9,13-Dicis-Retinoic Acid as an Isomerization Product of 9-cis-Retinoic Acid

With great interest we have read the article by Marchetti et al. on oxidation and isomerization of retinoic acid (RA) isomers in vitro, which was published in this journal recently (1).

However, there are more misleading statements in that publication, which require clarification.

For instance, more than the indicated “few studies” on the metabolism of 9-cis-RA exist. Among the literature not referred to are the isomerization of 9-cis-RA as published in this journal recently (1).

Marchetti et al., both in vivo and in vitro comparisons of biotransformation of 9-cis-RA, 13-cis-RA and all-trans-RA are available (3, 5–7). For instance, a higher degree of glucuronidation of 9-cis-RA and 13-cis-RA was observed in NMRI mice in vivo as compared with the all-trans-isomer, whereas all-trans-RA was oxidized to all-trans-4-oxo-RA to a higher extent than the 9-cis and 13-cis-isomers.

We consider a major shortcoming of the publication by Marchetti et al. (1) that isomerization of 9-cis-RA to 9,13-dicis-RA is completely ignored and profound transformation to 13-cis-RA is proposed instead. 9,13-dicis-RA has been identified as a major plasma metabolite of administered 9-cis-RA in mice and rats (3, 8, 9). Horst et al. demonstrated that 9,13-dicis-RA is a physiological constituent of neonatal calf plasma as well as of bovine plasma during the periparturient period (9, 10). In addition, 9,13-dicis-RA was found as a profound plasma retinoid in rabbit and human plasma after administration of retinyl palmitate and liver consumption, respectively (11, 12). 9,13-dicis-RA is also formed from 9-cis-RA in vivo after incubation with rat and bovine microsomes (6, 13).

Based on those results, it has to be expected that in the experiments described by Marchetti et al., a significant part of 9-cis-RA is transformed into 9,13-dicis-RA in analogy to the isomerization of 13-cis-RA to all-trans-RA, whereas the extent of the formation of 13-cis-RA (requiring two steps of isomerization) has probably been overestimated in this study (1). Although most other reversed-phase HPLC methods do not allow separation of 9,13-dicis-RA from 13-cis-RA (or 9-cis-RA), this can be achieved with a reversed-phase HPLC method (14), which enables the determination of four RA isomers as well as of three retinoyl-β-glucuronides and has already been applied for analysis of biological samples including microsomal preparations (4, 6, 12, 14). If HPLC methods that do not allow reliable separation of isomers are used, simultaneous detection at least two wavelengths is essential to detect peak impurities as caused by coeluting isomers. In addition, recording the UV-spectrum of the putative cis-isomer would provide valuable information on the identity of this retinoid, as has been done previously (3, 9, 11, 12). Doing so would certainly have indicated that the so-called “13-cis-RA” peak of Marchetti et al. (1) also contains great amounts of 9,13-dicis-RA.

It is questionable whether 9,13-dicis-RA has biological activity on its own or exerts activity via isomerization to 9-cis-RA and/or all-trans-RA only. In vivo formation of 9,13-dicis-RA is especially marked after administration of high doses of 9-cis-RA. This has led to the speculation that 9,13-dicis-RA might represent a detoxification product of 9-cis-RA or excess vitamin A (6).


Response to Letter

We have read with great interest Dr. Sass’ letter to the editor and we have the following comments:

First, concerning our statement on the “few studies” that exist on the metabolism of 9-cis-RA, we just wanted to say that 9-cis-RA has been the subject of many fewer studies than the two other isomers.

Second, when we stated that no other study on the comparative metabolism of all-trans-, 13-cis-, and 9-cis-RA existed, we were referring to the formation of oxo-metabolites. Among the publications cited by Dr. Sass (1–4), only that of Genchi et al. (4) has compared the metabolism of the three isomers simultaneously and only with respect to the formation of conjugated metabolites.

We were also aware that 9,13-di-cis-RA is the predominant polar plasma retinoid after administration of 9-cis-RAL, and a major metabolite, although not the main one, after administration of 9-cis-RA (2). Unfortunately we were unable to quantify this compound as we could not obtain any reference material. We therefore cannot assert that our method (5) allowed us to differentiate between 13-cis-RA and 9,13-di-cis RA. However, 9,13-di-cis is a minor metabolite in man, whereas 4-oxo metabolites are major ones (3). In addition, the microsomal model is more interesting for the study of oxidative metabolism than for the study of isomerisation. We therefore are focusing our interest on the formation of oxo-metabolites. The analytical method, developed in our laboratory (5), allows for the separation of the three 4-oxo metabolites, 4-oxo-all-trans-RA, 4-oxo-13-cis-RA, and 4-oxo-9-cis-RA.

Finally, we agree that a possible isomerization of 9-cis-RA to 9,13-di-cis-RA was not taken into account in our work. However, our study was not performed to answer this question. The aim of our study was to perform an intersex and interstrain comparison of rat metabolic behavior to validate the use of Hairless rats in pharmacokinetics and metabolic studies rather than to establish an exhaustive description of in vitro RA-metabolism.

Laboratoire de Toxicologie et Pharmacologie Clinique (M.-N.S.-M., E.S., H.S., B.L., A.D.)
Laboratoire de Pharmacocinetique et Toxicocinetique, (H.B.)
Faculte de Pharmacie Marseille, France

References


