

## PYRAZOLE AND METHYLPYRAZOLE FOR THE TREATMENT OF 2-BUTOXYETHANOL POISONING

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**Abstract:** 2-Butoxyethanol (BE) is a one member of a family of ethylene glycol monoalkyl ethers that are used in a variety of industrial and household products. The clinical features of human and animal BE intoxications mainly include metabolic acidosis, CNS depression and coma, hemolytic anemia, hematuria, and renal injury. It is believed that metabolic activation of BE to butoxyacetic acid (BAA) is responsible for these pathologic changes. The treatment of BE poisoning have been based on an inhibition of the metabolic pathway enzymes which convert BE to toxic metabolites. Therefore, a comparison was made between antidotal properties of pyrazole (PY) and 4-methylpyrazole (MP) in rats subcutaneously intoxicated with BE. It was found that both antidotes effectively protected animals against appearance of hemolytic anemia signs induced by BE. MP appears to be more efficient than PY. These data confirm the beneficial role of alcohol dehydrogenase (ADH) inhibitors in BE intoxication.

**Keywords:** ethylene glycol monoalkyl ethers, poisoning, intravascular hemolysis, pyrazole, methylpyrazole, treatment.

Ethylene glycol monoalkyl ethers (EGMEs), such as 2-butoxyethanol (BE), are a major class of industrial chemicals extensively used in the manufacture of a wide range of industrial and domestic products (1).

These chemicals are readily absorbed to the organism following inhalation, oral, and dermal exposure (2-4). EGMEs are metabolized primarily via alcohol (ADH) and aldehyde (ALDH) dehydrogenases with the formation of appropriate alkoxyacetaldehydes and alkoxyacetic acids (3, 5). In addition, parent compounds are partially conjugated with both glucuronic and sulfuric acids. These latter metabolic pathways are alternative for oxidation of this chemicals (3).

EGMEs cause a wide range of toxic effects in humans and laboratory animals including reproductive and developmental toxicity, as well as hematotoxicity and nephrotoxicity. Alkoxyacetic acids are considered to be the proximate toxic metabolites. Poisoning with EGMEs cause clearly marked metabolic acidosis and intravascular hemolysis as a primary effects (6-9) and renal failure as a secondary effect (10).

Methanol and ethylene glycol poisonings are generally treated with the combination of ethanol as antidote, and hemodialysis. Fomepizole (4-methyl-

pyrazole, MP), a competitive inhibitor of ADH, has more recently been used for the treatment of methanol and ethylene glycol intoxications due to its capability of blocking the toxic metabolism of these chemicals (11, 12). Also, MP is used in cases of severe disulfiram-ethanol reactions. The signs of these reactions are tachycardia, hypotensia and ECG disturbances of repolarization and also myocardia ischemia as a complication (13). There are few data indicating that MP may be powerful for the treatment of EGMEs poisoning (14, 15).

The current investigation was undertaken to compare the efficiency of both pyrazole (PY) and MP in male rats acutely intoxicated with BE. The intravascular hemolysis induced by BE as a critical effect was taken under consideration.

### EXPERIMENTAL

#### Chemicals

BE, PY, and MP were purchased from Sigma-Aldrich Ltd., Poland. Other chemicals were supplied by commercial firms operating in Poland.

#### Animals

Male Wistar Krf: (WI) WU rats (12-14 weeks old) obtained from the Jagiellonian University

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\*\* The co-author is a scholar of the project which is co-financed from the European Social Fund and national budget in the frame of The Integrated Regional Operational Program.

Faculty of Pharmacy Breeding Laboratory (Kraków, Poland) were maintained on standard diet (Murigran, Poland) and water *ad libitum*, and allowed a minimum 7 days acclimatization to appropriate facilities (12 h dark/light period, 20-23°C ambient temperature, and 40-60% relative humidity) prior to inclusion in the present experiment. In this experiment the Polish law on the protection of animals was followed (16).

### Experimental design

BE, PY, and MP solutions were prepared immediately before dosing by mixing these chemicals with 0.9% saline to obtain a dose volume of 2 mL/kg body weight (b.w.), and were administered to rats by subcutaneous (BE) and intraperitoneal (PY and MP) injection, respectively. Rats were randomly assigned to groups of 5 animals each. Rats in one group were treated with BE alone at a single dose of 1.25 mmol/kg b.w. The animals in other groups, beside the same dose of BE, received PY or MP at a single dose of 0.045, 0.09, 0.18 or 0.36 mmol/kg b.w. These antidotes were injected simultaneously, 2 h or 5 h after BE administration. Control animals received 2.0 ml of 0.9% saline and served as vehicle control.

### Hematologic analysis

At the end of the required period after BE administration, i.e. 0, 3, 6, 24, and 48 h, blood samples from end tail vein of rats were collected into heparinized test-tubes. Blood samples were analyzed by means of hematologic analyzer Cobas Micros ROCHE. The following indices were measured: red blood cells (RBCs) and reticulocyte counts, packed cell volume (PCV), mean cell volume (MCV), total hemoglobin concentration (HGB), plasma hemoglobin concentration (HGB<sub>p</sub>), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). Hematologic analyses were systematically checked by means of standard human blood CBC-3D Hematology Control (R & D System Inc., Minneapolis, USA). A day-to-day precision of measurement of RBC, PCV, and HGB in blood was 4.2%, 4.5%, and 7.2% (n = 30), respectively.

### Statistical analysis

All results are expressed as mean  $\pm$  S.D. of values obtained in five individual rats. The results were statistically evaluated by means of one-way ANOVA followed by Tukey's test or by unpaired t-test as appropriate after assessment of a normal distribution of variables by means of Shapiro-Wilk's W

tests. The regression equations and correlation coefficients were calculated with the STATISTICA – version 6.0 computer program.

## RESULTS

Subcutaneous injection of BE to rats at a single dose of 1.25 mmol/kg b.w. caused distinct intravascular hemolysis expressed by the reduction in the RBC count, decrease in the HGB concentration and increase in the free HGB in plasma and rise in the reticulocyte numbers in peripheral blood. All hematologic parameters demonstrated time dependence. Both RBC count and HGB concentration gradually decreased, whereas reticulocyte numbers increased with time after dosing. The maximum value of free HGB in plasma occurred at 6 h after BE administration (Figure 1). RBC count and HGB concentration correlated well with time of the experiment.

Both PY and MP administered simultaneously with BE protected rats against appearance of hemolytic anemia signs. PY at the doses of 0.09, 0.18, and 0.36 mmol/kg b.w. completely abolished the hemolytic effect of BE, whereas at the lowest dose (0.045 mmol/kg b.w.) only partially exerted protective effect (Figure 1).

MP at each dose level (0.045-0.36 mmol/kg b.w.), injected to rats simultaneously with BE, acted against hematotoxicity of BE (Figure 2). Both PY and MP at highest dose level (0.36 mmol/kg b.w.), given 2 or 5 h after BE administration, did not protect rats against hemolytic anemia signs (Figure 3 and 4). The lack of protection effect of PY and 4-MP in these rats was also expressed by the negative correlation between RBC count or HGB concentration and time of the experiment (Table 1).

## DISCUSSION

BE is readily absorbed from skin, and both respiratory and gastrointestinal tracts. This chemical is metabolized in rats by three pathways in the liver, skin, and testes (14, 16, 17). The major metabolic pathway is via ADH and ALDH to the primary metabolite butoxyacetic acid (BAA) (14, 17, 19). BAA is then excreted in the urine (8, 14). This metabolite is believed to be responsible for the hematotoxic manifestations of BE poisoning. The other two pathways involve BE conjugation with glucuronic acid and sulfate. These two pathways are believed to be primary detoxifying metabolism of the parent compound (14). The fraction of BE converted to the glucuronide and sulfate conjugates has

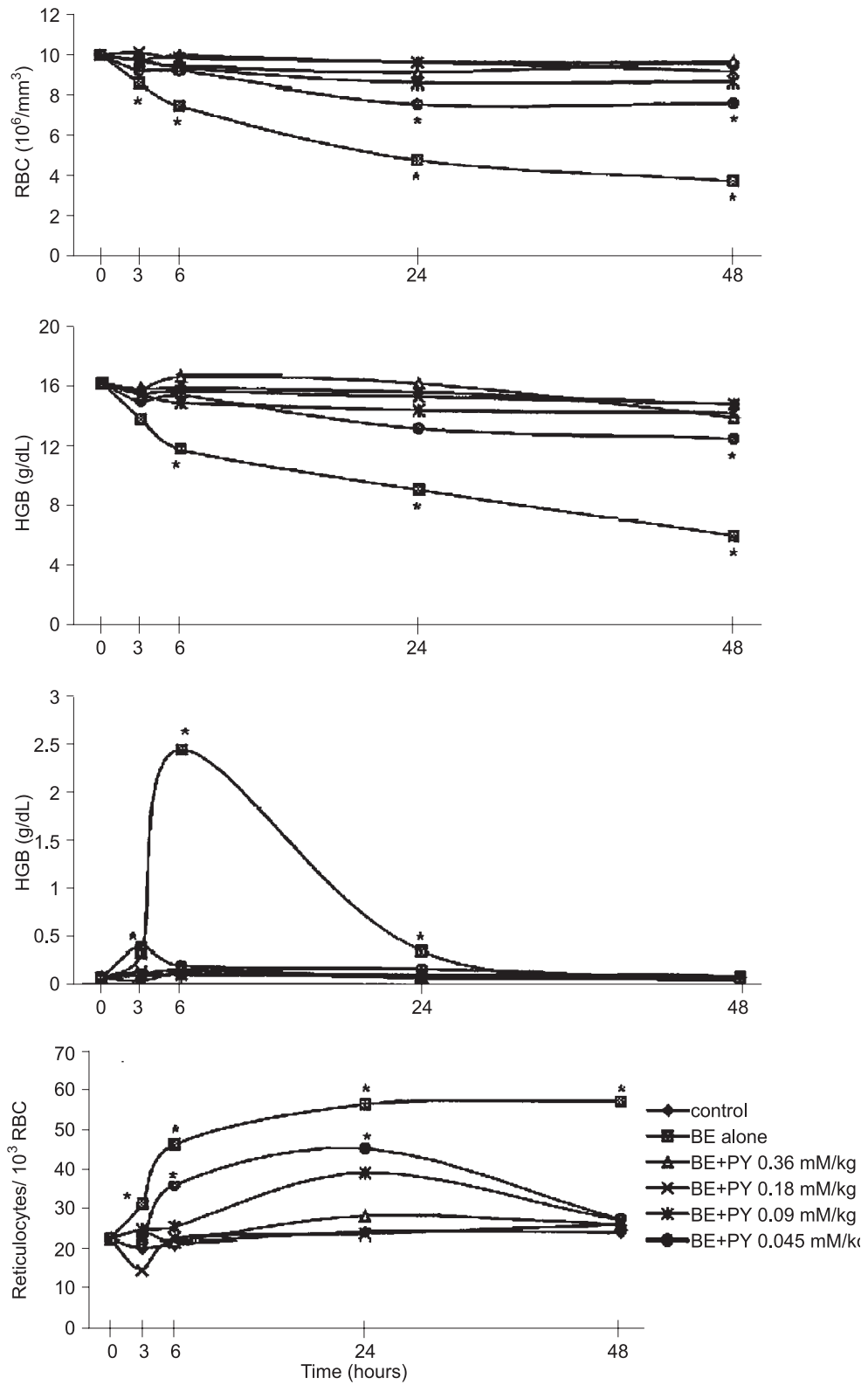


Figure 1. Time-course (for 48 h) of the effects of BE alone at the dose level of 1.25 mmol/kg b.w. and its combination with PY at the dose levels of 0.045-0.36 mmol/kg b.w. on RBC, HGB, HGB<sub>p</sub>, and reticulocyte counts after simultaneous administration. \*The values significantly different from control group.

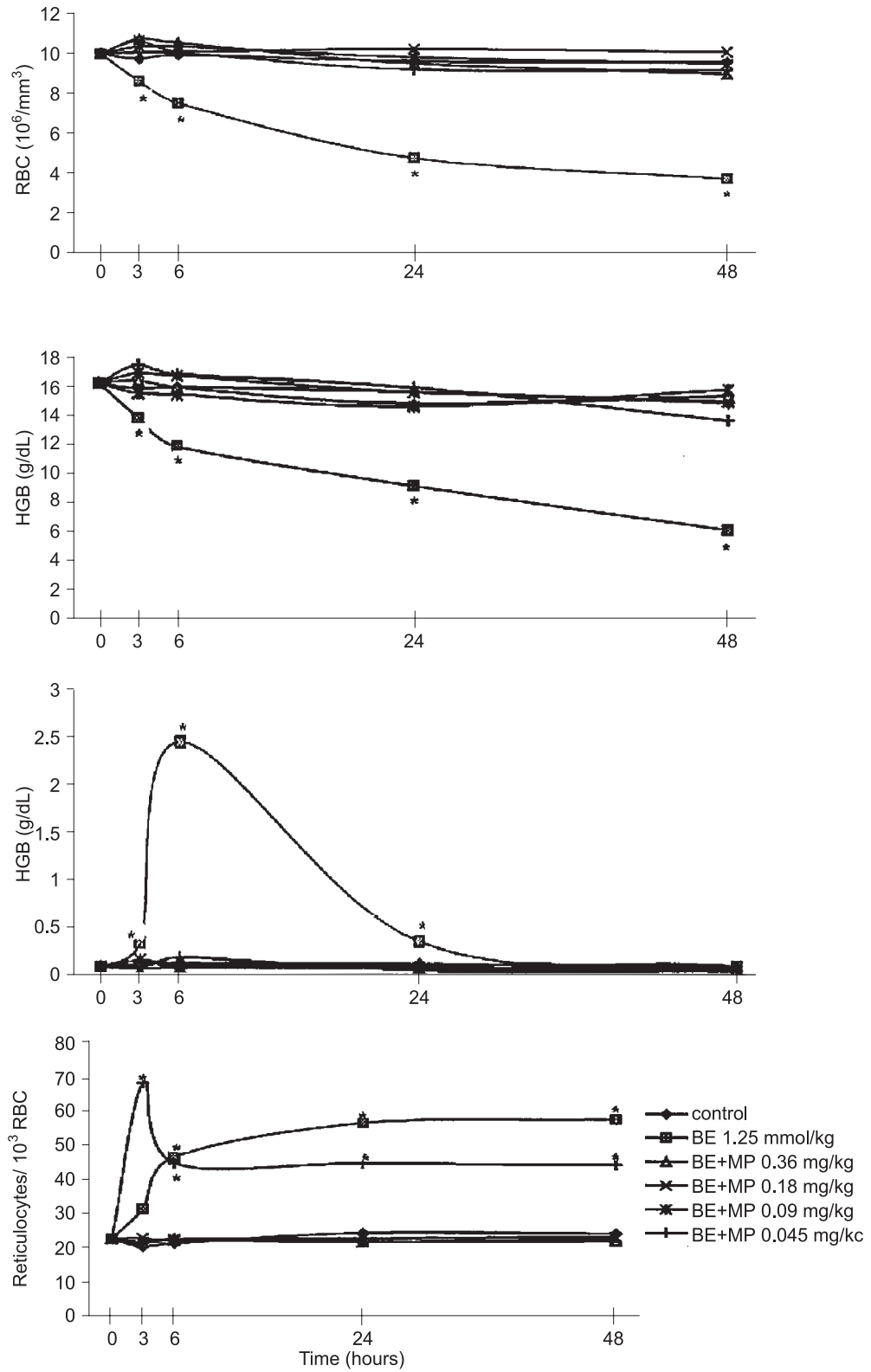


Figure 2. Time-course (for 48 h) of the effects of BE alone at the dose level of 1.25 mmol/kg b.w. and its combination with MP at the dose levels of 0.045-0.36 mmol/kg b.w. on RBC, HGB, HGB<sub>p</sub>, and reticulocyte counts after simultaneous administration. \*The values significantly different from control group.

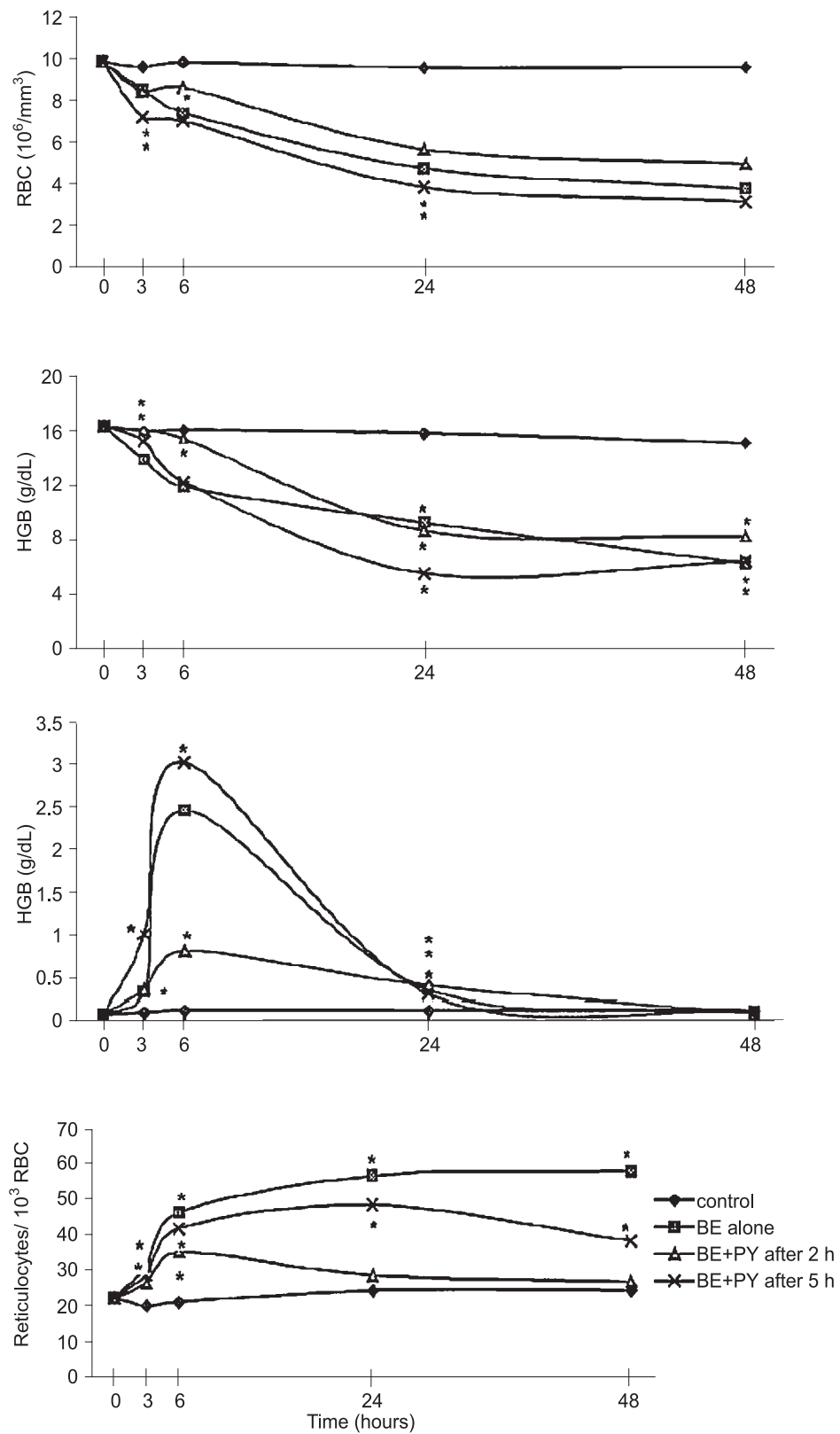


Figure 3. Time-course (for 48 h) of the hematologic effects of BE (1.25 mmol/kg b.w.) alone and its combination with PY (0.36 mmol/kg b.w.) administered 2 or 5 h later. \*The values significantly different from control group.

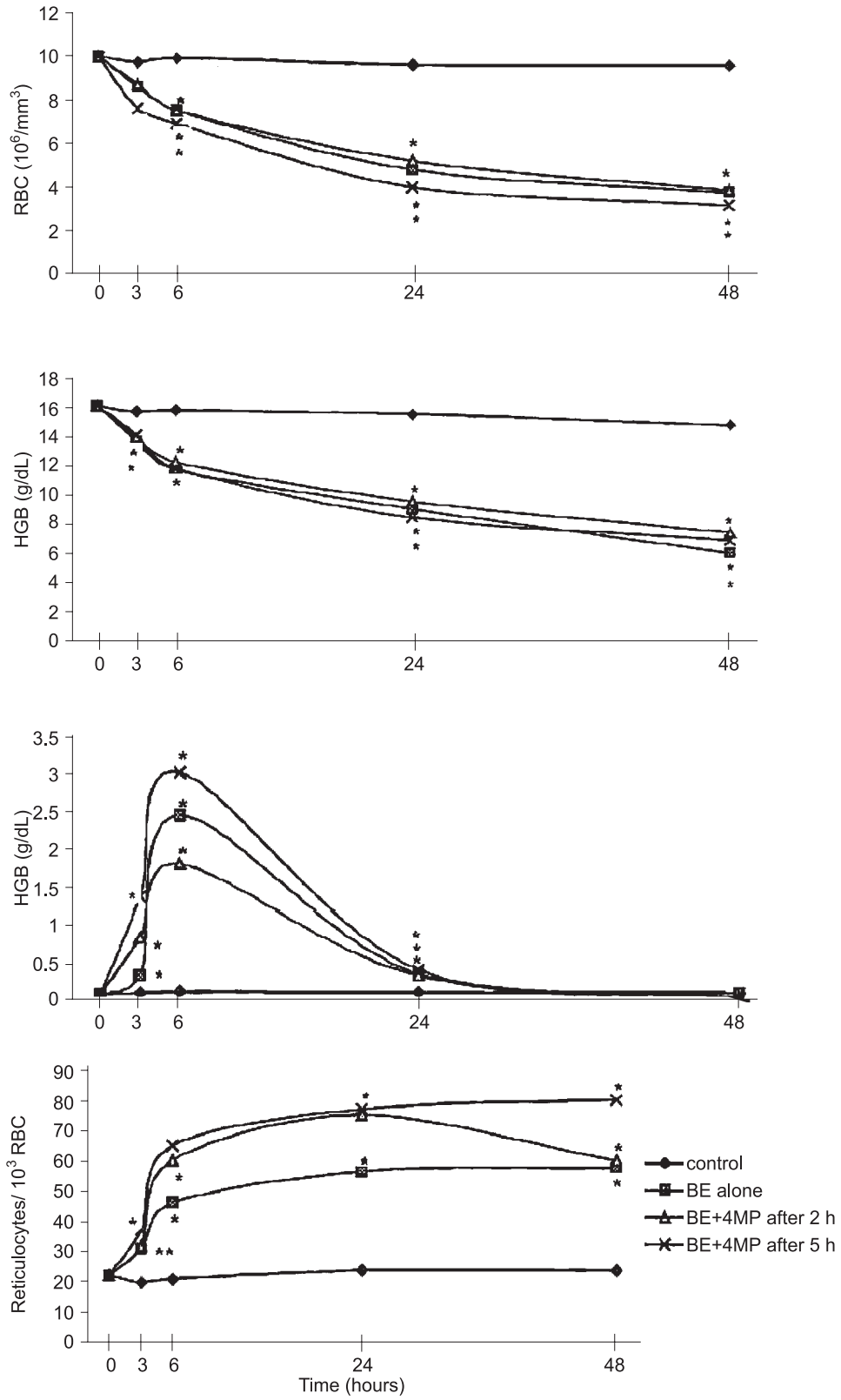


Figure 4. Time-course (for 48 h) of the hematologic effects of BE (1.25 mmol/kg b.w.) alone and its combination with MP (0.36 mmol/kg b.w.) administered 2 or 5 h later. \*The values significantly different from control group.

been shown to increase significantly after inhibition of BE metabolism to BAA by PY or ethanol (14).

The occurrence of intravascular hemolysis in animal models of BE poisoning has been well evidenced. *In vivo* and *in vitro* studies have ascribed a potent hemolytic activity to BAA but not to BE (20, 21). Hemolytic activity of BAA leads to a decrease in the number of circulating RBC and HGB concentration, and an increase in the concentration of free hemoglobin in plasma of peripheral blood. Subsequently, reticulocyte counts considerably increase as a result of regenerative process (8, 14, 15). Although human erythrocytes are resistant to the hemolytic action of BAA, even at concentrations several times higher than those producing hemolysis in rats (20, 22), some reports described a decline in number of RBC, hemoglobin concentration in blood and hematuria in humans following suicidal ingestion of BE or occupational exposure to this chemical (9, 23-25).

Treatment of BE poisoning have been based on the hypothesis that block of the metabolic activation of this compound prevents organism against its toxic effects. Data in the available literature indicate that inhibition of ADH and ALDH with PY and cyanamide (ALDH-inhibitor), respectively, prevented animals treated with BE at toxic doses against hematotoxicity (14, 15). Other animal studies have shown that ethanol therapy may prevent BE-induced hemolysis by competitive inhibition of its metabolism (15, 26). These data confirm the role of metabolic activation in BE hematotoxicity.

The results obtained in the present study indicate that both PY and MP exert distinct protective effect in rats subcutaneously treated with BE. These antidotes administered simultaneously with BE protected animals against onset of hemolytic anemia signs. This protection effect was dose-dependent. PY at doses of 0.09, 0.18 and 0.36 mmol/kg b.w., similarly as the MP at each dose level (0.045-0.36 mmol/kg b.w.), completely abolished the hemolytic action of BE. PY at the lowest dose (0.045 mmol/kg b.w.) only partially exerted protection effect in the rats treated with single dose of BE. Both PY and MP injected intraperitoneally 2 or 5 h after BE administration did not protect rats against onset signs of hemolytic anemia. The correlations between RBC counts or HGB concentration and time of the experiment confirmed the lack of a protective effect of these antidotes in rats intoxicated with BE. The reason for the lack of efficiency of these antidotes is mainly toxicokinetics of BE. BE is rapidly metabolized to BAA with its maximal levels in peripheral blood at 10-30 min after intraperitoneal or intravenous BE administration (27, 28).

The data obtained in the present study suggest that PY and MP may decrease the metabolic consequences of BE poisoning and may be of therapeutic value when administered early during the course of the intoxication before hemolysis have occurred. This suggestion is consistent with literature data which indicate that MP is a potent competitive inhibitor of ADH, especially ADH1 and ADH2 in rat liver and ADH1, ADH3 and ADH4 in rat skin (17). Additionally, MP in combination with hemodialysis or without hemodialysis is safe and effective in treatment of confirmed ethylene glycol or methanol poisoning (29, 30). During ethanol intoxication, in humans given MP a 30-40% decrease in the ethanol elimination rate was observed (31). It was found that the inhibition of ethanol metabolism by pretreatment with MP may lead to prolongation of its neurobehavioral toxicity in mice (32).

In conclusion, it is clear that BE is a strong hemolytic agent after metabolic activation by ADH and ALDH. Both PY and MP, as selective inhibitors of ADH, may decrease the hemolytic effect of BE and may be of therapeutic value when administered early during the course of intoxication by this chemical.

#### Acknowledgment

This study was supported by a KBN Grant No. WPR-002/CIOP/2005, Research Task No. 2.A.08.

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*Received: 7.06.2006*

### Erratum to *Acta Pol. Pharm. Drug Res.* Vol. 63, No. 5:

in the pages "Contents" (327-328) the following corrections should be made:

p. 391 author's name should be Elżbieta L. Anuszevska

p. 452 in the title should be: bortezomib (Velcade®)

addition: p. 465. Monika Grudzień, Franciszek Pluciński, Aleksander P. Mazurek

Molecular properties of econazole and sulconazole relevant to bioavailability. (the title and authors names should be also added in the Index for vol. 63, 2006)