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Commentary

The mechanisms of Fenretinide-mediated anti-cancer activity and prevention of obesity and type-2 diabetes

Nimesh Mody *, George D. Mcilroy

Institute of Medical Sciences, College of Life Sciences & Medicine, University of Aberdeen, Aberdeen, UK

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ABSTRACT

Fenretinide remains the most investigated retinoid compound for the prevention of cancer. Its clinical use remains a genuine possibility due to a favourable toxicological profile and accumulation in fatty tissues. Like other well-characterised pharmacological therapies, Fenretinide has been shown to affect multiple signalling pathways. Recent findings have discovered additional beneficial properties the synthetic retinoid was not intentionally designed for, including the prevention of high-fat diet-induced obesity and insulin resistance. These preclinical findings in rodents are timely since obesity has reached pandemic proportions and safe effective therapeutics are severely lacking. Recent investigations have proposed various mechanisms of action for the beneficial effects of Fenretinide. This review covers the current knowledge about Fenretinide's use as a therapy for cancer and potential to treat obesity, insulin resistance and glucose intolerance. An overview of the signalling pathways manipulated by Fenretinide including retinoid homeostasis, reactive oxygen species generation and inhibition of ceramide synthesis will be presented and insights into apoptosis and/or autophagy induction by Fenretinide will also be discussed. The largely unexplored area of Fenretinide metabolites as alternative therapeutic options and how these may be relevant will also be presented. Fenretinide shows great promise, but unfortunately evidence is lacking from clinical trials on Fenretinide's effectiveness in humans. Finally we identify what action can be taken to further progress the investigation of this extremely important retinoid.

1. Introduction

Obesity, the condition of being overweight or to carry excess body fat is widespread in today's society and is often stated as having reached pandemic status. Systematic analysis of health examination surveys and epidemiological studies has estimated that worldwide, more than 1.46 billion adults were overweight with a body mass index (BMI) of $\geq$25 kg/m^2 in the year 2008 [1]. Moreover, around a third of these individuals were classed as obese with a BMI of $\geq$30 kg/m^2 [1]. Of greater concern, obesity poses additional serious detrimental health consequences associated with perturbations to metabolic homeostasis. These include, but are not limited to, chronic diseases such as type II diabetes [2], cardiovascular disease [3] and the development of certain types of cancers [4]. Moreover, despite extensive preclinical and clinical research into these complex diseases, they continue to be the major causes of death worldwide. It is therefore imperative that efforts are made to reduce the levels of obesity and obesity-associated metabolic disturbances that are currently observed today in both the developed and developing world.

Unfortunately, education through the promotion of healthy lifestyles along with well-balanced diets appears to have had little impact on reversing the ever-increasing numbers of overweight and obese individuals. Thus, like the approach to target cancer with pharmaceutical therapy and/or prevention, an alternative approach to combat levels of obesity would be through the development of safe and effective pharmacological treatments. Despite the scale of the present situation, unfortunately very few therapeutic options are available [5]. More drugs are approved for the treatment of type II diabetes, however potentially dangerous side-effects are still encountered with their use [2,6]. Promisingly, vitamin A and its derivatives known as retinoids have been evaluated and used for the treatment of some types of cancer and more recently, preclinical studies have suggested they may be useful for the prevention and/or treatment of obesity and type II diabetes.

2. Retinol metabolism and all-*trans*-retinoic acid signalling

Vitamin A (or retinol) is the parent compound of all bioactive retinoids and is convertible to other natural forms through the retinol metabolism pathway. Active metabolites of retinol,

Abbreviation: FEN, Fenretinide.

* Corresponding author.
E-mail address: n.mody@abdn.ac.uk (N. Mody).

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primarily all-*trans*-retinoic acid (RA), act as important signalling molecules with the ability to induce gene expression through specific nuclear hormone receptors [7]. RA-receptor (RAR)s form heterodimers with retinoid-X receptors (RXR)s and bind to RA-response elements (RARE)s present in the promoters of target genes *via* the DNA-binding domain present within each receptor. As a result, the metabolism of vitamin A has been shown to play essential roles in the preservation of immune function, continued promotion of good vision and the development, growth and maintenance of multiple body tissues. Acquiring and maintaining a sufficient quantity of this fat soluble vitamin is therefore essential for life. Animals however do not have the capability to generate vitamin A *via de novo* synthesis. Vitamin A must therefore be obtained from dietary sources, stored in the liver and mobilised as required.

Dietary intake of vitamin A can be achieved through the absorption of pigments known as carotenoids from fruits and vegetables. These pro-vitamins can then be enzymatically cleaved and converted to compounds with the biological activity of retinol [7]. Alternatively, intake can be achieved by consuming animal material such as the liver, where pro-vitamin A carotenoids have already been processed and stored in the form of retinyl esters. Although vitamin A is essential, excessive intake can be equally detrimental to life. Hypervitaminosis A can lead to toxicity of the liver, decreased bone mineral density and induce teratogenic effects in the developing embryo [7]. Additional concerns arise with the use of retinoid therapy in women of child bearing age, as these compounds have the capability of inducing teratogenic effects in the developing conceptus. Vitamin A is a lipophilic, fat soluble molecule and therefore requires specific binding proteins in order to be transported in the circulation and within the cell. Despite this necessity, retinoid compounds are soluble in aqueous solutions at relatively low concentrations. For example, RA is water soluble up to concentrations of 210 nM at room temperature and pH 7.3 [7]. This makes retinoid compounds ideal morphogens. The generation of morphogen concentration gradients through diffusion allows for selective cellular differentiation to occur and determine tissue pattern during development [8]. As a result, the administration of retinoid compounds has been shown to provoke teratogenic effects in both animal models and humans. It has been suggested that chemical modification of the terminal-polar group of the retinoid molecule would offer a useful way to reduce toxicity but also modify activity, metabolism and tissue distribution of this class of compounds [9,10].

3. N-(4-hydroxyphenyl)retinamide; a synthetic retinoid

3.1. Structural and advantageous properties of N-(4-hydroxyphenyl)retinamide

N-(4-hydroxyphenyl)retinamide, otherwise known as 4-HPR or Fenretinide (FEN, used hereafter), is one such synthetic retinoid that was first synthesised in 1960s by R.W. Johnson Pharmaceuticals, now part of Johnson and Johnson [11]. FEN shares a similar chemical structure with RA however it contains an amide linked 4-hydroxyphenyl group, which replaces the carboxyl polar end group of RA (Fig. 1). It is the addition of this bulky 4-hydroxyphenyl group which is thought to be responsible for a number of beneficial properties associated with FEN treatment, compared to alternative retinoid compounds such as RA.

Since naturally derived vitamin A compounds such as RA and retinyl-acetate supplemented in large doses show liver toxicity with prolonged exposure, this restricts their potential use as medicinal agents. FEN on the other hand displays a decreased toxicological profile, which may be due to a number of reasons. Chronic retinyl-acetate treatment results in the deposition of

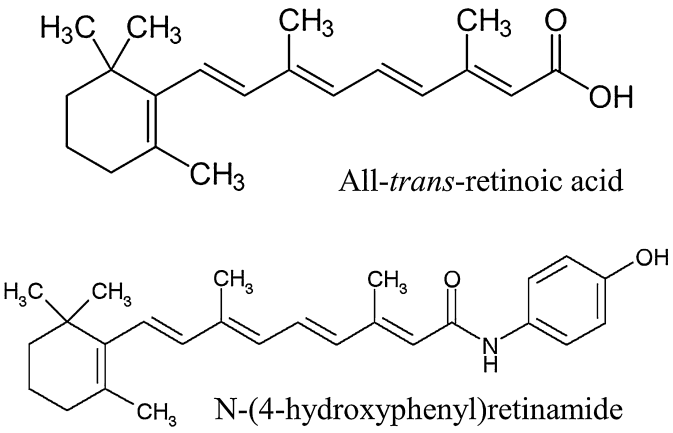

Fig. 1. The chemical structure of all-*trans*-retinoic acid and N-(4-hydroxyphenyl)retinamide. FEN is identical to RA except for the modification to the carboxyl functional group, which is replaced with an amide linked 4-hydroxyphenyl group.

retinyl esters in the liver and subsequently causes hepatic toxicity. In contrast, FEN does not appear to be stored in the liver of rats [11]. This may be due to the observation that FEN and its metabolites are preferentially stored in fatty tissues such as mammary gland, which has been observed in both animal models and human studies [11,12]. Therefore, this characteristic appears to prevent FEN treatment leading to hepatotoxic accumulation and is highly advantageous compared to the use of natural forms of vitamin A as a therapeutic option. The specific accumulation of FEN in fatty tissues is also a beneficial property for the prevention/treatment of breast cancer, obesity and type II diabetes [11–14].

Encouragingly, studies performed in rats and rabbits have revealed that when FEN was given orally at 20 mg/kg/day, no adverse effects were observed in either species. At higher doses of 125-800 mg/kg/day, FEN was deemed to be only weakly teratogenic in these species [15]. Studies in hamsters dosed with up to 130 mg/kg of 13-*cis*-N-(4-hydroxyphenyl)retinamide also failed to induce a teratogenic response [10]. Genotoxic studies (the Ames mutagenicity test, a mouse lymphoma assay and a rat bone marrow cytogenetic assay) with FEN treatment all reported negative results [16]. Together, these findings indicated that FEN is unable to induce point mutations or chromosomal aberrations and is therefore not a genotoxic compound.

3.2. Cancer chemoprevention trials

These desirable properties make the use of FEN as a therapeutic agent a genuine possibility. In agreement with this, due to the beneficial chemopreventive potential that FEN treatment has displayed during its early investigation in pre-clinical animal models [11], human clinical trials, predominantly for breast cancer chemoprevention, have demonstrated that FEN is well-tolerated and compatible with long term treatment schedules [17]. In a large randomised trial of FEN to prevent second breast malignancy in almost 3000 women with early breast cancer, overall, FEN treatment for 5 years appears to have no statistically significant effect on the incidence of second breast malignancies of women with breast cancer [17]. A possible benefit was detected in premenopausal women, results that persisted in a 15-year follow-up [17,18]. These effects are potentially through an associated lowering of circulating IGF-1 levels, a potent stimulator of cell growth [17]. Combination therapy with low dose tamoxifen also did not reduce breast cancer events compared to placebo or single agents alone [19]. Unfortunately, overall these trials have yielded only preliminary data and new untested hypotheses.