INFLUENCE OF ACETYLSALICYLIC ACID ON HEMATOTOXICITY OF BENZENE

JOANNA KOWALÓWKA-ZAWIEJA, BARBARA ZIELIŃSKA-PSUJA, JĘDRZEJ PRZYSTANOWICZ, and KARINA SOMMERFELD

Poznan University of Medical Sciences, Poznań, Poland
Department of Toxicology

Abstract
Objectives: The aim of the study was to evaluate the influence of acetylsalicylic acid (ASA) on benzene hematotoxicity in rats. Materials and Methods: The study was carried out on rats exposed for 2, 4 and 8 weeks to benzene vapour at a concentration of 1.5 or 4.5 mmol/m$^3$ of air (5 days per week, 6 hours per day) alone or together with ASA at the doses of 5, 150 or 300 mg/kg body weight (per os). Results: Benzene at a concentration of 4.5 mmol/m$^3$ caused a slight lymphopenia, granulocytosis and reticulocytosis in blood. In bone marrow traits of megaloblastic renewal, presence of undifferentiated cells and giant forms of granulocytes as well as an increase in myeloperoxidase and decrease in chloroacetate esterase activity and lipids content were noted. ASA (150 and 300 mg/kg b.w.) influenced some of hematological parameters, altered by benzene intoxication. ASA limited the solvent-induced alteration in blood reticulocyte count and in the case of bone marrow in the erythroblasts count. Traits of megaloblastic renewal in bone marrow were less pronounced. Besides, higher activity of myeloperoxidase and the decrease in the level of lipids in granulocytes were noted. Conclusion: Our results suggest that ASA limited the benzene-induced hematotoxicity.

Key words: Benzene, Acetylsalicylic acid, Interactions, Hematotoxicity, Blood, Bone marrow

INTRODUCTION

Occupational exposure to benzene is a frequent cause of chronic toxicity, which may result in induction of aplastic anemia and neoplastic processes, including leukemias, as well as breast and lung tumors. Proliferative disorders of the hemopoietic system, which most frequently develop in humans exposed to benzene, include chronic myeloid leukemia, acute myeloid leukemia, lymphoblastic leukemia, malignant lymphoma and multiple myeloma. Development of tumors of the hemopoietic system reflects the damage to bone marrow pluripotent stem cells, which leads to anemia, leukopenia or thrombocytopenia and, then, to fully symptomatic aplastic anemia or myeloid leukemia [1,2].

The mechanism of benzene hematotoxicity is not clear yet. One theory stresses the importance of active benzene metabolites and cellular DNA adducts, particularly inherited in somatic cell lines, which cause inability of the cells to react to cytokines, resulting in excessive proliferation. Chromosomal aberrations induced by binding active benzene metabolites to DNA may also lead to oncogenes activation or antioncogenes inactivation [3–5].

Another hypothesis is associated with stimulatory functions of the bone marrow microenvironment. Cytotoxic damage of bone marrow stromal cells and macrophages, in particular induced by benzene and its metabolites, affects their capacity to control proliferation and differentiation.
of stem cells. Failure to synthesize normal signaling polypeptides results in uncontrolled proliferation of cells which would otherwise be inhibited or the cells would be forced to differentiate into individual cell lines. In a similar way, a disturbed immune system including lymphocytes and stromal macrophages may also lead to development of neoplastic cells, which in physiological conditions would be detected and selected out by the immune surveillance mechanisms [6–10].

Hematotoxic activity of benzene involves both, the solvent and its metabolites. Induction of the cytochrome P-450 system, especially CYP 2E1 and CYP 2B1, which participates in metabolic activation of benzene, may lead to intensification of the observed alterations [11–13]. It has been demonstrated that acetylsalicylic acid (ASA), a widely used non-steroid anti-inflammatory drug, induces cytochrome P-450. Therefore, its prolonged administration for the purpose of prevention of cardiac and cerebral infarcts may lead to metabolic interactions with other xenobiotics, including benzene [14–16].

ASA may also modify toxic effects of benzene by inhibition of transcription factor κB, which participates in activation of genes involved in synthesis of inflammatory factors as well as being responsible for proliferation and maturation of T lymphocytes [17,18]. Hydroquinone, a benzene metabolite, exhibits a similar activity – it inhibits transcription and translocation of the κB factor to the cell nucleus by suppression of the translocation pathway composed of protein kinase B (Akt), kinase IκBβ (IKKβ) and inhibitor α of κB protein (IκBα) [10,19,20].

Secondary activation of benzene metabolites in bone marrow to the highly reactive compounds is paralleled by formation of free radicals and is responsible for cell membrane damage and genotoxicity [3,5,21]. ASA-induced inhibition of prostaglandin G2 transformations, resulting from repression of endogenous synthase, may lower activity of macrophage peroxidase and, thus, the capacity to form active metabolites [22–24].

ASA inhibits inflammatory processes by prostaglandin and nitric oxide production, which may result in reduction of hematotoxic effects of benzene [17,22,25].

The aim of our study was to investigate the effect of oral acetylsalicylic acid administration on hematotoxicity of benzene after inhalation exposure.

**MATERIALS AND METHODS**

**Animals**

The studies were performed on male Wistar rats. The animals originated from the breeding farm of the Department of Toxicology, Poznan University of Medical Sciences. The body weight of each rat was 230±15 g. The rats were housed in controlled light conditions (12 h light : 12 h dark), at 22°C and relative humidity of 60±10%. The animals were fed with standard laboratory chow with free access to tap water.

**Treatment protocols**

The animals were subjected to inhalatory exposure to benzene (Sigma-Aldrich) vapour at a concentration of 1.5 mmol/m³ (37 ppm) or 4.5 mmol/m³ (112 ppm). The exposure took place in a dynamic toxicological chamber for 5 days a week, 6 h every day, for a period of 2, 4 or 8 weeks. Concentration of the solvent inside the toxicological chamber was monitored by gas chromatographic analysis of air samples with 30 min intervals (packed column Supelco: 5% DIDP/5% Bentone 34 on 80/100 Chromosorb W NAW, 6’×1/8” SS, column temperature 80°C, carrier gas flow 20 cm³/min, FID detector, sample volume 10 cm³). Before each exposure, the rats received an aqueous suspension of ASA (Sigma-Aldrich) administered p.o. at a dose of 5 mg/kg b.w. (1/300 DL₅₀) or 150 mg/kg b.w. (1/10 DL₅₀) or 300 mg/kg b.w. (1/5 DL₅₀). On the 3rd day after exposure cycle termination, blood was sampled from the heart of superficially anesthetized rats. In the blood, hemoglobin concentration, hematocrit
value and number of reticulocytes (Ret) and leukocytes were established. Peripheral blood and bone marrow smears were evaluated after staining by the use of the May-Grünwald-Giemza (MGG) technique. Leukocyte alkaline phosphatase (LAP) activity in the smears of blood and the activity of acid phosphatase (ACP), myeloperoxidase (MPO), naphthol AS-D chloroacetate esterase (CAE), α-naphthyl acetate esterase (ANAE) and the content of lipids (reaction with Sudan Black B), and mucopolysaccharides (PAS reaction) were evaluated in smears of the bone marrow using standard methods described in Wintrobe’s Clinical Hematology 11th Ed [26]. Assessment of reactions for LAP, ACP, MPO, CAE and reactions with Sudan Black B involved a semi-quantitative technique, i.e. 100 granulocytes were counted, classifying the enzyme activity in every cell on a 5-grade scale:

- 0 – negative reaction,
- I – weakly positive reaction,
- II – positive reaction (numerous fine or single coarse granules),
- III – positive reaction (numerous coarse granules),
- IV – strongly positive reaction (coarse granules filling the whole cell).

The results were given in the score representing the sum of products of granulocyte count and the number of a given class. Activities of α-naphthyl acetate esterase and mucopolysaccharide content were expressed as the percentage of cells with a positive reaction. All experimental procedures were approved by the Local Ethical Committee for Experiments on Animals (3/2004).

Statistical analysis
When normal distribution of the experimental data was established using the Kolmogorov test, significant differences (p < 0.05) between experimental groups were evaluated using ANOVA-test. If ANOVA detected significant differences (p < 0.05), Dunnett’s test was used to compare the experimental groups to the control groups. Time impact on inter-group differences was tested by the use of the interactive Tukey’s test. The results of blood and bone marrow smears, which did not follow normal distribution, were tested using the non-parametric Kruskal-Wallis or Mann-Whitney U tests.

RESULTS
Red blood cell system
Hemoglobin concentration
Two weeks of exposure to benzene at concentrations of 1.5 mmol/m³ and 4.5 mmol/m³ decreased or increased blood hemoglobin level by about 11%, respectively (Table 1). In the rats, which were subjected to 4 and 8 week exposure to benzene, hemoglobin level resembled that noted in the control group. In the rats treated with the highest dose of ASA (300 mg/kg b.w.) for 2 weeks, blood hemoglobin concentration was elevated (by about 16%). No effect of the lower doses of ASA was detected. Parallel exposure to both xenobiotics did not affect hemoglobin levels.

Hematocrit
Benzene and ASA did not change hematocrit value, irrespective of the type and duration of the experiment.

Reticulocyte count
Blood reticulocyte number was increased after 2 weeks of inhalatory exposure to benzene at a concentration of 4.5 mmol/m³ and reached a maximum (around 98‰) after 4 weeks of exposure (Table 1). In the same period, elevated Ret levels were also noted in the group of animals exposed to lower concentrations of the solvent. None of the examined ASA doses affected Ret levels. However, in the rats exposed to benzene and ASA for the period of 4 weeks, Ret level was lower than in the rats exposed to benzene alone. After 8 weeks of exposure, Ret levels in all experimental groups resembled the level determined in the control group.
Table 1. Hematological parameters in the control rats and the rats exposed to benzene alone or in combination with acetylsalicylic acid

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Hemoglobin (mg/dm$^3$)</th>
<th>Reticulocytes (%)</th>
<th>Leukocytes ($10^3/\mu l$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$±SD</td>
<td>$\bar{x}$±SD</td>
<td>$\bar{x}$±SD</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>4 weeks</td>
<td>8 weeks</td>
</tr>
<tr>
<td><strong>Control group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.5±0.5</td>
<td>15.5±0.2</td>
<td>15.8±0.3$^a$</td>
</tr>
<tr>
<td><strong>Study groups</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA 5 mg/kg b.w.</td>
<td>14.2±0.3</td>
<td>15.2±0.6</td>
<td>15.0±0.4</td>
</tr>
<tr>
<td>ASA 150 mg/kg b.w.</td>
<td>14.9±0.4</td>
<td>15.6±0.2</td>
<td>15.3±0.3</td>
</tr>
<tr>
<td>ASA 300 mg/kg b.w.</td>
<td>16.9±0.9$^a$</td>
<td>16.0±0.8</td>
<td>16.0±0.8</td>
</tr>
<tr>
<td>Benzene 1.5 mmol/m$^3$</td>
<td>12.9±0.4$^a$</td>
<td>15.7±0.6$^a$</td>
<td>15.2±0.7$^a$</td>
</tr>
<tr>
<td>Benzene 1.5 mmol/m$^3$, ASA 5 mg/kg b.w.</td>
<td>13.4±0.7</td>
<td>14.7±0.4$^a$</td>
<td>15.2±0.4$^a$</td>
</tr>
<tr>
<td>Benzene 1.5 mmol/m$^3$, ASA 150 mg/kg b.w.</td>
<td>13.9±0.7</td>
<td>14.2±0.9$^a$</td>
<td>15.6±0.4$^a$</td>
</tr>
<tr>
<td>Benzene 4.5 mmol/m$^3$</td>
<td>16.1±1.0$^a$</td>
<td>17.1±0.6$^a$</td>
<td>16.2±0.5</td>
</tr>
<tr>
<td>Benzene 4.5 mmol/m$^3$, ASA 5 mg/kg b.w.</td>
<td>15.3±0.6$^d$</td>
<td>15.7±0.3$^d$</td>
<td>16.1±0.4$^c$</td>
</tr>
<tr>
<td>Benzene 4.5 mmol/m$^3$, ASA 150 mg/kg b.w.</td>
<td>15.8±0.8</td>
<td>15.8±0.6</td>
<td>16.2±0.6</td>
</tr>
</tbody>
</table>

Each study group consisted of 6 rats.
$x$ – mean; SD – standard deviation.
$^a$ As compared to the respective control group, difference significant at $p < 0.05$.
$^b$ As compared to the respective benzene group, difference significant at $p < 0.05$.
$^c$ As compared to the respective 2-week group, difference significant at $p < 0.05$.
$^d$ As compared to the respective 4-week group, difference significant at $p < 0.05$.
$^e$ As compared to the respective ASA group, difference significant at $p < 0.05$.
$^f$ As compared to the benzene group exposed to 1.5 mmol/m$^3$ versus 4.5 mmol/m$^3$, difference significant at $p < 0.05$.

Table 2. Activities of leukocyte alkaline phosphatase (LAP) in peripheral blood, acid phosphatase (ACP), myeloperoxidase (MPO) and naphthol AS-D chlороacetate esterase (CAE) in bone marrow cells of the control rats and the rats exposed to benzene alone or in combination with acetylsalicylic acid

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>LAP (score)</th>
<th>ACP (score)</th>
<th>MPO (score)</th>
<th>CAE (score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$±SD</td>
<td>$\bar{x}$±SD</td>
<td>$\bar{x}$±SD</td>
<td>$\bar{x}$±SD</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>4 weeks</td>
<td>8 weeks</td>
<td>2 weeks</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>184±9</td>
<td>182±14</td>
<td>188±15</td>
<td>198±14</td>
</tr>
<tr>
<td><strong>Study groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA 5 mg/kg b.w.</td>
<td>199±10</td>
<td>176±7</td>
<td>192±13</td>
<td>200±15</td>
</tr>
<tr>
<td>ASA 150 mg/kg b.w.</td>
<td>214±21</td>
<td>169±11$^a$</td>
<td>198±12</td>
<td>188±23</td>
</tr>
<tr>
<td>ASA 300 mg/kg b.w.</td>
<td>217±18</td>
<td>160±15$^a$</td>
<td>218±22</td>
<td>165±10$^a$</td>
</tr>
<tr>
<td>Benzene 1.5 mmol/m$^3$</td>
<td>183±15</td>
<td>170±11</td>
<td>198±12</td>
<td>194±14</td>
</tr>
</tbody>
</table>

$^a$ As compared to the respective control group, difference significant at $p < 0.05$.
Table 2. Activities of leukocyte alkaline phosphatase (LAP) in peripheral blood, acid phosphatase (ACP), myeloperoxidase (MPO) and naphthol AS-D chloroacetate esterase (CAE) in bone marrow cells of the control rats and the rats exposed to benzene alone or in combination with acetylsalicylic acid – cont.

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>LAP (score) x±SD</th>
<th>ACP (score) x±SD</th>
<th>MPO (score) x±SD</th>
<th>CAE (score) x±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
<td>4 weeks</td>
<td>8 weeks</td>
<td>2 weeks</td>
</tr>
</tbody>
</table>
| Benzene 1.5 mmol/m$^3$, ASA 5 mg/kg b.w. | 193±12 | 166±16 | 194±10 | 190±9 | 192±11 | 242±14 | 226±18 | 240±22 | 256±8 | 169±13 | 173±7 | 178±13
| Benzene 1.5 mmol/m$^3$, ASA 150 mg/kg b.w. | 193±14 | 170±12 | 198±8 | 194±14 | 199±12 | 226±12 | 233±21 | 219±19 | 246±19 | 129±13 | 154±9 | 167±13
| Benzene 1.5 mmol/m$^3$, ASA 300 mg/kg b.w. | 193±9 | 157±22 | 196±10 | 210±16 | 197±18 | 190±10 | 220±14 | 116±22 | 137±17 | 166±17 | 89±11 | 62±13
| Benzene 4.5 mmol/m$^3$, ASA 5 mg/kg b.w. | 192±22 | 156±28 | 209±13 | 189±21 | 197±20 | 182±13 | 178±16 | 130±22 | 112±14 | 166±13 | 104±10 | 94±15
| Benzene 4.5 mmol/m$^3$, ASA 150 mg/kg b.w. | 192±26 | 132±12 | 197±15 | 194±21 | 196±15 | 205±10 | 192±21 | 130±14 | 140±12 | 152±12 | 116±14 | 83±15
| Benzene 4.5 mmol/m$^3$, ASA 300 mg/kg b.w. | 193±9 | 157±22 | 196±10 | 210±16 | 197±18 | 190±10 | 220±14 | 116±22 | 137±17 | 166±17 | 89±11 | 62±13

Abbreviations as in Table 1.

Table 3. Activities of α-naphthyl acetate esterase (ANAE) and the content of lipids (Sudan Black B – SBB) and mucopolysaccharides (PAS) in bone marrow cells of the control rats and the rats exposed to benzene alone or in combination with acetylsalicylic acid

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>ANAE (%) x±SD</th>
<th>SBB (score) x±SD</th>
<th>PAS (%) x±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
<td>4 weeks</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Control group</td>
<td>81±7</td>
<td>84±5</td>
<td>82±3</td>
</tr>
<tr>
<td>Study groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA 5 mg/kg b.w.</td>
<td>83±5</td>
<td>78±5</td>
<td>84±4</td>
</tr>
<tr>
<td>ASA 150 mg/kg b.w.</td>
<td>83±6</td>
<td>83±6</td>
<td>80±2</td>
</tr>
<tr>
<td>ASA 300 mg/kg b.w.</td>
<td>87±4</td>
<td>85±6</td>
<td>83±6</td>
</tr>
<tr>
<td>Benzene 1.5 mmol/m$^3$</td>
<td>85±7</td>
<td>82±5</td>
<td>86±3</td>
</tr>
<tr>
<td>Benzene 1.5 mmol/m$^3$, ASA 5 mg/kg b.w.</td>
<td>86±6</td>
<td>82±7</td>
<td>84±4</td>
</tr>
<tr>
<td>Benzene 1.5 mmol/m$^3$, ASA 150 mg/kg b.w.</td>
<td>82±8</td>
<td>84±7</td>
<td>89±2</td>
</tr>
<tr>
<td>Benzene 4.5 mmol/m$^3$</td>
<td>85±4</td>
<td>88±3</td>
<td>86±2</td>
</tr>
<tr>
<td>Benzene 4.5 mmol/m$^3$, ASA 150 mg/kg b.w.</td>
<td>88±2</td>
<td>87±4</td>
<td>91±1</td>
</tr>
<tr>
<td>Benzene 4.5 mmol/m$^3$, ASA 300 mg/kg b.w.</td>
<td>88±3</td>
<td>81±3</td>
<td>88±2</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.
Morphology of peripheral erythrocytes
In the rats, which were exposed to benzene, slight hypochromia, polychromatophilia and slight anisocytosis with the prevalence of microcyte population were observed. The alterations became more pronounced along with increasing concentration of the solvent and with increasing exposure duration. In blood smears of the rats exposed to benzene at a concentration of 4.5 mmol/m³ for 4 or 8 weeks, presence of individual erythroblasts was noted. In the animals that were given ASA alone, the smears of peripheral blood did not show alterations in erythrocyte morphology. Changes induced by benzene exposure were less pronounced after simultaneous exposure to both xenobiotics.

Bone marrow myeloid cells
Benzene exposure at 4.5 mmol/m³ increased the content of cells of the erythroid system in bone marrow. Traits of megaloblastic renewal appeared in the 4th (4.5 mmol/m³) or in the 8th week (1.5 mmol/m³) of exposure. Similarly, the number of cells in karyokinesis was increased, depending on duration of the experiment and concentration of the solvent. As compared to the animals exposed exclusively to benzene, the animals exposed in parallel to the solvent and ASA manifested a reduced number of cells in karyokinesis and less pronounced signs of megaloblastic renewal.

White blood cells
Blood leukocytes
Blood leukocyte number was decreased by around 49% only in the group of rats exposed for 2 weeks to benzene at a concentration of 4.5 mmol/m³ and administered with ASA at a dose of 300 mg/kg b.w. (Table 1). In other experimental groups, leukocyte count varied, but the observed differences proved insignificant.

After exposure to any of the two concentrations of benzene, disturbances in the white blood cell pattern were detected, including an augmented proportion of neutrophilic granulocytes and a lowered content of lymphocytes. The alterations became more pronounced along with increasing duration of exposure. Moreover, exposure to a higher concentration of benzene induced the occurrence of undifferentiated cells. ASA at a dose of 300 mg/kg b.w. increased a proportion of neutrophilic and acidophilic granulocytes, and the occurrence of individual cells of altered morphology was noted. Lower doses of the drug failed to affect proportions of the white blood cell types. In the animals exposed to both xenobiotics in parallel, lymphopenia and granulocytosis were manifested but the alterations did not reach significant levels.

Bone marrow myeloid cells
In none of the experimental groups significant changes in bone marrow cell content were detected. The evaluated smears were rich in cells.

Giant forms of granulocytes were detected after 4 or 8 weeks of benzene exposure at 1.5 mmol/m³ and after 2, 4 or 8 weeks of exposure to benzene at 4.5 mmol/m³. In the rats exposed to the higher concentration of benzene, undifferentiated cells were present. Simultaneous administration of ASA (150 or 300 mg/kg b.w.) and benzene (4.5 mmol/m³) slightly elevated the content of granulocytes. As compared to the rats exposed to benzene alone, the rats given both xenobiotics in parallel manifested lower numbers of giant cells.

Cytochemical stains
Changes in LAP, ACP, ANAE activities and in mucopolysaccharide content observed in the animals treated with benzene and acetylsalicylic acid alone or in combination were nonspecific (Tables 2 and 3).

Activity of myeloperoxidase
Benzene at a concentration of 4.5 mmol/m³ inhibited MPO activity by 21%, 44% and 53%, after 2, 4 and 8 weeks.
of exposure, respectively (Table 2). ASA in any dose and any studied period did not significantly influence MPO activity. Simultaneous exposure to ASA (150 or 300 mg/kg b.w.) and benzene (4.5 mmol/m³) for 2 weeks increased MPO activity to the values observed in the control group. After prolonging the exposure period to 4 or 8 weeks, the protective effect of the drug was not found.

Activity of naphthol AS-D chloroacetate esterase

Inhibition of CAE was observed in the animals exposed to benzene vapour at a concentration of 4.5 mmol/m³ by about 16, 46 and 52% after 2, 4 and 8 weeks, respectively (Table 2). In the case of each ASA dose activity of CAE was decreased. ASA at doses of 150 and 300 mg/kg b.w. decreased CAE activity after 2, 4 and 8 weeks of administration by about 46%, 49% and 54%, and 55% and 62%, respectively. A similar effect was observed in the rats treated with the lowest ASA dose (5 mg/kg b.w.) for 4 and 8 weeks. Both xenobiotics caused a decrease in CAE activity in all experimental groups.

Content of lipids

Exposure to the highest concentration of benzene (4.5 mmol/m³) decreased the content of lipids after 4 and 8 weeks by about 45% and 63%, respectively (Table 3). Simultaneous administration of ASA at any tested dose caused a slight increase in the content of lipids which was less pronounced only after exposure to the solvent.

DISCUSSION

Toxic activity of benzene may lead to functional disturbances in stem cells and, in effect, to cytopenia (aplastic anemia) or excessive proliferation (leukemia). In humans, decreased levels of hemoglobin and erythrocyte number as well as an altered count of reticulocytes represent the first alterations indicating chronic intoxication with benzene. Furthermore, exposure to the solvent was followed by morphological alterations in the form of toxic granules, traits of degeneration in the cytoplasm and in the cell nucleus [2,27]. In our studies we have detected no significant effects of benzene on the level of hemoglobin and the value of hematocrit, but in smears of peripheral blood we have noticed slight hypochromia, polychromatophilia, poikilocytosis and microcytosis. The alterations have been intensified by prolongation of exposure time, which is connected with the release of less mature erythrocytes from bone marrow to the blood. In our study high reticulocytosis was noted after 2 weeks or 4 weeks of exposure to the solvent alone. However, after 8 weeks a lowered reticulocyte content and occurrence of traits of megaloblastic renewal were observed. It may be connected with depletion of compensatory capacities of bone marrow because of the fact that occurrence of erythroblasts in the rats’ blood was noted. Parallel administration of acetylsalicylic acid has significantly reduced the benzene-induced elevated content of reticulocytes.

Administration of ASA together with benzene has resulted in lower numbers of blood reticulocytes and bone marrow erythroblasts. It may be associated with the influence of ASA on benzene biotransformation to phenol which was shown in our previous study [16]. Phenol, one of benzene metabolites, undergoes secondary bioactivation in bone marrow, so its lowered concentration may limit benzene toxicity to the erythroid system.

Additionally, ASA-inhibited metabolism of arachidonic acid and free radical formation can counteract the bone marrow stromal cells damage caused by benzene. Occurrence of benzene myelotoxicity was prevented by simultaneous administration to mice of indomethacin (2 mg/kg b.w. i.p.) or aspirin (50 mg/kg b.w. i.p.) or meclofenamate (4 mg/kg b.w. i.p.) [22]. In our studies, the decreased number of leukocytes has been detected only in the group of animals exposed for 2 weeks to benzene at a concentration of 4.5 mmol/m³.
and ASA at a dose of 300 mg/kg b.w. In other experimental groups the unchanged levels of leukocytes were noted. Analysis of blood smears has revealed an increased content of neutrophilic granulocytes, which has been accentuated by exposure prolongation. Changes in the number of neutrophilic granulocytes have been accompanied by structural disturbances, involving cell nuclei, presence of toxic granules as well as traits of cytoplasmic degeneration. Granulocytosis was observed in the rats exposed to benzene (300 ppm) for 13 weeks and in the mice exposed to benzene (100 and 300 ppm for the entire lifetime), and they related it to the intensified proliferation of promyelocytes and myelocytes [28,29].

In contrast, Hazel et al. [30] suggested that hydroquinone created in benzene biotransformation inhibited differentiation of bone marrow granulocytes at the stage of myelocytes. ASA itself at a dose of 300 mg/kg b.w. increased the content of both neutrophilic and acidophilic granulocytes. However, in the rats exposed simultaneously to benzene and ASA no intensification of granulopoiesis was noted. It may have been expected due to the combination of the effects of both compounds and a distinct target of each of them: benzene is responsible mainly for the damage of precursor cells, while ASA modifies functions of cytokines. In our studies, we have detected lymphopenia which has been intensified by prolongation of exposure to benzene. Studies with benzene demonstrated lowered numbers of peripheral blood lymphocytes and B and T cells in spleen, thymus and bone marrow as well as dose-dependent decreases in CD4⁺-T cells, CD4⁺/CD8⁺ ratio and B cells [9,20,28,31]. The observed alterations may have been linked with a hydroquinone- and catechol-induced decrease in the number of precursor cells, and damaged structure of DNA as well as ribonucleotide reductase activity [32]. Moreover, benzene metabolites may affect cytokines synthesis and their release from macrophages and, therefore, influence lymphocytes proliferation, maturation and function [10,20]. Hydroquinone inhibits the effects of TNF-α and, thus, of the nuclear factor κB (NF-κB), disturbing activation of both T and B lymphocytes. It may also inhibit production of cytokines (IL-1β, IL-2, IL-3, IL-6, IL-10, IL-12) and inflammatory mediators (NO, PGE₂) [10,19].

Qualitative evaluation of bone marrow in the benzene-exposed animals has demonstrated alterations in morphology of nucleated cells. The presence of giant granulocytes and undifferentiated cells has been probably related to benzene metabolites such as phenol and quinone, which may affect synthesis of nucleic acids through inactivation of DNA and RNA synthases or through production of free radicals, finally resulting in a disturbed structure of nucleic acids and chromosomal aberrations [6,20,33]. In the animals receiving ASA exclusively, the quality and quantity of the parameters tested in bone marrow were similar to those observed in the control animals. ASA administration to the benzene-exposed rats has resulted in a decrease in the number of giant granulocytes, which has probably reflected ASA-induced alteration in benzene biotransformation, i.e. decreased production of phenol, and inhibition of its quinone metabolite turnover in bone marrow.

Cytoenzymatic examinations have been performed to characterize functional status of granulocytes and to complete cytomorphological examinations. Peroxidases are hemoprotein enzymes occurring in neutrophils and eosinophils, starting at the promyelocyte, and their enzymatic activity increases along with the maturation of cells. There are reports that the peroxidase of macrophages of the marrow stromal may take part in oxidizing quinone metabolites of benzene and, thus, mediate its hematotoxicity [12,34,35]. Liberation of lipoxin A₄ by ASA decreases activity of myeloperoxidase and also influences chemotaxis and stimulation of leukocytes [17,36]. Moreover, the secondary bioactivation of benzene metabolites by myeloperoxidase is inhibited by a quinone oxidoreductase present in fibroblasts of the marrow which reduces quinone biotransformation to nonreactive forms [21,37].
Naphthol AS-D chloroacetate esterase shows its activity only in the granulocytic cell line, mostly in monocytes. No information concerning the influence of the studied xenobiotics on activity of the enzyme is available. The observed decrease in CAE activity after ASA administration might be caused by its involvement in biochemical transformations of acetylsalicylic acid. Total influence of both studied compounds is difficult to interpret because the mechanism of inhibition of the activity of this esterase is unknown. Nevertheless, the observed changes do not seem to be beneficial for the correct maturation of granulocytic cells. Sudan Black B stains neutral lipids, phospholipids and cerebrosides present in the largest amount in the cells of the granulocytic system. Concentration of lipids increases along with maturation of the cells. In rabbits exposed to benzene vapour in subacute (14 days, 16 mg/dm$^3$, 6 h/day) and chronic (40 days, 10 mg/dm$^3$, 3 h/day) poisoning, a decrease in the lipid content in both peripheral blood granulocytes and in the bone marrow was found. In our experiment, ASA itself did not influence the content of lipids, but when administered to the rats exposed to benzene it increased the percentage of sudanophilic granulocytes, which may indicate a protective effect of the drug [38]. Cytochemical and cytoenzymatic examinations indicate benzene inhibition of granulocytic cell maturation. Simultaneous administration of acetylsalicylic acid has limited the observed changes. In summary, our results show that higher doses of acetylsalicylic acid limited benzene induced hematotoxicity, influenced the contents of reticulocytes, neutrophilic granulocytes with structural disturbances as well as lipids level and myeloperoxidase activity.

REFERENCES


8. Renz JF, Kalf GF. Role for interleukin-1 (IL-1) in benzene-induced hematotoxicity: inhibition of conversion of pre-IL-1α to mature cytokine in murine macrophages by hydroquinone and prevention of benzene-induced hematotoxicity in mice by IL-1α. Blood. 1991;78:938–44.


