Sodium Diethyl Dithiocarbamate and the Mononuclear Phagocytic System in Guinea Pigs

P.J.		Neveu ^a
P.		Vincendeau ^b

^aGroupe de Recherche de Biochimie Hématologique, UER de Médecine, Poitiers,

^bLaboratoire d'Immunologie et de Biologie Parasitaire, Université de Bordeaux II, France

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 ^{99m}Tc 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.

Correspondence to: P. J. Neveu, Groupe de Recherche de Biochimie Hématologique, UER de Médecine, 34, rue du Jardin des Plantes, F-86034 Poitiers (France)

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

277

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

Group Liver

Spleen

Lung Kidney Skin Muscle cpm/g cpm/g cpm/g cpm/g

weight/g cpm/g cpm/organ cpm/organ weight/g cpm/g cpm/ cpm/organ
organ

cpm

injected, '

cpm injected, %

19.25 1,184,000 22,797,847 96.56 ± ±

2.10 263,986

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

6,736 2,677 2,209 1,174

± ± ± ±

3,535 1,642 1,093 612

6,990 3,777 1,567 1,114

± ± ± ±

2,893 1,309 1,007 540

6,220 2,047 1,157 1,281

± ± ± ±

2,220 563 245 777

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. ^{99m}Tc 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 μ Ci 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 ^{99m}Tc sulfur colloid uptake and enzymatic markers were determined in peritoneal macrophage monolayers. The uptake of ^{99m}Tc 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- β -glucuronide 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 ^{99m}Tc sulfur colloid as macrophages from untreated control animals (table II). On the other hand, macrophages from untreated and DTC-

278
Neveu/Vincendeau

5 1

Acknowledgements

We wish to thank Prof. G. Renoux for his critical review of the manuscript. This work was supported by Inserm grant No. 79.3.047.2 and by the Fondation Langlois.

2 3-

References

- 1 Renoux, G.; Renoux, ML: Roles of the imidazole or thiol moiety on the immunostimulant action of levamisole; in Chirigos, J. - Control of neoplasia by modulation of the immune system, pp. 67-80 (Raven Press, New York 1977).
- Neveu, P.J.: The effects of thiol moiety of levamisole on both $\kappa = 0.11 \pm 0.01$ cellular and humoral immunity during the early response to a hapten-carrier complex. Clin. exp. Immunol. 32: 419-422 (1978).
- 3 Neveu, P.J.; Perdoux, D.; Lafleur, L.: In vivo enhancement of \mitogen induced lymphocyte DNA synthesis by sodium diethyl-dithiocarbamate. Int. J. Immunopharmacol. 4: 9-13 (1982).
- 4 Haugen, J.; Bassøe, H.H.; Flood, P.R.: Phagocytosis in rabbits treated with oxyphenbutazone and cortisone, studied by gold clearance test and electron microscopy. J. reticuloendoth. Soc. 6: 184-193 (1969).
- 5 Cooper, G.N.; Stuart, A.E.: Susceptibility of mice to pneumococcal infection after modification of the reticuloendothelial
- 3 5 10

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 (—).

15 mm system with simple lipids. J. Path. Bact. 83: 227–243(1962).

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 ^3H 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.

Zilversmit, D.B.; Boyd, G.A.; Brucer, M.: The effect of particle size on blood clearance and tissue distribution of radioactive gold colloids. J. Lab. clin. Med. 40: 255–620 (1952).

Hoebecke, J.; Franchi, G.: Influence of tetramisole and its optical isomers on the mononuclear phagocytic system. Effect on carbon clearance in mice. J. reticuloendoth. Soc. 14: 317–322 (1973).

Vincendeau, P.; Vincendeau-Scherrer, C; Ducassou, D.; Bez-ian, J.H.: Uptake of $^{99\text{m}}\text{Tc}$ -sulphur colloid by mononuclear phagocytes. Int. J. nucl. Med. Biol. 7: 383–385(1980).

Nathan, C.F.; Root, R.K.: Hydrogen peroxide release from mouse peritoneal macrophages. Dependence on sequential activation and triggering. J. exp. Med. 146: 1648–1662(1977).

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J.: Protein measurement with the Folin phenol reagent. J. biol. Chem. 193: 265–275(1951).

Talalay, P.; Fishman, W.H.; Huggins, G.: Chromogenic substrates. II. Phenolphthalein glucuronic acid as substrate for the assay of glucuronidase activity. J. biol. Chem. 166: 757–768 (1946).

Andersh, M.A.; Szczypinski, A.J.: Use of / > -nitrophenyl phosphate as the substrate in determination of serum acid phosphatase. Am. J. clin. Path. 17: 571–577(1947).

Neveu, P.J.; Lagrange, P.H.; Thierry, D.; Perdoux, D.: Diethyl-dithiocarbamate de sodium et système réticulo-endothélial. Nouv. Revue fr. Hémat. 22: C139 (1980).