Sodium Diethyl Dithiocarbamate and the Mononuclear Phagocytic System in Guinea Pigs

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Abstract
Sodium diethyl dithiocarbamate (DTC) is a low molecular weight sulfur compound which has been previously shown to have immunomodulatory properties. In the present report, the effects of DTC on the mononuclear phagocytic system were studied in guinea pigs. The colloidal gold clearance was enhanced in animals which received an i.v. injection of 10 mg DTC on day – 13, but not in animals treated on day – 8 with DTC. DTC treatment modified neither 99mTc sulfur colloid uptake nor activation markers in peritoneal macrophage monolayers. These results show that DTC which modulates both delayed hypersensitivity and antibody synthesis only enhances nonspecific binding of macrophages but leaves unaffected endocytosis and metabolic status of macrophages.

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Sodium diethyl dithiocarbamate (DTC) is a low molecular weight sulfur compound which has previously been chosen to exemplify the role of the thiol moiety of the levamisole molecule [1]. It was demonstrated to have immunomodulatory properties in mice [1] and in guinea pigs [2]. In guinea pigs, DTC enhanced delayed hypersensitivity reactions [2] and mitogen-induced proliferation of T and B spleen lymphocytes [3] only after a lag period suggesting that DTC, acting possibly at the macrophage level, modulates the immune responses through indirect pathways.

In the present report, the effects of DTC on the mononuclear phagocytic system were studied in guinea pigs. The clearance of colloidal gold was determined according to the method described by Haugen et al. [4]. Guinea pigs were injected with 2 mg/kg body weight of colloidal radioactive gold (198Au; diameter of particles 300 Å; CEA, Gif-sur-Yvette, France). Blood samples were collected by retroorbital sinus puncture on heparinized tubes 3, 5, 10 and 15 min after gold injection. The activity of 100-µl samples was measured within an interval of 1 h, using a SAIP γ counter. The phagocytic index was calculated according to the formula:

\[ \kappa = \log C_0 - \log C | T_1 - T_0 \]

where \( C_j \) and \( C_0 \) represent the gold concentration at time \( T_1 \) and \( T_0 \) (in min) expressed as cpm. Cpm were plotted in a semilogarithmic scale in relation to time. The best fitting curve for these points, called the elimination curve, was drawn and the phagocytic index calculated.

In a first experiment, guinea pigs were treated i.v. with 10 mg of DTC, 6 h before or at the same time they were injected i.v. with radioactive colloidal gold. Whether guinea pigs were treated with DTC 6 h before or at the time they were injected i.v. with radioactive colloidal gold, the phagocytic index was similar to that observed in untreated controls (K was 0.12 ± 0.01 , 0.13 ± 0.01 , and 0.13 ± 0.02, respectively). In a second experiment, groups of 4 guinea pigs were
treated i.v. with 10 mg of DTC 13 or 8 days before they were injected with colloidal radioactive gold (fig. 1). In guinea pigs treated with DTC 8 days before the injection of colloid, the phagocytic index was similar to that of controls, but animals treated 13 days before test exhibited an enhanced phagocytic index.

An increase or a decrease of the clearance rate induced by a variety of chemical or biological substances has been interpreted as a stimulation or blockade of the reticuloendothelial system (RES) activity [5]. From these results, DTC appeared to stimulate the activity of the RES after a lag period.

Effect of DTC on the Mononuclear Phagocytic System

Table I. Organ distribution of colloidal gold in guinea pigs treated with DTC

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Spleen</th>
<th>Lung</th>
<th>Kidney</th>
<th>Skin</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/g</td>
<td>cpm/g</td>
<td>cpm/g</td>
<td>cpm/g</td>
<td>cpm/g</td>
<td>cpm/g</td>
</tr>
<tr>
<td>19.25</td>
<td>1,184,000</td>
<td>22,797,847</td>
<td>96.56</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>2.10</td>
<td>263,986</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±</td>
</tr>
</tbody>
</table>

0.72  796,032 556,471 2.36 ± ±
0.05  184,000
0.62  818,450 510,303 2.16 ± ±
0.08  88,577
0.77  860,102 668,457 2.83 ± ±
0.11  148,988
6.73  2,677 2,209 1,174 ± ± ± ±
3.53  1,642 1,093 612
6.99  3,777 1,567 1,114 ± ± ± ±
2.89  1,309 1,007 540
6.22  2,047 1,157 1,281 ± ± ± ±
2.22  563 245 777

Guinea pigs were treated with an i.v. injection of 10 mg of DTC 13 days (group 1) or 6 h (group 2) before test. Guinea pigs of group 3 were untreated controls.

Table II. 99mTc sulfur colloid uptake, H2O2 release, protein content and enzymatic activities in resident adherent peritoneal cells from guinea pigs treated with DTC
The organ distribution of colloidal gold in animals injected i.v. with 100 µCi of the radioactive gold colloidal solution 13 days (group 1) or 6 h (group 2) after they were treated with 10 mg of DTC was similar to that observed in controls (group 3) which received only colloidal gold (table I). More than 90% of the injected colloidal gold was recovered in liver and spleen as previously reported [6]. Furthermore, the weight of liver and spleen was not increased after DTC treatment. These results, similar to those obtained using le-vamisole as immunostimulant [7], suggested that the effects of DTC were not due to hyperplasia or to hypertrophy of the RES but rather to an activation of the mononuclear phagocytic system. The 99mTc sulfur colloid uptake and enzymatic markers were determined in peritoneal macrophage monolayers. The uptake of 99mTc sulfur colloid was performed as previously described [8]. Hydrogen peroxide release by peritoneal adherent cells was studied using a fluoro-metric assay described by Nathan and Root [9]. The amount of macrophage monolayer protein was determined by the method of Lowry et al. [10] using bovine serum albumin (Biomérieux, Lyon, France) as standard. Enzymatic activities were measured in 0.1 % Triton X-100 adherent cell lysates. β-Glucuronidase (EC 3.2.1.31) activity was determined by the method of Talalay et al. [11] using phenolphthalein-/lucuro-nide as substrate, and acid phosphatase (EC 3.1.3.2.) activity by the method described by Andersh and Szczypinski[12] using 4-nitrophenyl phosphate in sodium acetate buffer as substrate. Peritoneal macrophage monolayers from groups of 5 guinea pigs treated with 10 mg DTC 14 or 6 days before test, incorporated as much 99mTc sulfur colloid as macrophages from untreated control animals (table II). On the other hand, macrophages from untreated and DTC-278

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2 3-
References
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Fig. 1. Kinetics of phagocytosis of colloidal gold (phagocytic indices (K values) in untreated guinea pigs (-- in guinea pigs treated i.v. with 10 mg of DTC on day -8 (---) or on day-13 (-).

Au) and )and treated guinea pigs did not spontaneously release significant amounts of hydrogen peroxide. In the presence of PMA (12–0-tetradecanoyl-phorbol-13-acetate; Sigma, St. Louis, Mo.), macrophages from DTC-treated animals released as little ¾C⅛ as macrophages from control animals. Protein content as well as acid phosphatase and β-glucuronidase activities were similar in macrophages from DTC-treated and from untreated control animals. Direct effects of DTC on the mononuclear phagocytic cells resulting in their activation could be excluded.

From the results reported here, it could be speculated that in DTC-treated guinea pigs, the membrane of macrophages is altered so that nonspecific binding is enhanced whereas endocytosis and metabolic status of macrophages are unchanged. This can explain the greater number of Listeria monocytogenes forming colonies in spleen and liver of DTC-treated as compared to control animals [13]. The trapping of listeria is enhanced while phagocytic activity is not. The mechanisms by which DTC can enhance the nonspecific binding of macrophages are under investigation.


