Vitamin A – lost in Research and Bedside
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Neonatus 2016; Poznan, Poland

1. Physiology of Vitamin A in lung development
2. Effects of prenatal administration of Vitamin A
3. Effects of Vitamin A in infancy and childhood
4. Postnatal Effects of Vitamin A on BPD in very preterm Neonates
5. Summary

Actions of Vitamin A (ATRA) on Lung Development

- Initial remark: When discussing on vitamin A it has to be considered, that except in the ocular system, the biologic active form is the all-transretinoic acid (ATRA); receptor systems: retinoic acid receptors (RARs) and retinoic acid X receptors (RXRs).
- 1. Lung development in utero
- A. At various gestational ages, vitamin A acts on different steps of lung development
- B. Action on extracellular matrix proteins- mainly elastin
- C. Promote formation of terminal sacules and alveolarization
- D. Decreases the susceptibility for inflammatory lesions
- 2. Consequences
- A. Lack of vitamin A leads to a reduced number of alveoli and at least in animals to an emphysema like histology
- B. Reduced overall elastin content
- C. Increases the susceptibility for infectious lung disorders
Normal Lung Development

- canalicular Period
- saccular Period
- Alveolarisation

Gestational age - weeks: 20, 24, 28, 32, 36, 40

Effects of Vitamin A on Lung Development

Regulation of alveolarisation and septation

Massaro D and Massaro GD. N Engl J Med 2010
Metabolism and Targets of Vitamin A

Pleiotropic Function of Vitamin A-ATRA –System: Effects apart from developmental Aspects
Role of Vitamin A in Lung Development and postnatal Function

Summary of experimental trials on alveolarisation and induced lung disorders

- Developmental Alveoli Formation
  - required for (53,56), regulate (53-56), increase (33), protect against inhibitors (33,53,57), allow posthac catch-up alveolar formation and partially rescue failed formation (33,39,40) but fail to rescue lung function (41)

- Prostatose-induced Emphysema
  - reverse anastomosis abnormality (42), increase (42,43), FEV 0.5 (45)

- Cigarette Smoke-Induced Emphysema
  - slow development (44-49), reverse in rats (46), fail to reverse in human lungs (47)

- Retinoids
  - increase (63,64)

- Pneumonectomy
  - improve functional recovery in dogs (34), increase lung volume in rats (90)

- Alveolar Epithelial Cells
  - protect replication (55-61), prevent elastase-induced injury (1)

Massaro D and Massaro GD. Am J Respir Cell Mol Biol 2003

Clinical Implications of therapeutic pre- and postnatal Administration of Vitamin A

- Prenatal administration:
  - Experimental and clinical studies
  - Model of bronchopulmonary dysplasia and congenital diaphragmatic hernia
  - Prenatal administration in pregnant woman with known vitamin A deficiency

- Postnatal administration:
  - Two types of controlled clinical trials:
    - A. Vitamin A in preterm neonates with bronchopulmonary dysplasia in very preterm neonates — metaanalysis slightly significant; details see below!
    - B. Vitamin A in deficient populations, a.o. 3rd world countries, obese in developed countries — metaanalysis on 3rd world countries will follow!
Effects of vitamin A on Infant and Childhood Mortality

Meta-analysis of postnatal administration of vitamin A on childhood mortality

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>log(Risk Ratio)</th>
<th>SE Weight</th>
<th>Risk Ratio IV, Fixed, 95% CI</th>
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<tbody>
<tr>
<td>1.1.1 Asian studies</td>
<td></td>
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<tr>
<td>West 1995 a</td>
<td>0.0676</td>
<td>0.2461</td>
<td>5.3% 1.07 [0.68, 1.73]</td>
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<tr>
<td>Humphrey 1996</td>
<td>-0.8416</td>
<td>0.4543</td>
<td>1.5% 0.39 [0.16, 0.99]</td>
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<td>Rahmatullah 2003</td>
<td>0.2464</td>
<td>0.1089</td>
<td>26.9% 0.78 [0.63, 0.97]</td>
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<tr>
<td>Kumir 2008</td>
<td>-0.1625</td>
<td>0.0629</td>
<td>40.4% 0.85 [0.73, 1.00]</td>
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<tr>
<td>Subtotal (95% CI)</td>
<td></td>
<td></td>
<td>80.1% 0.83 [0.73, 0.93]</td>
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<tr>
<td>Heterogeneity: Ch² = 4.23, df = 3 (P = 0.24), I² = 26%</td>
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<tr>
<td>Test for overall effect: Z = 3.03 (P = 0.002)</td>
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| 1.1.2 African studies |               |           |                             |
| Biworld 2008       | 0.1317         | 0.1048    | 8.4% 1.14 [0.78, 1.67]      |
| Malaba 2005         | 0.0164         | 0.167     | 11.4% 1.11 [0.60, 1.54]     |
| Subtotal (95% CI)   |               |           | 19.9% 1.12 [0.88, 1.44]     |
| Heterogeneity: Ch² = 0.01, df = 1 (P = 0.92), I² = 0% |
| Test for overall effect: Z = 0.91 (P = 0.36) |

Total (95% CI) 100.0% 0.88 [0.78, 0.98]

Heterogeneity: Ch² = 8.94, df = 5 (P = 0.11), I² = 44%
Test for overall effect: Z = 2.31 (P = 0.02)

L. Gortner, Homburg/Saar, 14.06.2012

According to: Larange A and Cheroutre M; Ann Rev Immunol 2016

Immune Response after high dose –Vitamin A: An Explanation for contradictory Results?

Health improvement  Health impairment

High-dose vitamin A capsules

Adequate vitamin A status

Adequate immune responses

Effect modifiers

Immunoparasitosis / Immunopathology

L. Gortner, Homburg/Saar, 14.06.2012

According to: Larange A and Cheroutre M; Ann Rev Immunol 2016
Modifiers of Vitamin A Effects

- Gender of study infants:
- If VA administration is given < 1 year, protective effects are superior in male children
- Previous vaccinations eg measles enhance the protective effects of vitamin A, however pertussis, tetanus and diphteria vaccination decrease the protective effect
- Previous supplementation of vitamin A decrease the effect of VA ie if 1st dose is given early postnataally, further administrations are less effective
- Discussion in depth see below!

According to: Benn CS et al.; Int J Epidemiol 2015
Summary: Vitamin A

- Prenatal administration of vitamin A under experimental conditions improves lung morphology, biochemical variables and lung function.
- Prenatal administration in pregnant vitamin A-deficient women from 3rd world countries improves lung function at follow up to school age.
- Postnatal administration in very preterm neonates improves survival without bronchopulmonary dysplasia in some studies after intramuscular administration. Details will be given!
- Vitamin A administration in early childhood reduces the frequency of severe infections and mortality in 3rd world populations. These effects however are not homogenous in various populations.

Transition of old vs. new Type of BPD

- Impaired alveolarization
- Impaired elastin formation
- Interstitial Fibrosis
- Bronchial/bronchiolar metaplasia
- Increased muscular diameter
- Reduced vascularization
- Pathologic vascularization

Variable:
- Gestational age: immature → extremely immature preterms
- Modifiers: Genetic variations, intrauterine milieu; treatment practices; stem cell repair?

(Gortner L., Intensivmed, 2007)
### Prevention of bronchopulmonary Dysplasia- current therapeutics or what had been improved?

- Smoke-free environment for pregnant woman
- Prenatal Corticosteroids – no clear cut data; mortality vs. risk of BPD
- Early therapy with surfactant in case of RDS
- Early i.v. hydrocortisone (O. Baud et al.; Lancet 2016; van Kaam et al.; Evid Based Med 2016)
- Inhalative budesonide (D. Basler NEJM 2015)
- **Vitamine A – substitution – modest effect**;
- Caffeine – already since decades administered in Europe
- Mechanical ventilation:
  - Indication for intubation and mechanical ventilation (INSURE or **LISA-procedure**; Metanalysis: Isayama T et al.; JAMA 2016.)
  - In case of mechanical ventilation- surfactant administration
  - Target: early extubation and CPAP
- Avoidance of: Fluid overload, nosocomial infections;

**Experimental Therapies**: Inhalative NO – application: no; late surfactant treatment: no; growth factors, stem cells a.o.- studies needed!!

### Radiographic Signs of BPD: Differential Diagnosis of old (classical) versus new Type

**Classical BPD**

Definition new type BPD: Oxygen requirements by 36 weeks' age for adequate oxygenation (according to Jobe et al., 2002)

**New BPD**
Is BPD a long term Disease? Answers from Histology of the Lung following BPD at 12 Years of Age left - right Control without Lung Disease at the same Age


L. Gortner, Homburg/Saar, 14.06.2012 17

Cochrane Vitamin A for BPD
Variable BPD 36 wks : OR 0.87 (CI 0.77-0.98) in favour of Vitamin A ; NNT 13
Observational Study on BPD/Mortality and Vitamin A Usage

Tolia VN et al.; JAMA Ped, Nov 2014

Neo VitaA Study - Basic Protocol Data

- Enrollment criteria: viable preterms without severe congenital malformations having a birth weight < 1000 grs;
- Parental informed consent
- Treatment started within 24 hrs following birth
- Intervention: High dose Vitamin A – enteral/oral 6000 IE per day
- Controls: Basic Vitamin A – enteral/oral 1000 IE per day both for 28 days
- Primary endpoint: survival without BPD at 36 weeks
- Hypothesis: High dose Vitamin A will increase survival without BPD at 36 weeks by 20%; BPD: oxygen supplement for SaO2 for >= 92% or CPAP or mechanical ventilation regardless of oxygen requirements
- Alpha: 95%; beta 80%
- Sample size calculation: a total of 914 ELBW infants will have to be enrolled
Schedule NeoVitaA Study I

- Clinical screening assessment
- Informed consent
- Baseline assessment
- Inclusion Criteria fulfilled? Yes / No
- Randomization
- Patients screened for eligibility
  - Placebo
  - Vitamin A 5,000 IU/kg/day

For 28 days:
- Measurement of endpoints

36 weeks gestational age:
- n = 1,100 patients randomized
- n = 914 patients to be analyzed

Schedule NeoVitaA Study II

- Start trial treatment:
  - Birth
  - VA via PN 1,000 IU/kg LV
  - Vitamin A 1,000 IU/kg p.o.

- End PN supplementation:
  - Vitamin A 1,000 IU/kg LV

- Placebo = Refined peanut oil

- End trial treatment:
  - Trial day 1
  - Trial day 14
  - Trial day 28

Summary

- Vitamin A has been shown to exert pleiotropic immunologic effects; apart from these and/or additionally it is active in regulation of lung differentiation among others septation/alveolarization
- Administration within the 1. year showed different effects in various populations: Favourable in Asian and unfavourable in African populations with respect to prevention of serious infectious disorders.
- The role of Vitamin A for prevention of BPD is further unclear- see metaanalysis; intramuscular administration has not been adopted in Europe.
- Thus a study is running in Germany to evaluate the effects of high dose Vitamin A given for up to 28 days iv and orally

Acknowledgement

- Animal experiments: Supported by HOMFOR 2013;
- Collaborators: D. Monz ; PhD. E. Tutdibi MD.
- Clinical Trial –NeoVitaA Study:
- Supported by: Deutsche Forschungsgemeinschaft ( Me 1/125 ):
- Collaborators: S. Meyer MD. T. Bay for the NeoVitaA Group
- Thanks to all parents and the clinical staff of 25 German NICUs!
Part II: Bronchopulmonary dysplasia – Definition and Differentiation

- Mild form: day 28 supplemental oxygen – week 36 room air
- Intermediate form: week 36 supplemental oxygen < 30%; further
- Severe form: supplemental oxygen > 30% and/or ventilation at 36 wks

Synonym: chronic lung disease; the disorder affects very preterm neonates i.e. < 32 weeks mostly after mechanical ventilation due to respiratory distress syndrome often preceded by prenatal insults i.e. infection and growth impairment.

Definition:
Oxygen supplement requirements and/or mechanical ventilation at 36 weeks postmenstrual age with further specifications.
(Shennan, 1984 and Modification Jobe and Bancalari 2001; Pediatrics 2004 and NIH-Statement 2005; intermediate form)
Clinical Consequences of BPD: Respiratory Symptoms up to School Age

After diagnosis of BPD during the neonatal period, sequelae are:

- A high rate of rehospitalisation during the 1. year of life - focussing on preterms discharged on supplemental oxygen (Lit. up to 73%); during the 2nd year, the risk is reduced – however still increased by the factor 2 (Doyle et al., ABC, 2001).
- The risk of severe viral infections a.o. RSV is considerably increased - thus preventive measures are indicated.
- Clinical signs of airway obstruction are described to be increased up to pre-school age (OR 2.7).
- Medications with bronchodilators are prescribed more than twice as often than in controls – a metaanalysis indicates an OR 2.4.
- At school age, a persisting high rate of respiratory symptoms was observed: 30-24% in preterms with or without BPD presented with airway obstruction compared with matched controls 7% (Gross SJ et al., J Ped, 1988)
- According to data from the Netherlands this difference persists up to the adolescence with higher rates in females (Vrijlandt et al. Respir Res 2005)

**Cave:** Strong association of BPD with adverse neurologic outcome i.e. spastic hemi-paraplegia (van Marter- NICHD network, Pediatrics 2011)

- Current therapies will be addressed.

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Cellular Actions of Vitamin A

Metaanalysis Vitamin A Metabolism depending on Polymorphisms of encoding Genes

Freucci L et al Am J HGEN 2011

Development of intestinal Immunity– Role of Vitamine A and D

Belkaid Y and Hand T. Cell. 2014
Endogenous vs exogenous Retinoic Acid
Brown and Noelle; Eur J Immunol 2016

Endogenous Retinoic acid

- Promotes Th1 differentiation
- Required for mucosal Th17 cells
- Promotes TGF-β mediated iTreg induction in the gut
- Required for Th2 responses to parasite infection

Exogenous Retinoic acid

- TGF-β mediated Th17 generation
- IFN-γ and IL-17 production by memory/effector CD4+ T-cells
- IL-4, IL-5, IL-10, and IL-13 in Th2 in vitro cultures. Dose dependent effects observed on in vivo Th2 responses

RA concentration