THE MISSING LINK IN DEHYDROEPIANDROSTERONE SULFATE-MEDIATED PEROXISOME PROLIFERATION

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Dehydroepiandrosterone sulfate (DHEAS) is the major circulating form of dehydroepiandrosterone (DHEA), a naturally occurring C_{19} adrenal steroid which serves as a precursor for major sex hormones, androgen and estrogen. In man, DHEAS attains a maximum level between 20-30 years of age, and thereafter the level declines markedly (1). Although the physiological effect of the steroid is not completely understood, several epidemiological studies suggest a beneficial role of DHEAS against important human diseases including cardiovascular disease, diabetes and cancer (2). Similar disease-modulating effects of the steroid were also observed in experimental rodent models (3-5).

Despite these potential beneficial effects, DHEAS belongs to a class of chemicals known as peroxisome proliferators (6). Peroxisome proliferators include a wide variety of chemicals such as hypolipidemic agents (e.g. clofibric acid, gemfibrozil, bezafibrate), industrial chemicals, herbicides, polyunsaturated fatty acids, and leukotrienes. These chemicals, when administered to rodents, produce several effects including an increase in the size and number of hepatic peroxisomes, a decrease in body weight and induction of lipid metabolizing enzymes (6-7). Following chronic treatment at a high dose, peroxisome proliferators are capable of producing liver tumors in those animals (6).

It is currently believed that peroxisome proliferators elicit their effects through activation of intracellular peroxisome proliferator activated- receptors (PPAR). Functionally, PPAR is a ligand-activated transcription factor, which is involved in gene expression in a tissue-, sex- and species-dependent manner (7-9). Upon activation, PPAR forms a heterodimer with retinoid X receptor alpha (RXRα) and regulates gene expression by binding to PPAR-response elements (PPRE) located upstream of target genes. Target genes for PPAR include lipid metabolizing enzymes and growth regulatory genes. To date, three subtypes of PPAR (α, β and γ) have been identified in several species including human. PPARβ often expressed at higher levels than PPARα and PPARγ, plays an important role in keratinocyte differentiation. PPARγ is essential for adipocyte and macrophage differentiation. Activation of PPARα, which is expressed predominantly in hepatocytes, by peroxisome proliferators such as DHEAS results in pleiotropic responses including peroxisome proliferation, induction of lipid metabolism and liver tumors in rodents.

The peroxisomal proliferation effects of DHEAS can be totally abolished in a knockout mice model where PPARα is ablated (6). In the wild-type mice, DHEAS administration caused an increase in the liver weight and induced enzymes such as acyl-CoA oxidase, bifunctional enzyme, thiolase, fatty acid binding protein (FABP) and CYP4A. These effects were totally absent in DHEAS-treated PPARα knockout mice (6). These findings clearly suggest that PPARα is required *in vivo* for DHEAS-mediated gene induction of peroxisomal and microsomal enzymes associated with lipid homeostasis.

In vitro, however, DHEAS failed to activate PPARα in transient transfection assays (6). It is a surprising result because other prototypical peroxisome proliferators such as Wy-14, 643 and clofibric acid markedly induced an increase in reporter gene activity in transient transfection experiments using COS-1 cells (6). Interestingly, DHEA and its reduced metabolite, 5-androstene-3β, 17β-diol (ADIOL, tested in sulfated form), which caused peroxisome proliferation *in vivo* (7), failed to transactivate PPARα in a cell culture model. Therefore, none of the aforementioned compounds can act as a direct ligand of PPARα. In fact, DHEAS also failed to transactivate other PPAR isoforms-namely PPARβ and PPARγ.

The apparent inactivation of PPAR by DHEAS led to consider several possibilities. Firstly, the entry of DHEAS into cells may require a specific transport protein that is only present in the hepatocytes. This assumption has been proven to be incorrect as later studies showed DHEAS can enter other cell lines such as breast cancer MCF-7 cells (8). Alternatively, a cofactor protein or a tissue-specific endogenous molecule may be required for trans-activation but only present in the liver of DHEAS-treated animals and not in cultured COS-1 cells. A third possibility could be that DHEAS may undergo further metabolism *in vivo* to generate a proximate activator of the receptor. DHEAS can also stimulate endogenous PPAR activators (for example- fatty acids or leukotrienes) by modulating intermediary metabolism. Finally, from an experimental standpoint, the transient transfection assay was not sensitive enough to detect activation of the receptor by DHEAS. This last possibility was addressed by utilizing a mutant PPAR vector, PPARα-G with a marked decrease basal reporter activity. Even with this modification, DHEAS could not activate PPARα (6).

Taken together, at this point, we do not have any information on any proximate metabolite of DHEAS capable of directly binding and activating PPARα. Co-activator protein and DHEAS stimulated endogenous PPARα activator are yet to be discovered. Therefore, extensive studies are necessary to delineate the receptor-mediated effect of this compound with several potential chemopreventive properties.

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