RETINOIDS – A PERSPECTIVE TO TERATOGENIC EFFECTS

E Auerkari
Bagian Oral Biologi Fakultas Kedokteran Gigi Universitas Indonesia

Abstract

Since the role of retinoids in normal growth, reproduction, maintenance and differentiation of epithelial tissue has been known, the therapeutic value of retinoids has been utilised in disorders resembling vitamin A (retinol) deficiency. Much effort has been directed to developing less toxic retinoids to increase this therapeutic potential, and now more than 2000 synthetic retinoids are known. However, the most severe side effect of retinoids is their teratogenicity, and a desired goal in the retinoids development is in dissociating the therapeutic benefits from teratogenic risk. It is not known if this result can be achieved, but increasing the understanding of the basic molecular mechanisms of retinoic action should offer one route to the attainment of this goal. This work reviews of the molecular biology of retinoids with particular reference with teratogenic effects, and summarises the strategies for managing the teratogenic risk with oral retinoid therapy.

Introduction

Retinoids or derivatives of vitamin A (retinol) are important in normal growth, vision, reproduction and maintenance as well as differentiation of epithelial tissue for all vertebrates. Vitamin A is a necessary dietary nutrient, but it is stored in the body and an overdose can be fatal.

Retinoid acid (RA) is a known control molecule of morphogen for vertebrate limb and nervous system morphogenesis as well as epithelial cell differentiation. During morphogenesis RA is believed to help establish the antero-posterior axis of the nervous system and limb buds. In the developing tissue, RA appears to be synthesized through enzymatic metabolism locally where needed from retinol which is widely available in the developing embryo. However, exposure to elevated levels of RA results in characteristics malformations.

Some of the dermatologic and oncologic effects of retinoids and lack of them have been known at least since the 1920s, when squamous metaplasia, increased cell proliferation, hyperkeratosis
and carcinoma was found in animals with vitamin A deficiency in diet. Although it was then clear that systemic vitamin A treatment could be beneficial in disorders (such as severe acne and psoriasis) resembling deficiency in vitamin A, its toxicity limited the therapeutic value. Since 1960s, increasing research into retinoids aimed largely to improve this value by developing less toxic synthetic retinoids. But also other research to retinoids expanded greatly. As a result, the overall metabolism of retinoids is generally seen as reasonably well understood, specific cellular binding proteins and nuclear receptors can be identified, and these receptors appear to be controlled by a gene family that also control many hormones. More than 2000 retinoid analogs have been synthesized, and the quest for improved retinoids continues.

The most severe side effect of retinoids is their teratogenicity. For example, increased incidence of stillborn and severely malformed children has been reported for women receiving dermatological RA treatment. The affected children have a characteristic pattern of malformations involving craniofacial, cardiac, thymic and central nervous system structures. Of the present commercial RA derivatives, at least isotretinoin (13-cis-retinoic acid, with the trade name Accutane) is as well known teratogenic agent. This effect is even aggravated in a newer retinoid derivative, etretinate, which remains in storage in adipose tissue up to 2 years after drug discontinuation, and hence requires contraception over this period.

One obvious desired goal in the further retinoid development is in dissociating the therapeutic benefits from teratogenic risk. It is not known if this result can be achieved, but increasing the understanding of the basic molecular mechanisms of retinoid action should offer one route to the attainment of this goal.

This work attempts to provide a review of the principles of teratology and the molecular biology of retinoids with particular reference to teratogenic effects, and to summarise the strategies for managing the teratogenic risk with oral retinoid therapy.

Principles of Teratology Applied to Retinoids

The susceptibility to teratogenic agents depends on the developmental stage of the embryo. Generally, earlier exposure results in more damage, and during the first few weeks of gestation the human embryo tends to react in an all or nothing fashion, with either death or complete recovery of the multipotent cells.

The period of organogenesis (18-60 days of gestation) is the most sensitive regarding teratogenic effects. Major malformations usually result when the insult occurs before 36 days for most organs, although the urinary tract, palate and central nervous system may be affected later. In the histogenesis stage (after the 8th week), teratogenic agents may cause a decrease in the number of cells or may alter function in a manner not easily recognizable by clear anatomical abnormalities.

Retinoids appears to be multifunctional since RA is believed to induce a cascade of events that regulate the transcription of many key genes needed for proper nervous system, limb, head and epithelial morphogenesis. However, for retinoids there is also a period of a maximum teratogenic effect: for mice this time appears to be about 8 to 10 days of gestation, and then a dosage of some 100 mg/kg is sufficient to cause craniofacial abnormalities in more than 90% of the cases.

Teratogens can show the following possible mechanisms:
- increase of cell death to a greater rate than the recuperative ability of the embryo or fetus;
- delay in mitosis;
- slowing down of stopping of the process of differentiation of particular cells;
- physical constraint or vascular insufficiency; and/or
- interference with histogenesis or inhibition of cell migration.
and carcinoma was found in animals with vitamin A deficiency in diet \(^3,4,5,6\). Although it was then clear that systemic vitamin A treatment could be beneficial in disorders (such as severe acne and psoriasis) resembling deficiency in vitamin A, its toxicity limited the therapeutic value. Since 1960s, increasing research into retinoids aimed largely to improve this value by developing less toxic synthetic retinoids \(^7,8,9\). But also other research to retinoids expanded greatly. As a result, the overall metabolism of retinoids is generally seen as reasonably well understood, specific cellular binding proteins and nuclear retinoids receptors can be identified, and these receptors appear to be controlled by a gene family that also control many hormones. More than 2000 retinoid analogs have been synthesized, and the quest for improved retinoids continues.

The most severe side effect of retinoids is their teratogenicity\(^7\). For example, increased incidence of stillborn and severely malformed children has been reported for women receiving dermatological RA treatment \(^10\). The affected children have a characteristic pattern of malformations involving craniofacial, cardiac, thymic and central nervous system structures. Of the present commercial RA derivatives, at least isotretinoin (13-cis-retinoid acid, with the trade name Accutane) is as well known teratogenic agent. This effect is even aggravated in a newer retinoid derivative, etretinate, which remains in storage in adipose tissue uf to 2 years after drug discontinuation, and hence requires contraception over this period.

One obvious desired goal in the further retinoid development is in dissociating the therapeutic benefits from teratogenic risk. It is not known if this result can be achieved, but increasing the understanding of the basic molecular mechanisms of retinoid action should offer one route to the attainment of this goal.

This work attempts to provide a review of the principles of teratology and the molecular biology of retinoids with particular reference to teratogenic effects, and to summarise the strategies for managing the teratogenic risk with oral retinoid therapy.

**Principles of Teratology Applied to Retinoids**

The susceptibility to teratogenic agents depends on the developmental stage of the embryo. Generally, earlier exposure results in more damage, and during the first few weeks of gestation the human embryo tends to react in an all or nothing fashion, with either death or complete recovery of the multipotential cells\(^10\).

The period of organogenesis (18-60 days of gestation) is the most sensitive regarding teratogenic effects. Major malformations usually result when the insult occurs before 36 days for most organs, although the urinary tract, palate and central nervous system may be affected later. In the histogenesis stage (after the 8\(^{th}\) week), teratogenic agents may cause a decrease in the number of cells or may alter function in a manner not easily recognizable by clear anatomical abnormalities.

Retinoids appears to be multifunctional since RA is believed to induce a cascade of event that regulate the transcription of many key genes needed for proper nervous system, limb, head and epithelial morphogenesis. However, for retinoids there is also a period of maximum teratogenic effect: for mice this time appears to be about 8 to 10 days of gestation, and then a dosis of some 100 mg/kg is sufficient to cause craniofacial abnormalities in more than 90% of the cases\(^2\).

Teratiogens can show the following possible mechanisms\(^10\):
- increase of cell death to a greater rate than the recuperative ability of the embryo or fetus;
- delay in mitosis;
- slowing down of stopping of the process of differentiation of particular cells;
- physical constraint or vascular insufficiency; and/or
- interference with histogenesis or inhibition of cell migration.
Also in this respect, retinoids function in multiple ways, such as through complex interference with the cell differentiation, but a particular feature in the craniofacial abnormalities appears to be local cell death as directed by RA.

Generally, the susceptibility to teratogenic agents is influenced by the genotype of the embryo as well as that of the mother. Mechanisms that account for the great variability in response between species and individuals include different rates of absorption, placental transfer, metabolism, and detoxification of the teratogenic agent or its metabolites.

On the other hand, teratogenic agents tend to show a dose response relationship, so that higher doses lead to more severe effects. In case of retinoids, the above-mentioned 90% incidence of malformations in murine fetuses after exposure to 100 mg/kg of RA shows that even at this high dose level some fetuses appear to escape at least the histologically obvious consequences. However, even much lower doses of 0.5 – 1 mg/kg that are commonly used to treat skin disorders, can apparently lead to a clearly observable and unacceptable rate of embryonic malformations in humans.

Molecular Biology of Retinoids

The mechanisms through which the effects of retinoids are mediated are not yet completely established, but retinoids certainly exhibit controlling role in normal embryonic development.

Following gavage of retinol (vitamin A), several natural derivatives can be found in the plasma. The chemical structure of these retinoids as well as a proposed metabolic pathway for them is shown in Fig 1.

A variety of specific retinoid-binding proteins exist. These are found in the blood and in the cytoplasm in addition to a specific cytoplasmic-binding protein for the vitamin A metabolite retinoic acid. The nuclear retinoic acid receptors (RAR) are strategically placed to provide the RA functionality for gene expression and development.

RARs belong to the thyroid/steroid superfamily of receptors, and hence are ligand-activated transcription factors. Three subtypes of RARs (α, β and γ) have been identified. Of the endogenous retinoids these receptors show highest affinity for all-trans-retinoid acid, on the other hand, isotretinoin (13-cis-retinoic acid) does not bind to these receptors in vitro.

There is another family of RA receptors, also with α, β and γ subtypes, called RXR, which is bound and activated by 9-cis retinoic acid. The RARs and RXRs control different gene pathways, and these receptor families and their subtypes show differences in tissue localization. For example, RARγ is found in adults only in the skin, whereas RARα is relatively ubiquitous in adult tissues.

There is interaction between the receptor families so that the action of the RARs and the action of other members of the thyroid/steroid hormone receptor family depends on the presence of a RXR. Hence there appears to be many different targets for different retinoids, and this may allow for improvement in the synthetic retinoids used for specific therapeutic purposes.

Although each cell has only an estimated 1000-1500 receptors, activation of the receptors by ligands can induce a cascade of events. The ligand-receptor binding produces transcriptional activation, which is the central event in the retinoid action in embryonic development as well as in therapeutics.

One proposed mechanism of the retinoid action through the RARs is shown in Fig 2. RARs are found in nucleus where they can bind retinoid acid response elements (RARE), a specific sequence of DNA in the untranslated region of the gene that is recognized by the RARs. The binding of the receptor-ligand complex to the RARE in the presence of appropriate cofactors such as RXR is followed by transcription of the mRNA from the corresponding gene. The mRNA moves
out of the nucleus to the cytoplasm, where it can be translated into the protein coded by the controlled gene.

There are several possible functions of the resulting proteins, eg as structural elements (like with collagen and laminin) or as new transcription factors that modulate the expression of other gene products. The latter possibility could produce easily a cascade of gene expression, substantially amplifying the original retinoid-receptor interaction. This also explains why inappropriate modulation of the retinoid-receptor gene pathway through eg addition of retinoids may lead to abnormal development.

**Vitamin A Metabolism in Monkey**

![Diagram of vitamin A metabolism]

---

**Retinoids: Risk Management**

Attempts to introduce new synthetic retinoids without the teratogenic side effects have been so far unsuccessful, although improvement in therapeutic effect has been realised. This is true for even the latest common commercial retinoids such as etretinate and acitretin.

At present doses of 0.5 – 1 mg/kg/day of retinoids that are used to treat skin disorders, lead to a clearly unacceptable rate of malformations in human embryos. This risk is mainly managed through contraception extended to about 2 years after discontinuation of retinoid therapy. It is possible though not established that when practical, topical rather than oral use of retinoids may reduce the side effects in some skin disorders such as photodamaged skin.

It would be prudent to remember that teratogenicity is not the only risk with retinoids. Although the present synthetic retinoids do not accumulate appreciably in the liver, and thus any similar liver overloading as in hypervitaminosis A is excluded, oral retinoids may be somewhat hepatotoxic, possibly promoting cirrhosis (all retinoids) and more rarely acute hepatitis (aromatic retinoids such as etretinate and acitretin). Therefore particularly for any long-term retinoid therapy, liver functions should be monitored, and patients with contraindications like previous history of retinoic-induced hepatitis not treated.

Other side effects of long-term retinoid therapy include skeletal hyperostosis and extraskeletal ossification, which may lead in severe cases to eg rigid
bridging of bones and premature epiphyseal closure in children. To reduce these effects, children under 10 years of age should not be subjected to long-term treatment, and otherwise doses should be minimised and/or administered intermittently. Again, regular patient monitoring is recommended. One more set of side effects are cardiovascular ones, stemming from the ability of retinoids to cause hyperlipidemia. Retinoids increase the triglyceride levels and also favor the formation low-density lipoproteins (LDL) over high-density lipoproteins (HDL), a shift that is a risk factor in developing ischemic heart disease (IHD). Also to manage these risks, mapping of individuals risk factors provides the basis for selecting options of therapy as above for skeletal side effects.

The above treatment of retinoids has concentrated on the disadvantages, adverse side effects and difficulties encountered with retinoid therapy. Nevertheless it is appropriate to see the retinoid family as a very useful group of drugs which is likely to find further clinical use in the future. In spite of the risk factors, few alternatives provide similar efficiency in eg skin disorders as well cancer treatment and prevention. Furthermore, as different retinoids tend to show very different effects in different disorders, it is highly likely that improved therapeutic effect is possible by careful targeting of a specific retinoid for a particular disorder complex.

Summary

To further improve the therapeutic value of retinoids in eg disorders resembling vitamin A (retinol) deficiency, much effort has been directed to developing less toxic retinoids. At present more than 2000 synthetic retinoids are known, though relatively few are in commercial use.

The most severe side effect of retinoids is their teratogenicity, and a desired goal in the retinoid development is in dissociating the therapeutic benefits from teratogenic risk. It is uncertain whether this result can be achieved, but improved understanding of the basic molecular mechanisms of retinoic action may offer a route to the attainment of this goal. At present doses of 0.5-1 mg/kg of retinoids that are commonly used to treat skin disorders, lead to a clearly unacceptable rate of malformations in human embryos. This risk is mainly managed through contraception extended to about 2 years after discontinuation of retinoid therapy.

References

4. Wolbach, SB & Howe, PR. Tissue changes following deprivation of fatsoluble A vitamin, J of Experimental Medicine 1925; 43, 753-77.
