

Dyslipidemia causes overestimation of plasma mitotane measurements

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Summary

Mitotane (*o,p'*-DDD) is the standard treatment for advanced adrenocortical carcinoma (ACC). Monitoring of plasma mitotane levels is recommended to look for a therapeutic window between 14 and 20 mg/L, but its positive predictive value requires optimization. We report the case of an ACC patient with a history of dyslipidemia treated with mitotane in whom several plasma mitotane levels >30 mg/L were found together with an excellent neurological tolerance. This observation led us to compare theoretical or measured *o,p'*-DDD and *o,p'*-DDE levels in a series of normolipidemic and dyslipidemic plasma samples to explore potential analytical issues responsible for an overestimation of plasma mitotane levels. We demonstrate an overestimation of mitotane measurements in dyslipidemic patients. Mitotane and *o,p'*-DDE measurements showed a mean 20% overestimation in hypercholesterolemic and hypertriglyceridemic plasma, compared with normolipidemic plasma. The internal standard *p,p'*-DDE measurements showed a parallel decrease in hypercholesterolemic and hypertriglyceridemic plasma, suggesting a matrix effect. Finally, diluting plasma samples and/or using phospholipid removal cartridges allowed correcting such interference.

Learning points:

- Hypercholesterolemia (HCH) and hypertriglyceridemia (HTG) induce an overestimation of plasma mitotane measurements.
- We propose a routine monitoring of lipidemic status.
- We propose optimized methodology of measurement before interpreting high plasma mitotane levels.

Background

Monitoring plasma mitotane levels is recommended in the follow-up of patients with unresectable adrenocortical carcinoma (ACC) to look for a therapeutic window of 14–20 mg/L to optimize benefit over risk and

avoid toxicities (1). Mitotane is a lipophilic drug that accumulates in lipoproteins and induces dyslipidemia (hypercholesterolemia (HCH) and/or hypertriglyceridemia (HTG)) (2, 3, 4, 5). Previous studies suggested that



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the lipoprotein profile may influence mitotane drug distribution (6). Moreover, high plasma mitotane levels have been described in dyslipidemic patients who did not exhibit any side effects, suggesting either methodological issues, or that plasma mitotane distribution in lipoprotein subtypes is a major determinant of its distribution in tissues (7). Indeed, we recently reported that plasma mitotane level was correlated with *o,p'*-DDD measured in HDL and LDL fractions (8).

Here, we report the case of an ACC patient with a severe dyslipidemia. Plasma mitotane monitoring revealed very high levels, that is, >30 mg/L, while our patient did not present any neurological side effects. Considering this observation, we demonstrate that dyslipidemia causes an overestimation of plasma mitotane levels explained by a so-called matrix effect.

Case presentation

Mr A, a 39-year-old man was referred in our institution in July 2007 for the therapeutic management of a functioning stage IV ACC with lung metastases. The main characteristics at the time of his first visit were a R0 ACC primary removal and a Weiss score of 6 including <5 mitosis per 50 high-power field (HPF). Previous history included obesity. During the 90-month of the follow-up period with mitotane therapy, 19 out of 103 plasma mitotane levels were found >20 mg/L including 9 >30 mg/L (up to 78 mg/L). Several sequences of radiofrequency or cytotoxic chemotherapies were used. Best morphological response according to response evaluation criteria in solid tumours (RECIST) 1.0 criteria was stable disease at

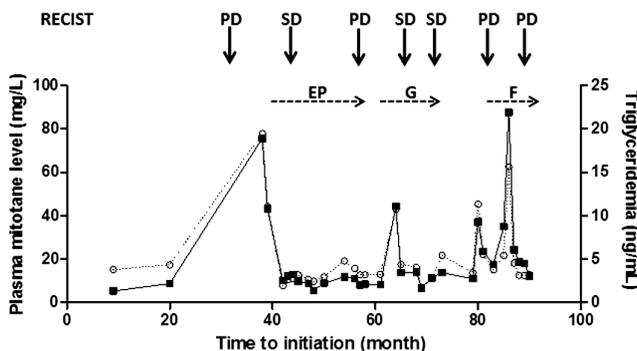


Figure 1
Monitoring of Mr A during mitotane therapy. Plasma mitotane level in mg/L (white circles) and triglyceridemia in ng/mL (black squares). Plasma mitotane measurements were performed using HPLC-UV; triglycerides measurements were performed using Unicel DXC Beckman Coulter Automate (Marseille, France). EP, etoposide-platin; F, fluorouracil; G, gemcitabine; PD, progressive disease; SD, stable disease.

the time of gemcitabine-based chemotherapy sequence as indicated in Fig. 1. Surprisingly, no single-grade 2–4 neurological adverse event was observed according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4 (NCI-CTCAE) criteria. At 3 months of mitotane therapy, an increase in LDL-cholesterol was found and resolved after the introduction of statins in September 2008. From 2008 onward, several triglyceride measurements were performed at the time of evaluation of plasma mitotane level. As shown in Fig. 1, a narrow parallelism was observed between the two curves. This observation led us to explore potential analytical issues responsible for an overestimation of plasma mitotane levels in patients with dyslipidemia.

Investigation

Influence of HCH and HTG on plasma *o,p'*-DDD and *o,p'*-DDE measurements

In routine practice, plasma mitotane concentrations are assayed by HPLC-UV using *p,p'*-DDE as an internal standard (IS). Plasma samples from normolipidemic (cholesterol <1 g/L and triglyceride <1 g/L) and dyslipidemic patients provided by various hospitals were spiked with fixed known concentrations of *o,p'*-DDD, *o,p'*-DDE, and *p,p'*-DDE. Mitotane and its metabolite *o,p'*-DDE concentrations were determined through the ratio of their peak surface area to the peak surface area of the known concentration of *p,p'*-DDE. After a liquid–liquid extraction, *o,p'*-DDD and *o,p'*-DDE measurements were determined and compared with the known theoretical concentrations. We showed that HCH (Fig. 2A) and HTG (Fig. 2B) led to an overestimation (from 10 to 75%) of measured plasma mitotane levels as compared with theoretical concentrations. The overestimation was similar (from 5 to 66%) for *o,p'*-DDE, as shown in Fig. 2C and D. *o,p'*-DDE is routinely performed at our institution for research purpose and was studied in an attempt to be exhaustive. By contrast, the measured concentration of *p,p'*-DDE, used as internal standard, gradually decreased as the amount of HCH and HTG increased (Fig. 2E and F).

To confirm the matrix effect, HCH and HTG plasmas were first diluted with normolipidemic plasma without mitotane. Second, Phree phospholipid removal cartridges were used. In comparison with theoretical values, *p,p'*-DDE values were found underestimated with a mean of –21.1% in eight plasma samples with high levels of lipids. By contrast, same samples analyzed after Phree extraction were found similar to theoretical values (mean = –2.3%).

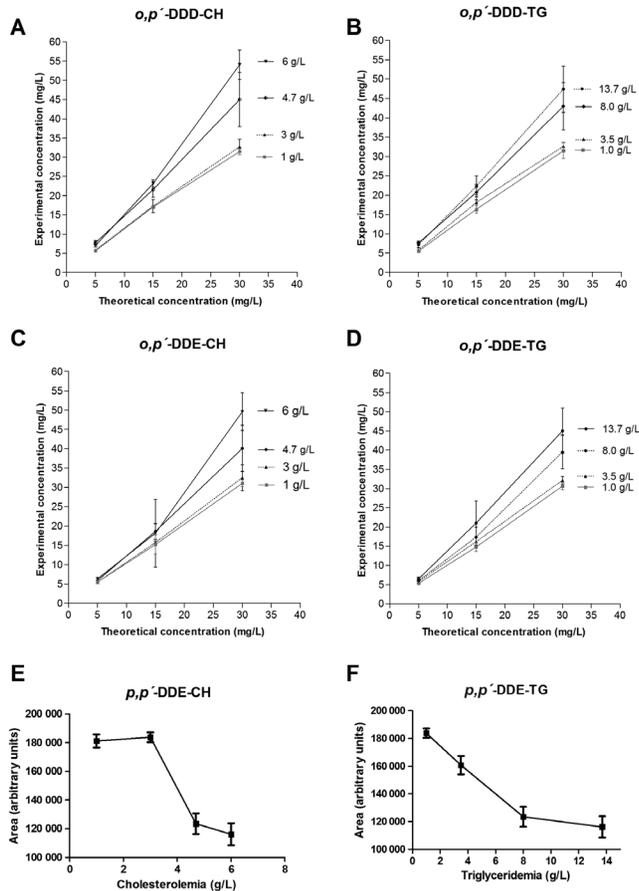


Figure 2 Impact of cholesterol concentration on measurements of plasma mitotane (*o,p'*-DDD: 2A) and its metabolite (*o,p'*-DDE: 2C) and the internal standard (*p,p'*-DDE: 2E) *in vitro*. Impact of triglyceride concentration on measurements of plasma mitotane (*o,p'*-DDD: 2B) and its metabolite (*o,p'*-DDE: 2D) and the internal standard (*p,p'*-DDE: 2F) *in vitro*. Measurements were performed in triplicate in each normal or dyslipidemic samples.

Discussion

We report for the first time an analytical overestimation of mitotane measurements in the plasma of dyslipidemic patients. From this case report, we hypothesized that a methodological issue could explain the absence of side effects in dyslipidemic patients with plasma mitotane levels >30 mg/L. Our study confirmed that mitotane and *o,p'*-DDE measurements were systematically overestimated by 10% when HCH was at 3 g/L and >50% when HCH was at 6 g/L. This overestimation was similar in HTG plasma (3.5–13.7 g/L). In clinical practice, these results indicate that cholesterol and triglyceride concentrations should be analyzed together with mitotane measurements on a regular basis to improve the accuracy of plasma mitotane measurements for a given

patient. Our results show that this so-called matrix effect phenomenon linked to a reduced extraction yield of *p,p'*-DDE is responsible for this overestimation. Indeed, the decrease in the area under curve of *p,p'*-DDE levels was proportional to the increases observed in *o,p'*-DDD and *o,p'*-DDE levels and normalized by diluting the plasma sample with normolipidemic plasma without mitotane or using phospholipid removal cartridges. At the end, mitotane-induced dyslipidemia may interfere with the tumor activity of the drug. Indeed, Hescot *et al.* previously supported that mitotane unbound to lipoprotein fractions is more efficient *in vitro* (8).

In conclusion, we demonstrate that HCH and/or HTG, a common side effect of mitotane, lead to an overestimation of its measurements suggesting that lipid alterations should be monitored in routine practice and optimized techniques should be implemented in patients with dyslipidemia.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this research reported.

Funding

S H was recipient of a fellowship from HRA Pharma Laboratories (Bourse CIFRE).

Author contribution statement

A P, S H, and E B designed the study. A P, S H, A S, C J, L M, and S B performed experiments and analyzed the results. A P, S H, D V, D D, M Q, M F, E B, M L, S L, S B, and E B contributed to interpretation of data and participated in discussion. S H, A P, and E B wrote the paper. All authors have read, revised, and approved the manuscript.

Acknowledgments

The authors are grateful to Lorna Saint-Ange for editing this manuscript.

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Received in final form 29 April 2016

Accepted 12 May 2016