EFFECT OF DICHLOROACETATE ON FEMALE RATS WITH ADJUVANT ARTHRITIS

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Abstract. The aim of the study was to investigate the prophylactic treatment with 25 mg/kg and 50 mg/kg of dichloroacetate (DCA) on 24 female Wistar rats with adjuvant arthritis (AA). Body weight, joint swelling, blood indices, pro-antioxidant status, histological changes in joints and liver were investigated. The results indicated a significant suppression of joint swelling. At the end of experiment it was lower by 49.3 % and 39.7 % in the groups respectively treated with 25 mg/kg and 50 mg/kg of DCA. The benefit effect was supported by histological studies showing a marked decrease of infiltration with inflammatory cells, suppression of edema and angiogenesis in periarticular tissues and synovium. The total antioxidant activity (AOA) of blood serum after the treatment significantly increased and was higher by 51.8 % and 55 % by using of 25 mg/kg and 50 mg/kg doses of DCA. Both doses of DCA did not show toxic effects on the liver and improved the histological changes in hepatic tissue induced by AA. Therefore, DCA may be successfully used to suppress the development of AA in female rats.

Keywords: adjuvant arthritis, dichloroacetate, antioxidant activity, rats

Introduction. The halogenated organic acid dichloroacetate (DCA) is an investigational drug used to treat metabolic disorders that involve mitochondrial dysfunction (Bonnet et al., 2007; Stacpoole et al., 2008). It increases mitochondrial aerobic energy production through the activation of pyruvate dehydrogenase (PDH) enzyme by inhibition of PDH kinase (PDK) (Kato et al., 2007; Michelakis et al., 2008).

In recent years, DCA has attracted attention as a novel anti-cancer agent to target glycolytic tumors without side effects in the healthy organs (Papandreou et al., 2011). It was found to act as an efficient tumor growth inhibitor in vitro and in vivo (Bonnet et al., 2007; Cairns et al., 2007; Cao et al., 2008; Wong et al., 2008), by shifting glucose metabolism from glycolysis to glucose metabolism in malignant cells, which resulted in the release of pro-apoptotic mediators and decreased proliferation in malignant cells. This leads to elimination of active tumor cells, while the normal cells are unaffected (Bonnet et al., 2007; Vella et al., 2012). It is proposed that DCA can reverse glycolytic phenotype in cancer cells through the inhibition of PDK (Sun et al., 2010).

Because rheumatoid arthritis (RA) is characterized by cartilage and bone destruction, and like a malignant tumor, by increased cell proliferation in synovium and pannus formation, DCA or its combination with the current anti-rheumatic drugs could be useful for the treatment of this systemic autoimmune disease. To our knowledge, only one study has been performed in which it was shown that DCA alleviates the development of collagen II-induced arthritis in female DBA/1 mice (Bian et al., 2009). The pro-apoptotic and anti-proliferative properties of DCA prompted us to investigate the effects of this compound in another model of RA, rat adjuvant arthritis (AA), that shares many histopathological features of the human counterpart and used to investigate new antiarthritis substances (Leonavičienë et al., 2006; Kazemekiai et al., 2008).

Oxygen metabolism and the increase in reactive oxygen species (ROS) play an important role in the pathogenesis of RA (Filipchin et al., 2008; Mirshafiey and Mohsenzadean, 2008). The pro-oxidant/antioxidant imbalance in RA and arthritis models develops due to acceleration of some cellular reactions or insufficiency of the antioxidant defense system (Minuz et al., 2006). The role of lipid peroxidation and antioxidant status of blood serum in rats with AA after the treatment with DCA has not been previously described. Therefore, we considered that it is important to analyze not only anti-inflammatory effect but also antioxidant activity of this compound.

Materials and Methods

Chemicals. Complete Freund’s adjuvant (CFA), 10 % formalin, spirı-formol, hematoxylin, eosin, picrofusin, toluidine blue, methyl-green-pyronin-y, acetic acid, trichloracetic acid, orthophosphoric acid, thioarbituric acid, nitric acid, ferrous sulfate, ascobic acid, ammonium molybdate, hydrogen peroxide, dichloroacetate (DCA) 99 % purity were obtained from Sigma-Aldrich Chemie and Fluka Chemie GmbH (Germany), ketamine and xylazine from Biowet (Poland). Tetrachloroauric acid (HAuCl₄·3H₂O) and tannic acid were obtained from Carl Roth GmbH & Co (Germany), sodium citrate – from Penta (Czech Republic).

Animals. The study was performed on 24 female Wistar rats 12 weeks of age obtained from the breeding...
unit of the laboratory of animal resources of the State Research Institute Centre for Innovative Medicine (Vilnius, Lithuania). All rats were maintained in plastic cages with rat chow and tap water ad libitum, at 20–22°C, and 50–70 % relative humidity with 12 hours light/dark cycle. All animals were used with the approval of the Lithuanian Laboratory Animal Use Ethics Committee under the State Food and Veterinary Service (No 0207–2010).

Adjuvant-induced arthritis, its evaluation and treatment. AA was induced by a single 0.1 ml injection of complete Freund’s adjuvant (CFA) into the left hind paw. Since AA inducing day two groups of rats (8 animals per group) were treated orally (5 times a week) with DCA in doses of 25 and 50 mg/kg dissolved in 0.5 ml of water. The control animals received the same volume of water. The development and progression of AA were monitored three times a week and clinical disease course was followed up for 18 days after the initial injection of CFA.

Blood and tissue collection. At the end of the experiments, animals were humanely killed by decapitation under ketamine-xylazine anesthesia. Their internal organs were examined macroscopically and weighed. The liver and injected joints were taken for histological analysis. The erythrocyte and leukocyte counts (made by using a Picoscale, Hungary) and the erythrocyte sedimentation rate (ESR) were determined in the blood. Blood samples were centrifuged at 800 g for 10 min to obtain the serum samples which were stored frozen at -20°C before testing.

Lipid peroxidation (MDA) level, catalase (CAT) and total antioxidant activity (AOA) detection in blood serum. The end product of lipid peroxidation malondialdehyde (MDA), expressed as nmol/ml, was determined by the thiobarbituric acid reaction at 535 nm and 580 nm by the method of Gavrilov and co-workers (1987). Catalase (CAT) activity, expressed in mmol/l/min, was measured at 410 nm as described by Koroliuk et al. (1988). The total antioxidant activity (AOA) was determined in the reaction with thiobarbituric acid, described by Galaktionova et al. (1998).

Histology. The liver and injected paws from AA rats were excised, followed by routine fixation, decalcification, and paraffin embedding. Histological 5 µm-thick sections of joints were stained with haematoxylin-eosin, picrofuchsin, toluidine blue, methyl-green-pyronin-y and safranin O. Histological assessment of changes in liver, synovium, soft periarticular tissues and earilage was performed in a blinded manner by two pathologists using the 0–3 scale, where 0 indicates the absence of changes and 3 means the most severe expression of a particular sign.

Statistical analysis. Statistical evaluation of the results was done by one-way analysis of variance ANOVA using PRISM Software (GraphPad Software, San Diego, CA, USA) and Student’s t test. The nonparametric Mann-Whitney U test was used to evaluate the histological changes. All data were expressed as mean ± SEM and were considered to be statistically significant at P<0.05.

Results

Body and organ’s weight. The total body weight of all rats increased during the experiment by 11.8 %, 17.4 % and 17 % respectively in the control and both treated groups. No significant differences between the groups were observed (data not shown). A postmortem examination of the internal organs revealed only a significantly lower relative weight of the spleen (P<0.05) in the II group of animals treated with 25 mg/kg DCA (0.28±0.02 g/kg – DCA; 0.35±0.02 g/kg – control). No decrease of the liver weight and 21 % higher thymus weight (0.51±0.04 g – DCA; 0.42±0.03 – control) was observed by using 50 mg/kg of DCA.

Histological changes in the liver. Both doses of DCA significantly decreased alteration of hepatic parenchyma in comparison with the control group (Table 1): dystrophic changes were lower by 42.2 % (P<0.002) and 27.2 % (P<0.001) and necrosis by 44.8 % (P<0.05) and 37.6 % (P<0.05) in the groups respectively treated with 25 mg/kg and 50 mg/kg doses of DCA.

<table>
<thead>
<tr>
<th>Pathomorphological changes</th>
<th>Groups</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II Control</td>
<td>DCA (25 mg/kg)</td>
<td>DCA (50 mg/kg)</td>
</tr>
<tr>
<td>Alteration of parenchyma</td>
<td>Dystrophy</td>
<td>2.06±0.06</td>
<td>1.19±0.21 *</td>
<td>1.50±0.12 *</td>
</tr>
<tr>
<td></td>
<td>Necrosis</td>
<td>1.25±0.16</td>
<td>0.69±0.16 *</td>
<td>0.78±0.12 *</td>
</tr>
<tr>
<td>Hypervolemia of V. centralis</td>
<td>1.19±0.16</td>
<td>0.62±0.12 *</td>
<td>0.61±0.07 *</td>
<td></td>
</tr>
<tr>
<td>Inflammatory infiltration of hepatic stroma</td>
<td>Lymphocytes</td>
<td>0.44±0.11</td>
<td>0.13±0.08 *</td>
<td>0.28±0.09</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>0.25±0.09</td>
<td>0.06±0.06</td>
<td>0.05±0.05</td>
</tr>
<tr>
<td></td>
<td>General</td>
<td>0.69±0.16</td>
<td>0.19±0.09 *</td>
<td>0.28±0.09 *</td>
</tr>
<tr>
<td></td>
<td>Penetration into the lobule</td>
<td>0.69±0.16</td>
<td>0.31±0.16</td>
<td>0.22±0.14 *</td>
</tr>
</tbody>
</table>

Note: * Differences are statistically significant in comparison with the control group

V. centralis hypervolemia was suppressed after the treatment with low (P<0.02) and high (P<0.01) doses of DCA. 25 mg/kg of DCA significantly diminished the inflammatory infiltration of hepatic stroma with lymphocytes (P<0.05), and general inflammatory reaction (P<0.02). The decrease of the latter was also found after the treatment with 50 mg/kg of DCA (P<0.05). This dose also markedly suppressed the penetration of inflammatory...
cells into the lobule (P<0.05). Therefore, both doses of DCA did not show toxic effects and improved the histological changes in hepatic tissue induced by AA. Low doses of DCA better suppressed the alteration of hepatic parenchyma, inflammatory infiltration of hepatic stroma with lymphocytes whereas high doses - the penetration of inflammatory cells into the lobule.

**Blood indices and pro-/antioxidant activity of blood serum of rats with AA treated with DCA.** The erythrocyte sedimentation rate (ESR) and leukocyte count for both groups of rats treated with DCA was markedly lower than in the control group (Fig. 1 (A)): ESR decreased by 54.1 % (P<0.001) and 57.8 % (P<0.001) and the amount of leukocytes - by 24.8 % (P<0.0001) and 25.4 % (P<0.0001) in the groups respectively treated with 25 mg/kg and 50 mg/kg of DCA. A higher count of erythrocytes as compared with the control group (P<0.05) was observed in rats that received 50 mg/kg of DCA.

Free radical formation resulting in lipid peroxidation, measured as the MDA level (Fig. 1 (B)) showed, that MDA was lower by 13.24 % in rats treated with 25 mg/kg of DCA (difference between the tested and control groups was near to significant; t = 2). Serum antioxidant enzyme CAT activities were found to be insignificantly higher by 11.2 % and 19.2 % in treated rats with lower and higher doses of DCA (the difference was near to significant by using 50 mg/kg of DCA; t = 2).

But AOA in comparison with the control group significantly increased by 51.8 % (P<0.01) and 55 % (P<0.001) after the treatment with 25 mg/kg and 50 mg/kg of DCA.

**Joint swelling and incidence of polyarthritis development.** The treatment with both doses of DCA (Fig. 2) significantly (P<0.05-0.001) decreased joint swelling during the experiment in a dose-independent manner. At the end of experiment, joint swelling was lower by 49.3 % and 39.7 % in comparison with the control group by using 25 mg/kg and 50 mg/kg of DCA.

Polyarthritis characterizing the generalization of the disease and exacerbation of the autoimmune process did not develop in the animals of the treated groups, whereas in the control group it was observed in 37.5 % animals.

**Histological features of arthritis.** Histological examination of the joints of injected paw on day 18 (Table 2) showed that in comparison with the control group both doses of DCA significantly diminished inflammatory infiltration with lymphocytes (P<0.05; P<0.0001), leukocytes (P<0.01; P<0.0001) and general inflammatory reaction (P<0.01; P<0.001) in the soft periarticular tissues.

The marked decrease of infiltration with macrophages by 36 % was found only after the treatment with 50 mg/kg of DCA (P<0.05).

Both doses of DCA significantly suppressed soft tissue edema (P<0.001; P<0.0001) and angiomatosis (P<0.01; P<0.0001). 50 mg/kg dose of DCA act more potently than lower dose on angiomatosis (P<0.01) and inflammatory infiltration of soft periarticular tissues with leukocytes (P<0.02).
Table 2. Histological changes in articular tissues after the treatment of adjuvant arthritis (AA) with dicloroacetate (DCA)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Index</th>
<th>Groups</th>
<th>I Control</th>
<th>DCA (25 mg/kg)</th>
<th>DCA (50 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft periarticular tissues</td>
<td>Inflammatory infiltration</td>
<td>Lymphocytes</td>
<td>2.19±0.09</td>
<td>1.57±0.23 *</td>
<td>1.19±0.09 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leukocytes</td>
<td>2.00±0.21</td>
<td>0.93±0.23 *</td>
<td>0.25±0.09 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Macrophages</td>
<td>1.75±0.13</td>
<td>1.43±0.13</td>
<td>1.12±0.22 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>General</td>
<td>2.44±0.11</td>
<td>1.78±0.15 *</td>
<td>1.44±0.11 *</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td></td>
<td>2.00±0.13</td>
<td>0.93±0.20 *</td>
<td>0.56±0.15 *</td>
</tr>
<tr>
<td></td>
<td>Angiomaotisis</td>
<td></td>
<td>1.75±0.13</td>
<td>1.07±0.17 *</td>
<td>0.44±0.11 *</td>
</tr>
<tr>
<td></td>
<td>γ-metachromasia</td>
<td></td>
<td>0.63±0.26</td>
<td>0.14±0.14</td>
<td>0.12±0.08 *</td>
</tr>
<tr>
<td>Synovium</td>
<td>Proliferation</td>
<td></td>
<td>1.38±0.18</td>
<td>0.71±0.10 *</td>
<td>0.69±0.09 *</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td></td>
<td>1.37±0.18</td>
<td>0.36±0.18 *</td>
<td>0.19±0.13 *</td>
</tr>
<tr>
<td></td>
<td>γ-metachromasia</td>
<td></td>
<td>0.50±0.16</td>
<td>0.21±0.15</td>
<td>0.06±0.06 *</td>
</tr>
<tr>
<td></td>
<td>Inflammatory infiltration</td>
<td>Lymphocytes</td>
<td>1.25±0.09</td>
<td>0.50±0.15 *</td>
<td>0.37±0.08 *</td>
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<tr>
<td></td>
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<td>0.25±0.16</td>
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<td></td>
<td></td>
<td>General</td>
<td>1.25±0.09</td>
<td>0.50±0.15 *</td>
<td>0.37±0.08 *</td>
</tr>
<tr>
<td></td>
<td>Angiomaotisis</td>
<td></td>
<td>1.38±0.18</td>
<td>1.43±0.13 *</td>
<td>0.44±0.15 *</td>
</tr>
<tr>
<td>Cartilage</td>
<td>Alteration</td>
<td>Erosion</td>
<td>0.88±0.12</td>
<td>0.64±0.18</td>
<td>0.25±0.16 *</td>
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<tr>
<td></td>
<td></td>
<td>Usura</td>
<td>0.50±0.19</td>
<td>0.07±0.07</td>
<td>0.06±0.06 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pannus</td>
<td>0.38±0.18</td>
<td>-</td>
<td>0.06±0.06 *</td>
</tr>
</tbody>
</table>

Notes: *Differences are statistically significant in comparison with the control AA group. †Differences are statistically significant between the treated groups.

Both doses of DCA similarly diminished synovium villi proliferation (P<0.01) and angiomaotisis (P<0.001; P<0.002). A markedly suppressed synovium edema by 73.7 % (P<0.002) and 86.1 % (P<0.001) was observed in the groups treated with 25 mg/kg and 50 mg/kg of DCA respectively. Only one animal from the group treated with 50 mg/kg of DCA had the traces of connective tissue disorganization (γ-metachromasia) what significantly differed from the control group (P<0.05), in which 3 of 8 animals showed a small disorganization (+1), and 2 – it’s traces.

The investigation of synovium infiltration with inflammatory cells showed a significant decrease of lymphocytes and general inflammatory reaction by 59 % (P<0.001) and 70 % (P<0.0001) after the treatment with 25 mg/kg and 50 mg/kg doses of DCA respectively.

The changes in the cartilage were small. Seven animals of the control group had slight erosions and 4 animals had small usuries. The number of animals with erosion and usuries was less after the treatment with DCA, and the dose of 50 mg/kg significantly diminished them in comparison with the control group (P<0.01 – erosion and P<0.05 – usuries). Any pannus formation in animals treated with 25 mg/kg of DCA was observed, and only minimal pannus in one animal that received 50 mg/kg of DCA was found. In the control group 3 of 8 animals had a small pannus (+1). Any thinning of cartilage was observed in both treated groups, but in the control group 2 animals had a minimal and small (+1) thinning.

Discussion

RA is an inflammatory disease characterized by chronic inflammation of the joints associated with proliferation of synovial cells and infiltration of activated immune inflammatory cells, leading to progressive destruction of cartilage and bone (Hitchon and El-Gabalawy, 2004). Another central feature of RA synovitis is the transformation of fibroblast-like synovial cells into autonomously proliferating cells with a tissue-infiltrating nature, forming hyperplastic tissue with potential for bone erosion and cartilage degradation known as pannus (Filippin et al., 2008). Because RA, like a malignant tumor, is also characterized by increased cell proliferation in synovium and pannus formation, the use of DCA or its combination with the current anti-rheumatic drugs could be useful for the treatment of this systemic autoimmune disease. It is shown that DCA was useful to treat some types of cancer in humans and rats (Bonnet et al., 2007) and the decrease in tumor growth by DCA was associated with an increase in apoptosis and a decrease in proliferation. A very attractive property of DCA resides in its tolerability and safety (Vella et al., 2012). Its selectivity is evident by a lack of any systemic toxicity in animal and human studies (Bonnet et al., 2007; Stacpoole et al., 2006). Although in some reports DCA was found to be hepatotoxic and hepatocarcinogenic in rodents and induced oxidative stress in hepatic tissues of mice after long term exposure (Daniel et al., 1992; Hassoun et al., 2010; Hassoun and Gearfoss, 2011), our studies did not show that. Conversely, both investigated doses of DCA did not show toxic effects and improved the histological changes in hepatic tissue induced by AA. Lower doses better suppressed the alteration in parenchyma, inflammatory infiltration of hepatic stroma with lymphocytes and higher doses - the penetration of inflammatory cells into the lobule.
Biomarkers of oxidative stress, such as production of lipid peroxidation, superoxide anion (SA) production, and DNA damage were found to be induced in the hepatic tissues of mice exposed to single high dose of the compound (Austin et al., 1996; Hassoun and Dey, 2008). It is also shown that serum MDA levels are significantly increased and the total oxidative status levels are decreased in patients with RA (Ozkab et al., 2007). Our investigation of pro-antioxidant activities of the blood serum of rats with AA treated with DCA showed insignificant decrease of MDA level, higher CAT activities and significant increase of the AOA levels in serum.

Treatment with both doses of DCA also improved blood indices such as ESR and leucocyte count which were markedly lower than in the control group.

The results of this study indicated the suppression of joint swelling in response to administration of DCA to female rats with AA. DCA effects on the joint swelling was immediate, with significant effects even after 3 days of the treatment which lasted during the experiment.

The benefit of DCA administration on joint swelling was further supported by histological studies showing a marked decrease of infiltration with inflammatory cells and significant suppression of edema and angiogenesis in periarticular tissues and synovium. Both doses of DCA similarly diminished synovium villi proliferation, but only 50 mg/kg of DCA significantly decreased infiltration with macrophages in soft periarticular tissues and diminished erosion and usurae in the cartilage. It should be noticed that higher dose of DCA acted more potent on angiogenesis and inflammatory infiltration of soft periarticular tissues with lymphocytes. Our findings are in keeping with data of previous study showing that DCA alleviates development of collagen II-induced arthritis in female DBA/1 mice (Bian et al., 2009). May be, the small size of DCA, after oral intake, results in excellent tissue penetration, including the joints tissue, especially the synovium, where the markedly diminished synovium villi proliferation, angiogenesis and suppressed synovium edema, were observed after the treatment with DCA.

In summary, we conclude that the current data provide evidence for the safety of DCA and its benefit effect in the treatment of female rats with AA. So, this preliminary study expands the possible use of DCA for RA therapy supporting the need of a detailed investigation of its anti-arthritis properties alone or in combination with other drugs.

References


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