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Short communication

The distribution of *o,p'*-DDD (Mitotane) among serum lipoproteins in normo- and hypertriglyceridemia

D. O. E. Gebhardt[1], A. J. Moolenaar[1], A. P. van Seters[2], E. A. van der Velde[3], and J. A. Gevers Leuven[4]

[1] Department of Clinical Chemistry and
[2] Department of Endocrinology, University Hospital, 2333 AA Leiden, The Netherlands
[3] Department of Medical Statistics, University of Leiden, The Netherlands
[4] Gaubius Laboratory, IVVO-TNO, Leiden, The Netherlands

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Summary. We found that the distribution of the lipophilic chemotherapeutic agent *o,p'*-DDD (mitotane) among serum (lipo)proteins was altered in hypertriglyceridemia, with relatively more *o,p'*-DDD accumulating in the chylomicron and very-low-density lipoprotein (VLDL) fraction. Intralipid, an artificial chylomicron emulsion, or isolated VLDL could extract *o,p'*-DDD from the other serum (lipo)proteins. There was an inverse relationship between the relative amount of *o,p'*-DDD found in the fraction exhibiting a density of <1.006 g/ml (chylomicrons plus VLDL) and the relative amount observed in the LDL or HDL fractions of serum. Our results indicate that hypertriglyceridemia may impede the entry of *o,p'*-DDD into the brain or the adrenals. For therapeutic monitoring of *o,p'*-DDD levels in severe hypertriglyceridemia, we recommend that the chylomicron and VLDL fraction first be removed from the serum by ultracentrifugation.

Introduction

The compound *o,p'*-DDD (mitotane or lysodren) is related to the insecticide dichloro-diphenyl-trichloroethane DDT [5]. It is used in the treatment of adrenal cortical carcinoma [6] and, like DDT, it is strongly lipophilic. In blood, such substances are often partitioned into the hydrophobic core of lipoproteins or are attached to albumin by means of low-affinity hydrophobic bonding [9, 11, 22].

The distribution of a variety of lipophilic substances such as benzo(a)pyrene [21, 25], cyclosporine [20], dioxin [10], and tocopherol [1] among serum lipoproteins has been determined both in normolipidemia and in hyperlipidemia. However, such data have not yet been published for *o,p'*-DDD. We therefore decided to study its distribution among the serum (lipo)proteins in normo- and hypertriglyceridemia.

Offprint requests to: D. O. E. Gebhardt, Department of Clinical Chemistry, Building 1, E-2-P University Hospital, 2333 AA Leiden, The Netherlands

Materials and methods

Serum samples were obtained following an overnight fast from 11 patients who were being treated with *o,p'*-DDD for adrenal cortical carcinoma. The samples were stored at –20°C for not longer than 5 months. It has been established that such storage does not change the lipoprotein profile by >15% [19]. Two to four samples were taken from each subject for the study, usually at 2-week or monthly intervals. Patients 1–5, 9, and 10 were considered to be normotriglyceridemic, exhibiting serum triglyceride concentrations of <2 mmol/l, and subjects 6–8 and 11 were considered to be hypertriglyceridemic, as their serum triglyceride levels lay above this reference value. Patient 8 displayed exceptionally high serum triglyceride values, which occasionally reached 15 mmol/l (Fig. 6).

For the determinations shown in Figs. 1–5, the serum lipoproteins were separated into three fractions: (1) the chylomicron plus very-low-density lipoprotein (VLDL) fraction; (2) the low-density lipoprotein (LDL) fraction; and (3) the high-density lipoprotein (HDL) plus rest fraction, the latter comprising albumin and all other serum proteins. Fractionation was performed by sequential flotation centrifugation using a Beckman TL-100 Tabletop ultracentrifuge according to our modification [4] of the method previously described by Naito [13]. Essentially, this procedure consists of adding 0.5 ml 0.9% NaCl solution to 0.5 ml serum and centrifuging the mixture in a 3-ml centrifuge tube at 20°C for 2 h at 541,000 *g* using the TL-100.3 rotor. The top 0.5 ml then contains the chylomicrons and VLDL. The 0.5-ml infranatant consisting of LDL and HDL plus the other serum proteins is mixed with 0.5 ml 16.8% NaCl solution and centrifuged for another 2 h at 541,000 *g*. This causes the LDL (0.5 ml) to float above the 0.5-ml infranatant in which the HDL plus rest fraction is present. The data shown in Fig. 6 were obtained following density-gradient ultracentrifugation [18], which separates HDL from the other serum proteins (rest fraction).

The *o,p'*-DDD content of the sera was determined by gas-liquid chromatography [12]. With the exception of sera from patient 8, whose *o,p'*-DDD concentration occasionally reached 70 mg/l, the concentration of *o,p'*-DDD lay in the range of 15–25 mg/l. (Such levels are usually attained after about 2 months of oral intake.) In general, higher serum concentrations cause severe neurological side effects [23, 24]. The extraction experiments using Intralipid or VLDL (Fig. 2) were carried out on serum samples from which the chylomicron and VLDL fraction had been removed by ultracentrifugation. To 1.9 ml of such a sample, which contained about 20 mg mitotane/ml, 50 µl Intralipid was added and the mixture was thoroughly vortexed.

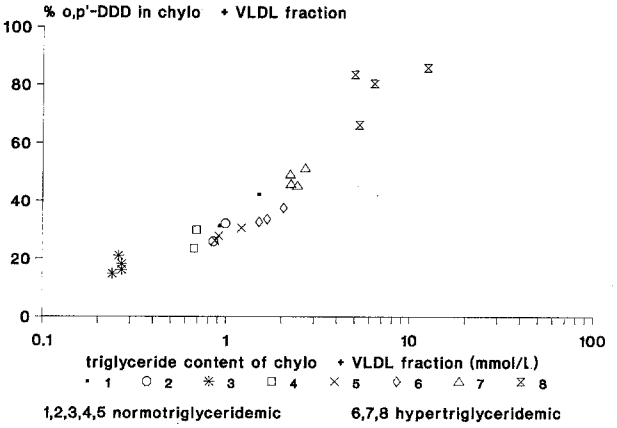

Fig. 1. Relationship between the percentage of *o,p'*-DDD in the chylomicron (*chylo*) plus VLDL fraction and the triglyceride content of this fraction

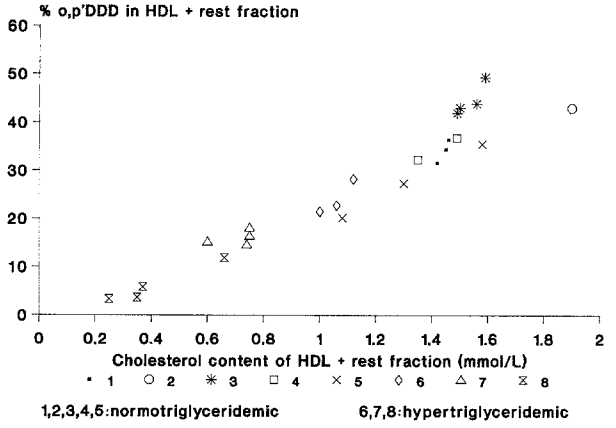

Fig. 3. Relationship between the percentage of *o,p'*-DDD in the HDL + rest fraction and the cholesterol content of this fraction

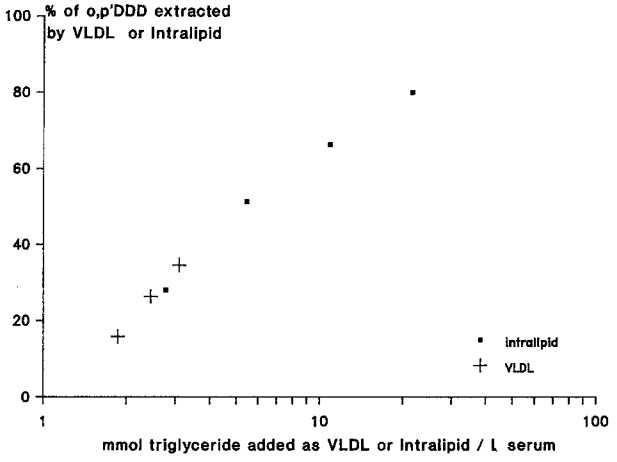

Fig. 2. Relationship between the percentage of *o,p'*-DDD extracted by VLDL or Intralipid and the amount of triglyceride added as VLDL or Intralipid

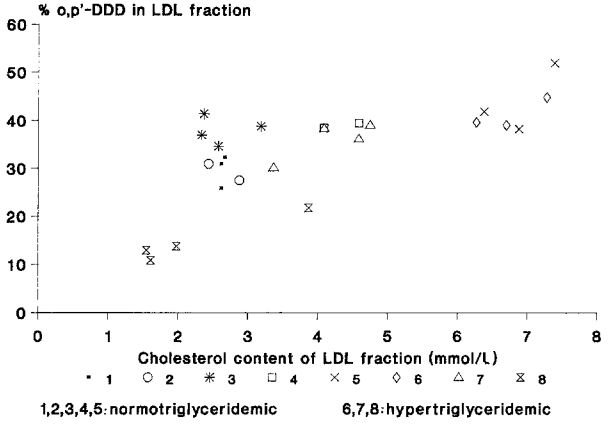

Fig. 4. Relationship between the percentage of *o,p'*-DDD in the LDL fraction and the cholesterol content of this fraction

The Intralipid emulsion used in these studies was prepared by diluting 1 ml Intralipid (20%) with 4 ml saline. Intralipid is an artificial chylomicron emulsion that was obtained from KabiVitrum, Stockholm. In the experiment using VLDL, the latter was first isolated from sera from normal individuals.

After the mixture of Intralipid (or VLDL) and serum had been kept at room temperature for 10 min, the chylomicron or VLDL fraction was again removed by ultracentrifugation. The *o,p'*-DDD content of the infranatant was then determined as described above. The cholesterol and triglyceride content of the various lipoprotein fractions was obtained using the Technicon RA-1000 system (Technicon Instruments Co., Tarrytown, N. Y.), which applies an enzyme analysis method.

To visualize the relationship, we constructed scattergrams in which all observations were plotted using a separate symbol for each patient's serum. For statistical analysis, the observed values were averaged for every patient and computation of the correlation coefficient was based on these means. In this way, each patient represented one observational unit in the correlation analysis.

Results

In Fig. 1, the percentage of the total serum concentration of *o,p'*-DDD in the chylomicron plus VLDL fraction is shown as a function of the triglyceride content of this fraction. The correlation coefficient between the two variables was $r = 0.94$ ($P < 0.001$). In view of this result, is seemed likely that chylomicrons and VLDL could extract *o,p'*-DDD from the other serum (lipo)proteins. The data presented in Fig. 2, in which experiment Intralipid or VLDL was used as the extraction agent, confirm this assumption.

The relationship between the percentage of *o,p'*-DDD in the HDL plus rest fraction is illustrated in Fig. 3 as a function of the cholesterol content of this fraction ($r = 0.96$; $P < 0.001$). Similarly, Fig. 4 shows the relationship between the percentage of *o,p'*-DDD in the LDL fraction and the cholesterol content of this fraction. In this case, the correlation coefficient was somewhat lower ($r = 0.75$; $P = 0.03$).

The relationship between the percentage of *o,p'*-DDD in the HDL plus rest fraction versus that in the chylomicron plus VLDL fraction is presented in Fig. 5 ($r = -0.91$; $P < 0.001$). A high negative coefficient of correlation was also found when the percentage of *o,p'*-DDD in the chylomicron plus VLDL fraction was plotted against that in the LDL fraction ($r = -0.84$; $P = 0.005$). Finally, Fig. 6 shows the absolute amount of *o,p'*-DDD in each lipo-

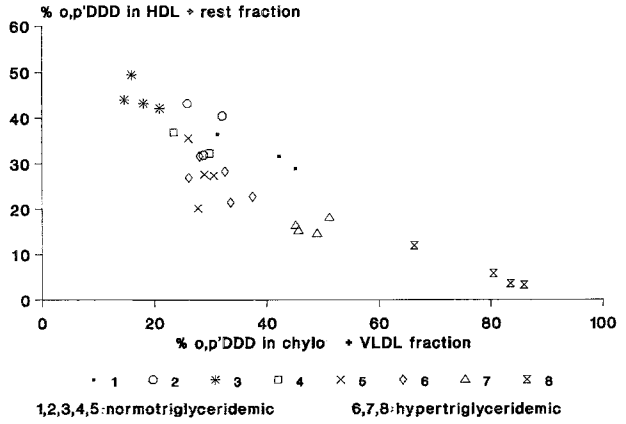

Fig. 5. Relationship between the percentage of *o,p'*-DDD in the HDL + rest fraction and that in the chylomicron (*chylo*) + VLDL fraction

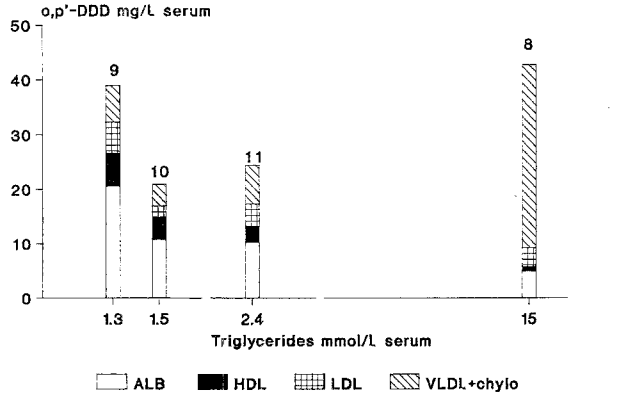

Fig. 6. Amounts of *o,p'*-DDD (mg/l) found in the various (lipo)protein fractions of the sera from patients 8–11 as a function of their triglyceride content. *ALB*, Albumin

protein fraction and in the albumin (or rest) fraction from four patients who differed in their serum triglyceride content. (The recovery of *o,p'*-DDD from the various (lipo)protein fractions relative to the total amount determined in the serum was in the range of 96%–103%.)

Discussion

Our results indicate that hypertriglyceridemia is associated with an increase in the percentage of *o,p'*-DDD in the triglyceride-rich lipoprotein fraction of serum. In this respect, our results are in agreement with the work of various other authors, whose studies concerned the distribution of a number of lipophilic agents among serum lipoproteins [1, 10, 20, 21].

It is interesting that there was an opposing tendency between the values shown in Fig. 1 and those presented in Fig. 3. For instance, the serum from patient 8 contained most of the *o,p'*-DDD in the chylo + VLDL fraction and only very little in the HDL fraction. This tendency, which is confirmed in Fig. 5, is caused by the negative correlation between HDL_2 levels and the degree of triglyceridemia [2]. Similarly, an inverse relationship has been reported between the concentrations of VLDL vs LDL [27], which explains the negative correlation coefficient found in the present study between the percentage of *o,p'*-DDD in the LDL vs VLDL fractions ($r = -0.84$).

Verril et al. [26] reported that toxic serum concentrations of the lipophilic drug cyclosporine did not cause any damage in type V hyperlipoproteinemia (hyperchylomicronemia). These authors state: "It was decided not to decrease the dosage because it was assumed that most of the cyclosporine in the plasma was bound to the chylomicrons and was therefore unavailable to produce nephrotoxicity." According to Fig. 6, patients 8 and 9 should have shown signs of neurotoxicity, since their serum concentration of *o,p'*-DDD exceeded 30 mg/l. In fact, van Slooten et al. [24] write: "At serum levels of over 20 mg/l a gradual increase in neuromuscular toxicity occurs. At levels [of] over 30 mg/l intolerable symptoms appear." On enquiry, we learned that severe neurotoxicity had occurred in patient 9 but not in patient 8. The following explanation for such a difference in response seems plausible.

Pardrige and Mietus [16] have shown that both the free (dialyzable) fraction of a steroid hormone and the albumin-bound fraction can cross the rat blood-brain barrier (BBB). In the case of *o,p'*-DDD, the free moiety is negligible (unpublished data); therefore, transport of the drug to the brain depends on the amount bound to the (lipo)proteins that can cross the BBB. In analogy with the rat BBB, it is most likely that the *o,p'*-DDD bound to albumin will cross the human BBB. Since the BBB is a sieve whose molecular-weight cut-off point is about 50,000 Da [14], *o,p'*-DDD bound to lipoproteins cannot easily cross this barrier. An exception might involve transport via the LDL receptor in the brain. Although Pardrige [15] has found little evidence for such a mechanism, it has recently been reported that in the hamster, small amounts of LDL do cross the BBB by means of the LDL receptor [8]. It is clear that in severe hypertriglyceridemia, in which the content of VLDL is much higher than that of LDL, the amount of *o,p'*-DDD in the VLDL fraction does not contribute to the neurotoxic side effects.

Related to this problem is the question as to which vehicle [i.e., which (lipo)protein] is most suitable for the introduction of the lipophilic, water-insoluble cytostatic agent *o,p'*-DDD into the adrenals. Under normolipidemic conditions, a substantial amount of the *o,p'*-DDD is bound to HDL and albumin (Fig. 3, 6). Pohland and Counsell [17] have shown that *o,p'*-DDD complexed to HDL can enter the adrenal cell of the rat, even if the drug partially prevents the recognition of the HDL receptor. The *o,p'*-DDD bound to albumin may pass into the adrenal cells by diffusion. However, the best mode of entry of *o,p'*-DDD most likely involves its adherence to LDL [3]. Furthermore, *o,p'*-DDD induces the formation of LDL [7], and this also probably promotes its uptake into the adrenals. On the other hand, VLDL is not incorporated into the human adrenal [3]. In cases of hypertriglyceridemia, which involves an increase in the amount of this lipoprotein and a decrease in the LDL concentration [27], much less *o,p'*-DDD is available for entry into the adrenals or the brain. Under such conditions, it seems advisable to monitor *o,p'*-DDD levels only after removal of the chylomicron plus VLDL fraction. A

favorable effect may be expected if the patient is also given a drug to alleviate the hypertriglyceridemia.

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