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To the editor:

Dietary eicosapentaenoic acid (EPA) to produce antileukemic cyclopentenone prostaglandin J_3?

We read with great interest the article by Hedge et al,[1] reporting that Δ^{12}-prostaglandin (PG) J_3 (Δ^{12}-PGJ$_3$) has antileukemic activity in mice. Anti-inflammatory and antineoplastic activity has also been reported for 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ$_2$).[2] We

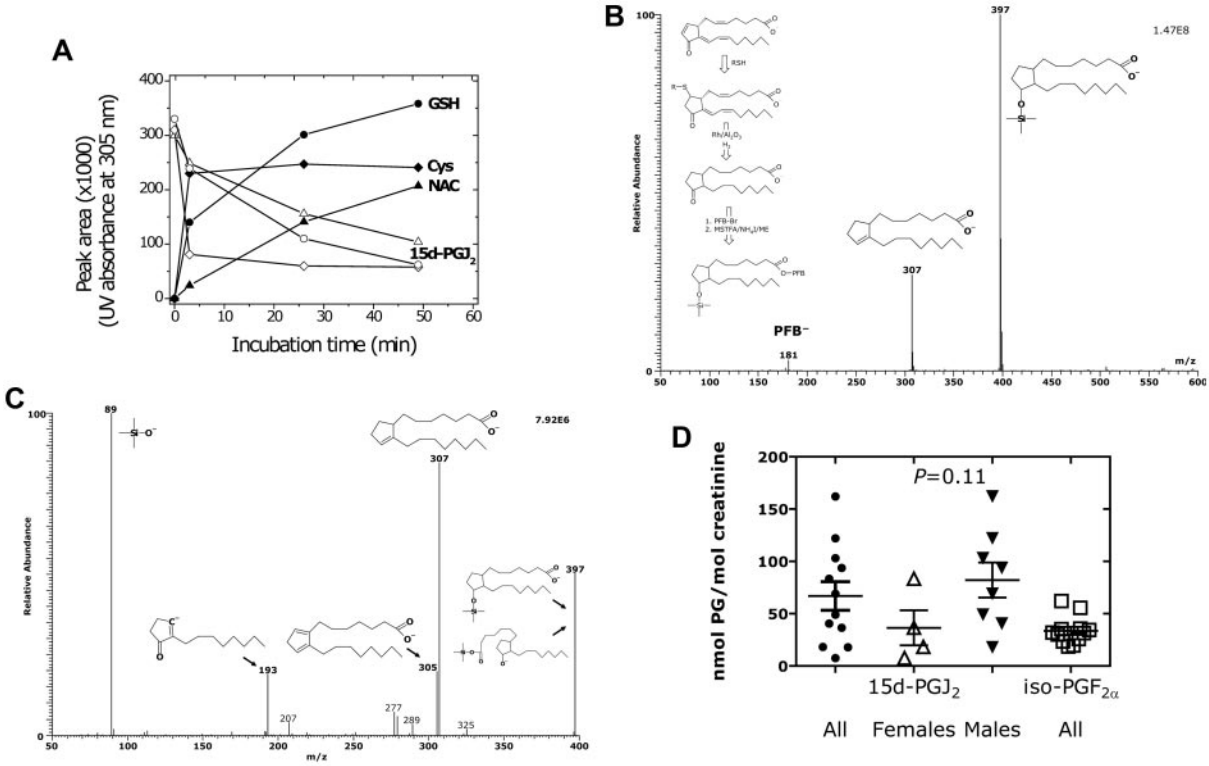

Figure 1. Excretion of 15d-PGJ$_2$ in human urine and its in vitro conjugation with glutathione, L-cysteine and *N*-acetylcysteine. (A) Reaction of 30μM 15d-PGJ$_2$ with each 1110μM glutathione (GSH), L-cysteine (Cys) or *N*-acetylcysteine (NAC) in 100mM phosphate buffer (pH 7.4) resulted in formation of the corresponding conjugates and concomitant decrease of 15d-PGJ$_2$ as measured by high-performance liquid chromatography (HPLC). Retention time was 12.7, 3.6, 2.8 and 1.2 minutes for 15d-PGJ$_2$ and the 15d-PGJ$_2$-NAC, 15d-PGJ$_2$-Cys, and 15d-PGJ$_2$-GSH conjugates, respectively. Reaction of 15d-PGJ$_2$ with Cys was accompanied by a shift of the maximum wavelength from 318 nm to 312 nm and an increase in absorbance at 230 nm. (B,C) The HPLC fractions of the above mentioned conjugates were collected and subjected to catalytical hydrogenation/desulfurization as described elsewhere for the cysteinyl leukotriene E_4.[5] After derivatization with pentafluorobenzyl (PFB) bromide (PFB-Br) followed by *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MTSFA) in the presence of NH_4I and 2-mercaptoethanol (ME), gas chromatography-mass spectrometry (GC-MS) spectra were generated in the electron-capture negative-ion chemical ionization mode (B). The precursor ion at *m/z* 397 [M-PFB]$^-$ was subjected to collision-induced dissociation (CID) to generate GC-tandem MS (GC-MS/MS) spectra (C). Expectedly, virtually identical GC-MS and GC-MS/MS mass spectra were obtained from all thiol (RSH) conjugates of 15d-PGJ$_2$. Inserts in panels B and C indicate schematically part of the analytical procedure used and the proposed structures for the ions obtained. (D) Excretion of 15d-PGJ$_2$ and the isoprostane 15(*S*)-8-*iso*-PGF$_{2\alpha}$ (iso-PGF$_{2\alpha}$) was measured in fresh spot urine samples of 12 healthy volunteers (4 females) by GC-MS/MS using $^2H_4$-15d-PGJ$_2$ and $^2H_4$-15(*S*)-8-*iso*-PGF$_{2\alpha}$ as internal standards. 15(*S*)-8-*iso*-PGF$_{2\alpha}$ was extracted from urine (1 mL) by immunoaffinity column chromatography.[6] 15d-PGJ$_2$ was extracted from acidified (pH 4.5) urine samples by solid-phase extraction and purified by isocratic reverse phase HPLC. In the urine samples no 15d-PGJ$_3$ was detectable. 15(*S*)-8-*iso*-PGF$_{2\alpha}$ was measured because it is considered a COX-independent metabolite, analogous to 15d-PGJ$_2$ and 15d-PGJ$_3$.

agree with Hedge et al[1] that one of the most important questions is whether sufficient quantities of Δ^{12}-PGJ_3 are formed in vivo to exert any biologic activity. Here, we comment on this eminently crucial issue from pharmacologic and nutrition perspectives.

PGJ_3 and PGJ_2 are the dehydrated products of PGD_3 and PGD_2 formed in vivo from eicosapentaenoic acid (EPA) and arachidonic acid (ARA), respectively, by the catalytic action of cyclooxygenase (COX). PGJ_3 and PGJ_2 are further dehydrated and isomerized to produce Δ^{12}-PGJ_3 and 15d-PGJ_3 and 5d-PGJ_2, respectively. Common feature of Δ^{12}-PGJ_3 and 15d-PGJ_2 is the highly reactive cyclopentenone ring, which is readily attacked by low- and high-molecular-mass thiols to form thioethers (Figure 1). Thiolation of Δ^{12}-PGJ_3 and 15d-PGJ_2 is likely to reduce both availability and bioactivity of Δ^{12}-PGJ_3 and 15d-PGJ_2. So far, there are no data about excretion of Δ^{12}-PGJ_3 and 15d-PGJ_3. We (Figure 1) and others[3] found only pM-concentrations of 15d-PGJ_2 in human urine, while PGJ_3 metabolites including 15d-PGJ_3 were below the detection limit of our method (30 pM) in urine. This may suggest that basal PGJ_3 biosynthesis from EPA is several fold lower than PGJ_2 from ARA. Dietary EPA has been shown to increase formation of prostaglandin I_3 (PGI_3) and thromboxane A_3 (TxA_3), but EPA, even at very high doses, did not increase PGI_3 and TxA_3 synthesis to a degree comparable with that of PGI_2 and TxA_2 from ARA.[4]

Δ^{12}-PGJ_3 and 15d-PGJ_2 are considered potentially useful therapeutic agents for the treatment of cancer.[1,2] Dietary EPA supplementation is unlikely to produce nM-concentrations of Δ^{12}-PGJ_3 required for antileukemic activity, but topical administration of considerable amounts of synthetic Δ^{12}-PGJ_3 would be required.

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Contribution: D.T. and D.O.S designed and performed the study, analyzed the data, and wrote the manuscript.

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Response:

Endogenous levels of D12-PGJ3 derived from eicosapentaenoic acid

In response to the comment by Tsikas and Stichtenoth,[1] we would like to provide clarification for their views and address the questions. First, while it is correct that the reactivity of the 2 electrophilic centers could make these classes of compounds less bioavailable, our data clearly demonstrates that intraperitoneal administration of D12-PGJ3 completely eradicates leukemia stem cells in the bone marrow and spleen. This suggests that the formation of Michael adducts does not affect their antileukemic activity nor systemic bioavailability. Second, it is not surprising to find that the pM concentrations of 2- and 3-series CyPGs (of the J class) in the urine. Our studies show (see Figure 1 in Hegde et al[2]) that macrophages cultured with 50μM EPA for a week, produce D12-PGJ3 in the cell culture media in quantities (nM) sufficient to target leukemia stem cells. The authors show very low levels (pM) of these metabolites in urine. However they did not measure levels in the serum and it would be difficult to infer serum concentrations from these measurements. Moreover, it is not surprising that given the low rate of conversion, the level of D12-PGJ3 from ARA-derived EPA is likely to be in the pM range as described. In the future, quantitation of these metabolites in the serum will be necessary to provide a true measure of their concentration, particularly in EPA-supplemented individuals. Unpublished studies from our laboratory confirm the metabolism of dietary EPA generates D12-PGJ3 at concentrations in the serum high enough to induce apoptosis in leukemia stem cells in vitro. A manuscript with these results is being currently prepared for submission.

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