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International Journal of Pharmaceutics 333 (2007) 1–4
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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# The effect of a high-fat meal on the pharmacodynamics of a model lipophilic compound that binds extensively to triglyceride-rich lipoproteins

Pavel Gershkovich, Daniel Shtainer, Amnon Hoffman *

*Department of Pharmaceutics, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

Received 9 October 2006; received in revised form 1 January 2007; accepted 13 January 2007
Available online 19 January 2007

**Abstract**

A high-fat meal induces transient hyperlipidemia characterized by elevated triglyceride-rich lipoproteins (TRL) which are composed mainly of chylomicrons. The purpose of this work was to investigate the effect of this transient hyperlipidemia on the pharmacodynamics of lipophilic drugs, using DDT as a model compound since it binds extensively to TRL and has a distinct neurotoxic effect. The postprandial hyperlipidemia in rats was induced by oral administration of peanut oil and was monitored by measurement of plasma triglyceride levels. The control group received water instead of oil. The rats received a continuous intravenous infusion of DDT (10 mg/h) until onset of a predefined pharmacodynamic endpoint (facial muscle tremor). Plasma and brain samples were then obtained and assayed for DDT. Rats with postprandial hyperlipidemia required higher dose of DDT to induce onset of facial muscle tremor. At the pharmacodynamic endpoint, oil treated rats had significantly higher concentrations of DDT in plasma and in the chylomicron fraction, but DDT brain concentrations were the same in both groups. In conclusion, a high-fat meal induces postprandial hyperlipidemia that may significantly alter the pharmacological profile of lipophilic compounds that bind to TRL. This is due to alteration of the distribution characteristics of the lipophilic compound through its association with postprandial lipoproteins. However, this pharmacokinetic phenomenon did not affect the concentration–effect relationship at the site of action in the brain.


*Keywords:* Pharmacodynamics; Pharmacokinetics; Food–drug interaction; Chylomicrons; Neurotoxicity; Lipophilic drugs

## 1. Introduction

The association of drugs with plasma lipoproteins is a widely studied phenomenon which may have pharmacokinetic and pharmacodynamic consequences (Wasan, 1996; Humberstone et al., 1998b; Wasan and Cassidy, 1998; Wasan et al., 2002, 2006; McIntosh et al., 2004b; Procyshyn et al., 2005; Shayeganpour et al., 2005; Brocks et al., 2006). Most of the work in this area has focused on association of drugs with lipoproteins that circulate in plasma under fasting conditions (i.e. LDL and HDL) that characterize different pathological dyslipidemias. However, the data on the acute effect of postprandial transient hyperlipidemia, characterized by transient elevation in triglyceride-rich lipoproteins (TRL) in plasma (mostly chylomicrons (CM) and VLDL), is scarce. This postprandial hyperlipidemia is a physiological phenomenon, and thus, a common condition, especially after consumption of high-fat meals. Although there are a small number of reports that have considered pharmacokinetic consequences of association of lipophilic compounds with TRL (mostly a decrease in volume of distribution and clearance) (Humberstone et al., 1998b; Brocks and Wasan, 2002; Brocks et al., 2006), the data that has taken into account the pharmacodynamic consequences of uptake of lipophilic compounds by TRL is extremely limited and controversial. In one study, it was found that probucol, a lipophilic drug that significantly binds to TRL, increased the QT interval in monkeys when given with a high-fat diet (Eder, 1982). In contrast, it was found in another work that hyperlipidemia caused by intralipid infusion to rabbits decreased the QT interval prolongation in comparison to the normolipidemic rabbits following intravenous administration of halofantrine (McIntosh et al., 2004a). In addition, it was found that high TRL levels in serum increased the IC50 of

*Abbreviations:* TRL, triglyceride-rich lipoproteins; TG, triglycerides; CM, chylomicrons; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; $f_u$, fraction unbound

* Corresponding author at: Department of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem, P.O. Box 12065, Jerusalem 91120, Israel. Tel.: +972 2 6757014; fax: +972 2 6757246.
*E-mail address:* ahoffman@cc.huji.ac.il (A. Hoffman).

0378-5173/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2007.01.012

halofantrine when tested in-vitro on a culture of *Plasmodium falciparum* (Humberstone et al., 1998a). Given these limited and controversial pieces of information, the purpose of this work was to explore the effect of association of lipophilic compounds with TRL in plasma following a high-fat meal on the kinetics of pharmacological action.

The experimental design utilized here is based on the long list of similar investigations by Levy on the kinetics of action of neuroactive compounds in disease states. It compares the concentration of the drug at various biological fluids and tissues at the onset of a predefined pharmacological endpoint (Hisaoka and Levy, 1985; Ramzan and Levy, 1985; Hoffman and Levy, 1993).

## 2. Materials and methods

### 2.1. Materials

Testosterone, 1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane (DDT) and peanut oil (Sigma–Aldrich, Rehovot, Israel) were all used as received. Diazepam was kindly provided by Taro Pharmaceutical Industries Ltd. (Haifa, Israel). All other chemicals were of analytical reagent grade, and solvents were of HPLC grade.

### 2.2. Animal studies

The research protocol was approved by the Animal Experimentation Ethics Committee of the Hebrew University Hadassah Medical School in Jerusalem. Male Wistar rats weighting 300–350 g (Harlan, Israel) were used in these studies. The right jugular vein was cannulated using PE50 tubing. Following the surgery, rats were transferred to metabolic cages and fasted overnight with free access to drinking water. On the next day, animals were allocated into two groups (oil treated and water treated groups) prior to the treatment. The oil treated group received 0.6 mL of peanut oil, followed by 1.4 mL of water by oral gavage 3, 2 and 1 h prior to the beginning of the infusion. The water treated group received 2 mL of water by oral gavage at the same time points. Twenty minutes prior to the experiment, rats were immobilized in a Broome rodent restrainer (Harvard Apparatus Inc., Holliston, MA) on a 37 °C isothermal pad. DDT solution (5 mg/mL in propylene glycol 90%, ethanol 10%) was infused into the jugular vein at a rate of 10 mg/h until onset of the pharmacodynamic endpoint presented as bilateral tremor of facial muscles in the area of eyes and nose (an early neurotoxic symptom of acute DDT intoxication (Black and Ecobichon, 1971)). This pharmacodynamic endpoint was predefined by several blinded investigators (i.e. unaware to which group each rat belonged). At the onset of facial tremor, rats were anesthetized quickly by isoflurane, and terminal blood collection (8–10 mL) was obtained from the caudal vena cava. The brain was removed and stripped of its external vasculature and meninges. The plasma was separated by centrifugation (800 × *g*, 5 min, 15 °C). Two millilitres of plasma were subjected to density gradient ultracentrifugation for separation of CM. The rest of plasma, brain tissue and CM fractions of ultracentrifuged plasma were stored at −20 °C until analysis.

### 2.3. Analytical procedures

The analysis was performed using a Waters 2695 Separation Module HPLC system with Waters 2996 Photodiode Array Detector (Waters Corporation, Milford, MA).

The determination of DDT concentrations in CM emulsion was performed as previously described (Gershkovich and Hoffman, 2005). For the determination of DDT concentrations in plasma samples, the same method as for CM emulsion was implemented. The minimum quantifiable concentration of DDT in plasma was 30 ng/mL. Calibration curves were linear in the range of 30–10,000 ng/mL. The intra- and inter-day coefficients of variation were below 2%. For determination of DDT concentrations in brain tissue, brain samples (approximately 250 mg) were accurately weighed. Seven hundred microlitres of acetonitrile containing internal standard were added to the brain tissue and homogenized by a Polytron PT 1200 homogenizer (Kinematica, Switzerland) for 2 min in the ice bath. After vortex mixing with 4 mL of hexane for 2 min and centrifugation (1050 × *g*, 7 min), the upper organic layer was decanted to a fresh test tube and evaporated similarly to the plasma and CM emulsion samples. The residue was reconstituted with 120 μL of acetonitrile and 80 μL were injected into HPLC system. The minimum quantifiable concentration of DDT in brain tissue was 20 ng/g. Calibration curves were linear in the range of 20–20,000 ng/g in brain tissue. The inter- and intra-day coefficients of variation were below 3%.

TG concentrations in plasma and plasma CM fraction were measured as previously described (Gershkovich and Hoffman, 2005).

### 2.4. Statistical analysis

The data is presented as mean ± S.E.M., if not specified otherwise. The statistical significance of the differences between groups was determined using unpaired Student *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

## 3. Results and discussion

The triple oral administration of peanut oil resulted in a significant increase in total plasma TG levels as compared to the water treated rats. This rise in TG was demonstrated at the pharmacodynamic endpoint (Fig. 1). The mechanism of peanut oil absorption is via assembly of CM in the enterocytes and subsequent transport to the systemic circulation by the lymphatic drainage. As evident in Fig. 1 the increase in TG concentration is contributed by both chylomicrons and TG derived from the chylomicron-free fraction of plasma. This result is expected, since the first peanut oil dose was administered 3 h prior to the initiation of DDT infusion, and other TRL (e.g. CM remnants or VLDL) were circulating in plasma at the moment of onset of pharmacodynamic endpoint. In addition, it is possible that non-lipoprotein associated TG could be also elevated in hyper-

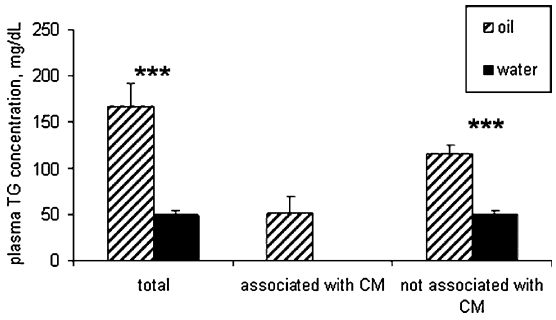


Fig. 1. Plasma TG concentrations (mean ± S.E.M.) at the time of the pharmacodynamic endpoint. After triple oral administration of peanut oil ($n = 11$) or water ($n = 11$) DDT was intravenously infused (10 mg/h) to rats until the onset of bilateral facial muscle tremor (pharmacodynamic endpoint), $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

lipidemic rats, since there is evidence that some amount of long chain fatty acids may bypass the lymphatic pathway (Thomson et al., 1989; Ramirez et al., 2001).

The effect of postprandial hyperlipidemia on the kinetics of action of DDT-induced neurotoxicity was examined using an experimental setup in which a predetermined pharmacodynamic endpoint (onset of facial muscle tremor) was used to compare hyperlipidemic versus normolipidemic rats. This experimental design allows efficient detection of the concentration–effect relationship since it uses a fixed pharmacodynamic endpoint. It enables the differentiation between pharmacokinetic effects imposed by the hyperlipidemia versus changes in the concentration at the site of action required to induce the pharmacological effect. We chose DDT as a model compound for the following reasons: (1) DDT has been shown to have a high degree of association with plasma derived CM (Gershkovich and Hoffman, 2005); (2) DDT induces acute neurotoxic symptoms that are clearly defined (Ecobichon and Saschenbrecker, 1968; Henderson and Woolley, 1970; Black and Ecobichon, 1971). The onset of facial muscle tremor was selected as a pharmacodynamic endpoint because it was found to be in good correlation with brain DDT concentrations (Henderson and Woolley, 1970).

Oil treated rats required a significantly higher dose of DDT in order to provoke the onset of bilateral facial muscle tremor in comparison to the control group (Fig. 2). The difference between total DDT plasma concentrations at the pharmacodynamic endpoint of the two groups is even more pronounced than the difference in cumulative dose (Fig. 3). This is probably due to the combined effect of higher dose and lower volume of distribution and clearance in oil treated rats due to the association of DDT with TRL in plasma. An attempt to measure the free concentrations of DDT was made in this study; however, DDT is a compound with very high plasma protein binding. As a result, the free concentrations were below the limit of quantification of the analytical method.

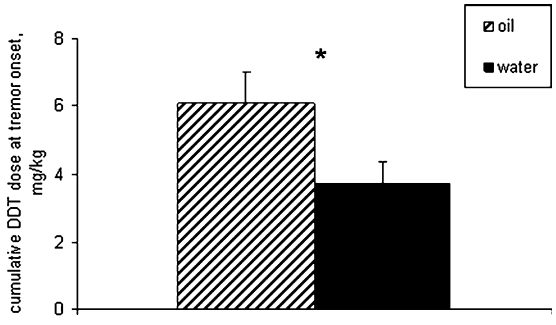


Fig. 2. Cumulative dose (mean ± S.E.M.) of DDT required to induce the pharmacodynamic endpoint in animals pretreated with peanut oil ($n = 11$) or water ($n = 11$). DDT was intravenously infused (10 mg/h) to rats until the onset of bilateral facial muscle tremor, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

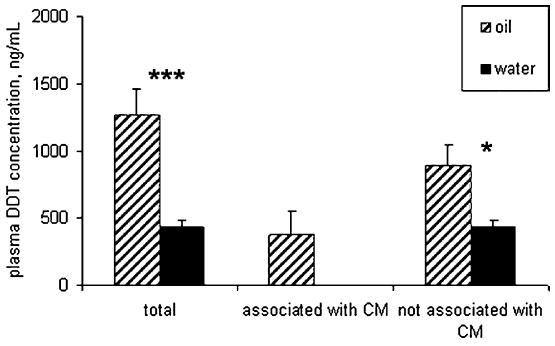


Fig. 3. Plasma DDT concentrations (mean ± S.E.M.) at pharmacodynamic endpoint. See details in Figs. 1 and 2, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

The apparent similarity in the pattern of the plasma DDT concentrations vs. time and TG concentrations vs. time profiles (Figs. 1 and 3) is noteworthy. Total DDT plasma concentrations as well as the concentrations associated with CM and those that are not associated with CM were all found to be significantly higher than the corresponding plasma concentrations in the water treated group. However, despite these pronounced differences in plasma DDT concentrations, the brain concentrations in the oil and water treated rats at the pharmacodynamic endpoint were not different (Fig. 4). These results are in agreement with another study, where lower dose of heptabarbital were needed to induce the same cerebrospinal fluid concentration and similar nervous system depression in hypoalbuminemic versus normal rats (Hoffman and Levy, 1989).

The increase in TRL levels in the plasma following oil administration enhances the degree of uptake of DDT by the lipophilic core of the lipoproteins and thus decreases the unbound fraction ($f_u$). As a result, higher total plasma concentration of DDT is needed in hyperlipidemic rats to maintain the same free concentration as in normolipidemic animals. The free DDT concentrations are in rapid equilibrium with the brain tissue as

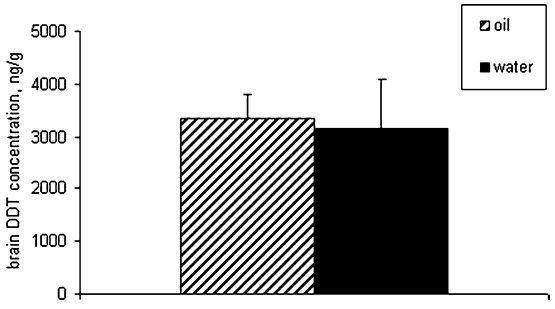


Fig. 4. DDT concentrations in brain at the pharmacodynamic endpoint. See details in Figs. 1 and 2, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

indicated by the equal total brain concentrations at the onset of the pharmacodynamic endpoint. Thus, higher total plasma concentrations of DDT are required to produce the same brain concentrations (and the same effect) in hyperlipidemic versus normolipidemic rats.

## 4. Conclusion

Taken together, these results show that for lipophilic compounds which bind extensively to TRL, significantly higher total plasma concentrations are needed in hyperlipidemic versus normolipidemic conditions to attain the same concentrations in peripheral tissues. Thus, following a high-fat meal, a larger dose of the lipophilic drug may be required to reach the same pharmacological effect. However, it should be noted, that lipoprotein receptor mediated uptake may also attenuate the pharmacological action of a lipophilic drug associated with TRL. In this case, the effect of postprandial hyperlipidemia on the pharmacological action of lipophilic compound will be more complicated and less predictable. Further work is needed to confirm these assumptions.

## Acknowledgements

This paper is a part of Pavel Gershkovich's PhD dissertation. This study was partially supported by Israeli Consortium of Pharmalogica. We would like to thank Joseph Fanous for excellent technical assistance and Dr. Josh Backon for constructive comments. Prof. Amnon Hoffman is affiliated with the David R. Bloom Center for Pharmacy at The Hebrew University of Jerusalem.

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