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Importance of vitamin-A for lung function and development

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Abstract

Vitamin-A is essential for growth and development of cells and tissues. In its active form, retinoic acid, it controls the regular differentiation as a ligand for retinoic acid receptors (RAR, RXR) and is involved in the integration (gap junction formation) of cell formations [Nature 37 (1994) 528; International Review of Cytology. San Diego Academic Press, 1–31]. Vitamin-A plays a substantial role, especially in the respiratory epithelium and the lung. During moderate vitamin-A-deficiency, the incidence for diseases of the respiratory tract is considerably increased and repeated respiratory infections can be influenced therapeutically by a moderate vitamin-A-supplementation [Aust. Paediatr. J. 22 (1986) 95; Lancet 338 (1991) 67]. In addition to the importance of the vitamin for the lung function, vitamin-A is also responsible for the development of many tissues and cells as well as for the embryonic lung development. Recent studies proved that the control occurs by different expressions of retinoid receptors as well as by time-dependent changes of the vitamin-A-metabolism respectively via cellular vitamin-A-binding proteins (CRBP: cytoplasmatic retinol binding protein; CRABP: cytoplasmatic retinoic acid binding protein).

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1. The influence of vitamin-A for the maturation and differentiation of the lung

The alveolar cells of type II are especially prepared to synthesize and secrete the surfactant (Zachman, 1989). Retinoic acid (RA) is able to stop, concentration-dependently (Metzler and Snyder, 1993) the expression of the surfactant-protein A (SP-A) in human fetal lung explants. Insulin, TGF- β and high concentrations of glucocorticoids can also down-regulate the SP-A-mRNA-expression (Weaver and

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Whitsett, 1991), but lower concentrations of glucocorticoids are stimulating the expression of these genes (Odom et al., 1988). In contrast, the SP-B-mRNA-expression is increased in human fetal lung explants both by hyperoxia (rats) (Metzler and Snyder, 1993) and by dexamethason (human fetal lung explant) (Metzler and Snyder, 1993). Consequently, the formation of some surfactant-proteins is regulated differently and selectively by RA together with glucocorticoids.

Prostaglandins of type PGE₂ are able to increase the surfactant-synthesis. Under the influence of EGF (epidermal growth factor) the formation of prostaglandin rises, especially of PGE₂. On the other hand, the expression of the EGF-receptor is increased by RA. EGF increases the proliferation of the lung tissues and this leads to an amplified formation of surfactant phospholipids. RA as well as EGF are both leading to an increase (40%, 80%) of the PGE₂-secretion in fetal lung cells of the rat in vitro (Haigh et al., 1989). The combination of RA and EGF though leads to a more than a six-fold increase of the PGE₂-secretion. Consequently, RA can interfere in the lung development by its modulating effect on the EGF-expression and the subsequent PGE₂-induced surfactant formation. A sufficient and continuous availability (either on the blood pathway or by local storage sides) is pivotal, especially for a time-dependent regulation of the lung-development and the related formation of the active metabolite RA.

2. Vitamin-A kinetic during fetal lung development

In fibroblast-like cells close to the alveolar cells, in type-II-cells as well as in the respiratory epithelium retinyl-esters, as local extrahepatic stores are present. The importance of these retinyl-esters as “acute reserve” during the development of the lung becomes apparent during the late phase of gestation and the beginning of lung maturation. During this period a rapid emptying of the retinyl-ester storage’s in the lung of rat embryos occurs (Geevarghese and Chytil, 1994). This depletion is the result of an increased demand in the process of the lung development, because the retinoic acid is “instantly” needed for the process of cellular differentiation (e.g. proximalization) and metabolic work (surfactant).

The prenatal lung development is also influenced by glucocorticoids. The steroid hormones have a similar effect on lung development as vitamin-A, i.e. the two factors complement each other. This is not surprising, because the receptors for steroids and retinoids belong to the same multireceptor-complex. The mode of action of glucocorticoids does not only come into action on the level of gene-expression, but seems to have an impact in a much earlier phase of the vitamin release. The application of dexamethason leads to an increase of the maternal and fetal retinol-binding protein. Thus, the vitamin-A-supply is improved via the regular hepatic export pathway. Such an increase of the vitamin-A-concentration in the systemic circulation diminishes obviously the morbidity and mortality of prematures due to bronchopulmonary dysplasia (Shenai et al., 1990). Dexamethason respectively glucocorticoids are not only leading to an improvement of the total vitamin-A-supply through a change of the release from the liver, but they also influence, as recently described (Gee-

varghese and Chytil, 1994), the metabolization of the vitamin-A-esters, which are stored in the lung. After administration of dexamethason, as well as after administration of steroids, a significant reduction of retinyl-esters in the maturing lung can be detected, together with a moderate increase of retinol, the hydrolyzation product of retinyl-ester. This observation may explain the therapeutical success with steroids respectively also their failures during the therapy of lung-distress-syndrome of prematures. As far as an insufficient supply is concerned, inappropriate retinyl-ester stores, caused by a shortage of supply of the fetal lung during the late pregnancy, the regulatory effect of glucocorticoids for the vitamin-A-metabolism of the lung cells cannot take place.

Very low plasma-vitamin-A-levels (Shenai et al., 1981) are recurrently found in prematures, especially in cases with lung-distress-syndrome. This can, amongst other things, be attributed to the relative immaturity of the liver for the synthesis of retinol-binding proteins. The neonate is almost exclusively dependent on the mother in its supply, this includes the lung retinyl-esters which are either absorbed by the cells directly (from chylomicrons) or by esterification of retinol after uptake into the cells. These lung retinyl-ester stores can only be sufficiently filled if the mother guarantees an appropriate vitamin-A-supply especially during the late pregnancy.

3. The influence of an insufficient vitamin-A-supply on the post-natal development of the lung

A disease seen recurrently in connection with vitamin-A-supply is the broncho-pulmonary dysplasia (BPD). The pathogenesis of BPD certainly depends on a multitude of factors. Some of the observed morphological changes are very similar to the ones seen in vitamin-A-deficiency of humans and animals. In particular, there is focal loss of ciliated cells with keratinizing metaplasia and necrosis of the bronchial mucosa as well as an increase of mucous secreting cells (Fig. 1) (Stahlman, 1984; Stofft et al., 1992).

Especially focal keratinizing metaplasia, as it may occur after a vitamin-A-deficiency, is strengthening the assumption of an impairment of the differentiation on the level of the gene-expression. Since vitamin-A regulates the expression of different cytokeratins and therefore influences the terminal differentiation, it seems obvious to suppose common mechanisms. Consequently, the premature but especially the neonate are dependent on a sufficient supply with vitamin-A, to ensure the regulation of the cellular differentiation of the respiratory epithelium and lung epithelium. The earlier a child is born before due date, the lower its serum-retinol-levels are (Mup-anemunda et al., 1994). Since a further decrease of the serum-retinol-level and RBP-level occur postnatally, the plasma value at the time of birth, is considered to be a critical parameter regarding the lung development.

Repeatedly it was shown that serum-retinol-level and RBP-level in prematures are significantly lower than in neonates (Shah and Rajalekshmi, 1984). In the liver of prematures significantly lower retinol levels can be found in comparison to neonates

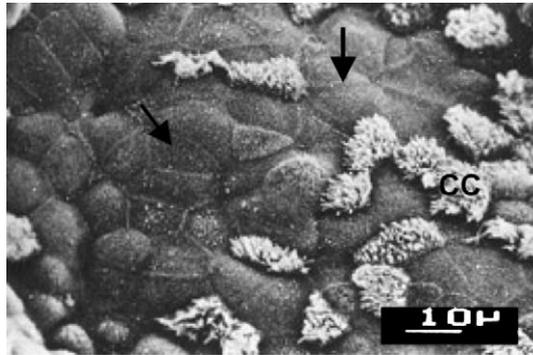


Fig. 1. High resolution micrograph of respiratory epithelium under vitamin-A-deficiency. Note the huge amount of squamous cells (arrow) having replaced most of the ciliated cells (CC).

(Shensi et al., 1985). Plasma values lower than 20 $\mu\text{g}/\text{dl}$ are not rare in this case and they should be taken as an indicator of a relative vitamin-A-deficit.

Reduced plasma levels during the first months of life have got a considerable influence on the overall development as well as on the susceptibility of infants to infections. With reduced retinol-plasma-levels repeated infections are more often described (Barreto et al., 1994; Filteau et al., 1993) and they are counted among the main complications of a poor vitamin-A-supply in developing countries. In addition, the serum vitamin-A-level during infectious diseases, particularly of the respiratory tract, continues to drop (Neuzil et al., 1994). On the one hand, this can be explained with an increased metabolic demand. On the other hand, an increased, and maybe additional renal elimination of retinol and of RBP during acute infections has been shown (Stephensen et al., 1994).

4. Possibilities of prevention and therapy

On the basis of the importance of vitamin-A as described above, the question arises as to what extent a therapeutical intervention can take place, especially in the case of imminent premature deliveries but also concerning preterm infants, to prevent the development of diseases and/or immaturities of the lung. One solution could be the intravenous administration of vitamin-A, but with the infusion-systems used so far, vitamin-A is almost completely adsorbed at the polyethylene tubes (Zachman, 1989) respectively is damaged by light. A possibility to improve availability consists of coating the infusion systems with foil to avoid a further reduction of the vitamin due to light. Since the solutions used so far are not available on the market any more, and on the other hand new parenteral vitamin-A-preparations are not available yet, the significance of supplying the mother with vitamin-A before delivery, must be pointed out.

The results of two randomized double-blind controlled studies (Shensi et al., 1985; Pearson et al., 1992) regarding premature infants show that the supplementation

with vitamin-A in one study (Shensi, 1985) lead to a considerable reduction (55%) of the risk to be affected by bronchopulmonary dysplasia. Another study though did not observe any changes. In a third study (Italian Collaborative Group on Preterm Delivery, 1993) 12 prematures received vitamin-A intravenously for a period of 28 days (400 IE/d), and during later development vitamin-A was also administered orally (1500 IE/d). In the process of supplementation a significant change of the initially reduced plasma- and RBP-values occurred. The latter is an indication for an actual vitamin-A-deficiency of prematures, because an increase of retinol-RBP can only be observed if a vitamin-A-deficiency really exists (principle of the relative-dose-response-test). A direct effect of the plasma concentration on the development of BPD could not be determined. The authors conclude that the plasma level (after delivery) hardly reflects the supply of the lung with vitamin-A (before delivery). Yet in this study it was again documented that especially prematures obviously feature a relative vitamin-A-deficiency. Thus, the attention should be directed to supplying them with vitamin-A. On the other hand, the vitamin-A-supply of the premature seems to be either not sufficient for achieving adequate concentrations in the lung or the vitamin is not sufficiently available to the corresponding cells of the lung. An alternative solution could be the inhalative administration of vitamin-A. The lung is hereby directly attained and retinyl-esters administered by inhalation can be absorbed into the cells and metabolized in a controlled way, as shown in different animal studies (Biesalski and Weiser, 1993b; Biesalski, 1996).

The quoted results show that retinyl-esters in respiratory epithelium and in alveolar cells form a pool of vitamin-A, which can be used physiologically by the tissue. The formation of retinol and at least retinoic acid from retinyl-esters is strictly controlled. So far an unphysiological formation of retinoic acid and a subsequent toxicity seems not possible. Retinyl-esters however, are biochemically inert with respect to gene expression or vitamin-A activity as long as they are not hydrolyzed. Consequently, the inhalative application, especially in cases of insufficient lung development, could represent a true alternative. Indeed, a study with preschool children in Ethiopia showed a significant increase of serum retinol as well as RBP after an inhalation therapy (Biesalski et al., 1999). In addition, a pilot study with 16 human volunteers with diagnosed dys- or metaplastic changes of the respiratory mucosa could show, that a three month inhalation of retinyl-palmitate led to a remission of the metaplasia in 44%, and a partial remission in 12%, while the other volunteers remained unaffected or showed a further progression in 18% (Kohlhäufel et al., 2002). Although these are small numbers, the results seem promising, especially because oral administration is little effective because of the poor RBP-synthesis of the liver and a parenteral solution is currently not available.

5. Significance of vitamin-A for structure and function of the maturing lung

As already described in the discussion concerning the inhalative application of vitamin-A-esters in bronchopulmonary dysplasia, it should be possible to treat squamous epithelial metaplasia and dysplasias of the human respiratory tract with

this type of administration. On the basis of a few reports it is assumed that a “local” vitamin-A-deficiency exists in meta- and dysplastic-areas. Measurements of vitamin-A-concentrations in metaplastic areas of the respiratory epithelium and the cervix epithelium actually proved that vitamin-A, in contrast to the surrounding healthy tissues, was no longer detectable.

At the moment it is difficult to distinguish between cause and effect. Studies carried-out by Edes and co-workers hint to an induction of metaplasia caused by a vitamin-A-deficit. These studies showed that a depletion of vitamin-A-ester storages in different tissues (Edes, 1991) is caused by toxins, that are present in cigarette-smoke (predominantly polyhalogenated compounds). Studies *in vitro* by Sobeck (2003) showed that incubation of bronchial epithelial cells for three days with Benzo[a]pyren, a substance present in cigarette smoke condensates, increases the amount of the cytochrome P450 Cyp26, which metabolizes RA into polar metabolites.

Epidemiological evidence supports the assumption that the development of obstructive respiratory diseases plays an important role in the scope of cancer mortality of smokers. It was shown that the relative risk for smokers to be affected by lung cancer, when they suffered from obstructive ventilation disorder (FEV 1% <60 (Melvyn et al., 1987) respectively 70) (Skillud et al., 1987), was significantly higher than that of comparative groups with normal lung-function-parameters.

A survey about the dietary habits within the scope of the “National Health and Nutritional Examination Survey” showed that an inverse correlation (Morabia et al., 1989) exists between obstructive respiratory diseases (COPD) and vitamin-A-supply as the only one of 12 examined dietary components. If a diminished supply of vitamin-A increases the appearance of obstructive respiratory diseases, a marginal or local vitamin-A-deficit could be responsible for the observed changes of the respiratory mucosa. Such a deficit results in a loss of cilia, an increase of secreting cells and finally the formation of squamous metaplasia (Shah and Rajalekshmi, 1984; Chytil, 1985; Biesalski et al., 1985).

Such changes (decrease of ciliated cells with simultaneous increase of the secretion) are noted for smokers (Gouveia et al., 1982; Mathe et al., 1983) and cause a reduction of the mucociliary-clearance. This reduction of the mucociliary-clearance, associated with an increased adsorption of the respiratory syncytial virus (RSV) (Donnelly, 1996), could explain the extraordinarily high morbidity and mortality for respiratory infections of children with vitamin-A-deficiency in developing countries (Sommer, 1993).

There is sound evidence from experimental studies that the alteration of the respiratory mucosa, caused by the vitamin-A-deficiency, can be redifferentiated into its functional original epithelium, *in vivo* as well as *in vitro*, following vitamin-A-supply (Biesalski et al., 1985; McDowell et al., 1987a,b, 1984a,b; Rutten et al., 1988a,b). Squamous metaplasia of the bronchial mucosa, which occurs in smokers in spite of a sufficient supply with vitamin-A as an effect of inhalative noxae could also be reversed through systemic application of high retinoid-concentrations *in vitro* (Lasnitzki and Bollag, 1982; Lasnitzki and Bollag, 1987) and in humans *in vivo* (Gouveia et al., 1982; Mathe et al., 1983) (Fig. 2).

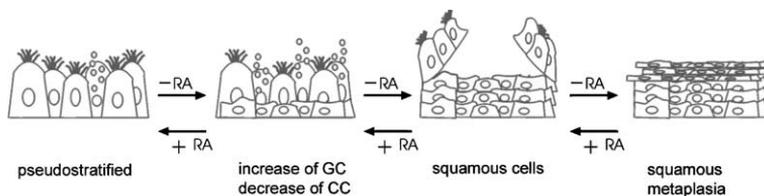


Fig. 2. Schematic drawing of changes of the respiratory epithelium in various stages of vitamin-A (retinoic acid: RA) deficiency. Note that most of the respective effects are reversible by adequate administration of vitamin-A.

The assumption of “local” vitamin-A-deficits as a basis for the inhalation approach is supported by studies, which showed that especially polyhalogenated compounds (e.g. TCDD) cause a local vitamin-A-depletion (Hakansson and Ahlborg, 1985; Thunberg and Hakansson, 1983; Thunberg et al., 1980), which again contributes to the development of metaplastic- and possibly to dysplastic-changes (Chopra and Joiakim, 1991). Thus, metaplastic changes are reversible by “topic” application (in vitro) of vitamin-A (retinoic ester and retinoic acid). Consequently, a topical (inhalative) in vivo-treatment of metaplasia of the respiratory tract could represent an efficient measure. In contrast, since retinoic acid is absorbed uncontrolled into the cells and a regulation of the CRABP-formation does not exist in that case, an application of inhalative retinoic acid is questionable with regards to potential toxic effects.

By application of an inhalable vitamin-A-ester, an accumulation in the target cells can be achieved by much lower than the toxicological concentrations. In these target cells the retinyl-esters, after controlled hydrolysis, are released as retinol. Under the same controlled and consequently physiological conditions retinol is re-esterified to the active metabolite retinoic acid. Consequently, the produced amount of retinol is adjusted to the respective amount of CRBP and along with it a corresponding amount of CRABP is expressed. Though many experiments in vitro as well as in vivo showed retinoic acid was effective in reversing squamous epithelial metaplasia (see above) an inhalation of retinoic acid would hardly be justified, because cellular regulation mechanisms are circumvented which is not the case for retinyl-esters.

6. Toxicological considerations

By inhalative application of vitamin-A an accumulation of peripheral vitamin-A stores is achieved. For the lung and the respiratory epithelium, concentrations in the range of 1–20 $\mu\text{g/g}$ were obtained (Biesalski, 1990). When comparing the concentrations in the respiratory epithelium and in the mixed epithelium of the nasal mucosa—after topical administration in different animal species—the vitamin-A-concentrations attained in the epithelium of the nose were 10–100 times higher (in humans 5–20 times higher) than in the respiratory mucosa (Lewis, 1973).

The therapy of atrophic rhinitis by means of vitamin-A-containing nose drops showed that high-dose topical application of the vitamin leads to the restitution of metaplastically modified nasal mucosa. Side effects, especially differentiation impairments, were not reported (Breuninger and Kahn, 1960; Duncan and Briggs, 1962).

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