology SAS, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

moravcova@ibp.cz

Introduction

The production of reactive oxygen species (ROS) by phagocytic cells belongs to basic microbicidal mechanisms of the body. However, extracellularly produced ROS are thought to be involved in the development of a number of pathological conditions and diseases, especially of the cardiovascular system1. Therefore, substances reducing production of or quenching extracellularly produced ROS could be used to prevent adverse effects of ROS.

Carvedilol has been in clinical use as an antihypertensive agent for several years and has been shown to possess antiarrhythmic, antiischemic and cerebroprotective effects2. These effects are suggested to be mediated through nonselective β-adrenoreceptor and α1-adrenoreceptor blockade by carvedilol. However, recent studies described carvedilol as a potent antioxidant3. The antioxidant potential of carvedilol could contribute to the clinically observed positive effects on cardiovascular pathological conditions. For its multifaceted therapeutic potential and for its beneficial antioxidant properties carvedilol itself could substitute the combination of different type of drugs used in the treatment of cardiovascular diseases and decrease the possibility of negative interactions of these agents. Therefore, we studied the potential antioxidant properties of carvedilol. The major aim of the present study was to evaluate the effect of carvedilol on the oxidative burst of blood leukocytes stimulated by various types of activators. Furthermore, direct scavenging effects of carvedilol on various types of ROS generated by cell-free systems were determined.

Materials and methods

Carvedilol [1-(carbazolyl-(4)-oxy)-3-(2-methoxyphenoxyethyl)amino]-2-propanol] (Zentiva, Czech Republic) was dissolved in tartaric acid solution (5·10−4 M) and used in the final concentration range of 1·10−4 – 1·10−7 M. Tartaric acid was tested not to have any effects on the CL signal. All other chemicals were obtained from Sigma-Aldrich (Germany) or local distributors in the highest quality.

Heparinized peripheral blood from Wistar rats was obtained by cardiac puncture as described previously4. Leukocyte rich plasma was prepared by dextran sedimentation. Obtained buffy-coat was washed and isolated leukocytes were resuspended in HBSS.

Luminol-enhanced chemiluminescence (CL) was employed to determine ROS production by blood leukocytes as described previously5,6. The principle of the method is based on luminol interaction with phagocyte-derived ROS, which results in large measurable amounts of light. The CL reaction mixture consisted of isolated leukocytes (1·106 cells ml−1) with or without carvedilol, 1 mM luminol, and one of the receptor-operating activators: N-Formyl-Met-Leu-Phe – fMLP (10−7 M), opsonized zymosan particles – OZP (0.1 g l−1) or one of the receptor-bypassing activators: phorbol myristate acetate – PMA (10−8 M), calcium ionophore A23187 – Ca2+ (5·10−6 M). Spontaneous (non-activated) CL of phagocytes was also measured. The maximum (peak) of the CL response was determined as relative light units (RLU). The scavenging ability of carvedilol was studied in various chemical systems producing individual ROS: peroxyl-radical produced by thermal decomposition of ABAP [2,2’-azo-bis(2-amidinopropan)], superoxide anion produced by hypoxanthine (1 mg ml−1)/xanthine oxidase (0.1 U ml−1) system, hydroxyl radical produced by hydrogen peroxide (2 mM)/ferrous sulphate (1 mM) system and hydrogen peroxide itself (2 mM).

All CL measurements were performed in a microtiter plate luminometer LM-01T (Immunootech, Czech Republic) and in a cuvette luminometer 1251 (Bioorbit, Finland).

Data are expressed as the mean ± standard error of the mean (SEM) of twelve different experiments. The assays were run in duplicates. Results were analyzed by ANOVA, followed by the Student’s t-test.

Results

Carvedilol dose-dependently decreased not only the CL of nonactivated leukocytes (data not shown) but also the CL of leukocytes activated by all used activators. The most efficient inhibition of CL by carvedilol was observed in leukocytes stimulated by fMLP and Ca2+ (fig. 1). Carvedilol dose-dependently scavenged individual ROS. Carvedilol most efficiently inhibited the CL signal generated by hydrogen peroxide and hydroxyl radical and to a lesser extent also by superoxide anion (Table I).

Discussion

All used activators are able to stimulate cells to generate ROS via various mechanisms. fMLP and OZP...
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The effect of carvedilol on the CL of isolated leukocytes activated by fMLP (a), OZP (b), CaI (c) or PMA (d)

Table I
Direct scavenging effect of carvedilol against individual ROS. Asterisks show the statistical significance against control at $P \leq 0.05$ (*) and $P \leq 0.01$ (**)³.

<table>
<thead>
<tr>
<th>Concentration of carvedilol</th>
<th>Peak CL [% of control]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>superoxide radical</td>
</tr>
<tr>
<td>Control</td>
<td>100 ± 0.02</td>
</tr>
<tr>
<td>$10^{-8}$ M</td>
<td>105 ± 0.77</td>
</tr>
<tr>
<td>$10^{-7}$ M</td>
<td>97 ± 0.51</td>
</tr>
<tr>
<td>$10^{-6}$ M</td>
<td>100 ± 0.02</td>
</tr>
<tr>
<td>$10^{-5}$ M</td>
<td>105 ± 0.19</td>
</tr>
<tr>
<td>$10^{-4}$ M</td>
<td>76 ± 0.12**</td>
</tr>
</tbody>
</table>

Carvedilol was shown to be a direct dose-dependent scavenger of hydroxyl radical, hydrogen peroxide and superoxide radical in our experiments. The antioxidant properties of carvedilol and some of its metabolites, which are not observed in most of other beta-adrenoceptor antagonists, are attributed to the presence of a carbazole moiety in the drug molecule.

This study was carried out within a research plan AVOZ50040507 and was supported by a grant No. 524/07/1511 of the Grant Agency of the Czech Republic.

REFERENCES