Avian Antibodies (IgY) to Fight Antibiotic Resistance

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Received date: March 02, 2015, Accepted date: April 21, 2015, Published date: April 28, 2015

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Keywords: Antibiotic resistance; Microbes; Disease; Cross-infection

Introduction

The alarming increase of resistant microbes (bacteria, viruses, fungi, protozoa) is today one of the biggest threats to both mankind and environment. This article will push for the use of avian antibodies as a replacement or complement to antibiotics and thereby diminish the development of antibiotic resistant microbes.

Antibiotic resistance and how to fight it

The big load of antimicrobials (antibiotics, antimycotics and antivirals) given both to man and animals is a threat to our environment with the menace to give a total imbalance between microbial species in humans, animals, plants, water and soil.

Antibiotics are today found ubiquitous in our environment in constantly growing amounts. Antibiotics are given to animals as “growth factors” - especially to piglets, calves and chickens - to avoid weaning diarrhoea and to promote growth [1]. It is forbidden in Europe since 2006 and also in some states in USA. But they are still used widely in other countries. Antibiotics are distributed everywhere in animals and humans-blood, flesh, urine, faeces. Antibiotic contaminated flesh is eaten both by humans and animals. Antibiotics from animals and humans are excreted to the environment by urine and faeces. Antibiotic factories spread out tons of antibiotics from their plants. Unused antibiotics are thrown away. Soil and water are enormous reservoirs for antibiotics. Soil-dwelling microbes encounter a myriad of antibiotics mostly in low concentrations. Low concentrations do not kill bacteria, but they will evolve strategies to resistance. Multi resistant bacteria are found in excrements of birds far out in the wilderness of Greenland. Genetic spread of multidrug resistant bacteria is the biggest problem for propagation of antibiotic resistance. The gastrointestinal canal of humans and animals are “mating” places for bacteria to exchange their genetic material. Hospitals - especially intensive care unit (ICU) and new-born wards - are important domains for spread of genetic resistance. Cross-infections are the major impediment for the control of resistance.

European Union has decided to give a strong support for depression of resistant bacteria by formulating guidelines to control use of antibiotics (European Union Official Journal, nr C, 14.11.2013). The Infectious Diseases Society of America (Bad bugs, no drugs: Public Health Crisis Brews, 2004) has proposed legislative and federal solutions to this emerging health problem: Well controlled hygiene, isolation of patients with severe infections, careful antibiotic prescribing.

The recommended measures are not enough! For an optimal antibiotic control we need to think in new directions or rather go back to old ideas - according to Rousseau: “Retournons a la nature”!

The best defence for humans, mammals and birds against all kind of infectious agents has always been the immune system. A powerful way to fight infections in our days will be to use this system as complement or replacement to antibiotics.

Antibodies

Antibodies have a high specificity for binding and inactivating foreign substances including different microbes (bacteria, viruses, fungi, protozoa). The immune system and microorganisms have coexisted for millions of years and microorganisms have not become resistant towards them.

All mammals produce antibodies. Lately, much interest has been turned to antibodies from eggs of hens (avian antibodies, IgY). The yield of antibodies from eggs is much larger than can be achieved from any mammal. The collection of antibodies from egg yolk does not comprise any bleeding. European centre for the validation of alternative methods (ECVAM) strongly recommends avian antibodies as alternative to mammalian antibodies.

Antibodies from egg yolk (IgY)

Antigen specific avian antibodies (IgY) have been used for numerous applications in medical and research fields. One of the most valuable and promising areas of IgY is its potential to be used for passive immunization to treat and prevent human and animal infections. Much research has been done both in vitro and on animals and humans. However, clinical applications of IgY in humans are very scarce. For references see below: “IgY in therapies”.

Avian IgY is the functional equivalent to mammalian IgG and is the predominant immunoglobulin in egg yolk [2]. IgY is actively transferred from the serum of the hen into the egg yolk to give immunity to embryos and chickens. This corresponds to placental IgG transfer in mammals to provide immunity to the fetus. The overall structure of avian IgY is similar to mammalian IgG (Figure 1), with two heavy and two light chains, but there are some important differences. The molecular mass of mammalian IgG is 150 kDa, whereas it is approximatively 180 kDa for avian IgY. The greater molecular mass is due to increased number of heavy chain constant domains and a pair of extra carbohydrate chains. The hinge region of IgY is shorter and less flexible.

The evolutionary spread means that there is no immunological cross reactivity between avian IgY and mammalian IgG. Avian antibodies do not activate the complement system.

Antibodies are absorbed from the intestine in young piglets and calves during the first 24 hours to 48 hours post natum [3]. After this time period there will be no absorption of active antibodies from the gastrointestinal tract in animals. All studies in humans, including...
infants, show, that there is no absorption from the intestine [4], but
studies on new-borns are scares or not done. Since IgY is not absorbed
from the intestinal canal, there will not be any reactions outside the
 canal and no risk for toxicity.

Figure 1: Immunoglobin Y and Immunoglobin G.

A hen usually lays ~280 eggs in a year and an egg yolk contains
100–150 mg of IgY antibodies. This results in a yield of 28–42 g IgY
per year per hen [5]. When a hen is vaccinated against a microbe, 
around 5%-10% of the IgY will react to this microbe. The production
is simple, efficient and economical. It involves separation of the egg
yolk from the white, followed by purification of antibodies from lipids
and other materials. Avain IgY for therapeutic use can be produced 
from the egg yolk without any other ingredients than sterile water. IgY
is stable at pH 4-9 and up to 65°C in aqueous condition. High salt
conditions and stabilizing regents (e.g. sucker) increases the stability to
heat, acid pH and high pressure.

IgY fractions have been stored in at +4°C for 20 years without any
significant loss of antibody titer. The yield can easily be scaled up to
produce enormous quantities of specific protective antibodies. Eggs
are an essential part of the normal diet of man. There is practically no
risk for side effects when taken orally. However, it is important to
consider egg allergy before starting therapy with egg yolk antibodies
[5].

IgY in therapy

There are thousands articles about the use of IgY both in vitro and
animal studies, but there are very few human studies.

Bacteria

Anti-pseudomonas IgY and Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder caused by
mutations in a gene seven, encoding the cystic fibrosis transmembrane
conductance regulator [6]. This genetic defect leads to abnormally
thick mucus [7] and predisposes to respiratory infections, which are
the major cause of morbidity and mortality in CF. Several bacteria may
cause respiratory infections in CF, but chronic Pseudomonas
aeruginosa (PA) infections ultimately occur in virtually all of them.
Once a chronic PA-infection has been established, eradication is
hardly ever possible, even with high doses of antibiotics. Patients with
CF are a group of patients, who has the highest incidence of multi-
resistant bacteria in the world.

Anti-pseudomonas IgY (IgYPseud) reduces PA adhesion to
epithelia. Flagellin is the major antigen to which IgYPseud binds. This
binding prevents PA [8] to invade the host.

Flagellin is the main protein of flagellae and is crucial for
establishing infections in hosts. Flagellin effects PA chemotaxis, 
motility, adhesion and inflammation. In an in vitro test with PMN
cells there was a statistically significant (p<0.05) higher bacterial killing
with IgYPseud compared to non-IgY controls. This protective activity
may be explained by an opsonising effect of IgYPseud to Pseudomonas
[9] Vaccination with other bacteria that have flagellae, will probably
give similar immunologic reaction, as we have found for PA.

The first big clinical study in humans is our on patients with CF
[10,11]. CF-patients have been gargling every night with IgYPseud and
thereafter swallowing the drug. IgYPseud prevents colonization of PA
(statistically significant, p=0.011) and reduces the number of antibiotic
treatments. Anti-Pseud IgY was approved to be sold on license to
name-given CF patients in Sweden for the prevention of PA infections 
in 2002 (Clinical trials.gov/NCT00633191) and got Orphan Drug
Designation by EU in 2008 (EMEA/COMP/325516/2008). The study is
still going on. Hitherto~100.000 doses have been taken with same
good results and without any advert events.

Single trials indicate that IgYPseud might be of help also for
infections in other locations. One boy, who had an outer ear infection
with PA, which did not respond to antibiotics, was promptly helped by
IgYPseud droplets administered in the outer ear (unpubl.). A patient
with severe burns was helped by local application of compresses with
IgYPseud solution (unpubl.).

Gastro-enteric infections

IgY can easily be administered orally for the treatment of
gastrointestinal infections [12]. A fraction of immune globulins retain
their neutralization activity in various segments of the gastrointestinal
tract. A stronger activity would be easy to accomplish by buffers or in
acid resistant capsules (see “chlostridium difficile”).

Helicobacter pylori: IgY against H. pylori has been studied ed together
with lansoprazol in human volunteers with good results [13,14].

Enterotoxig Escherichia coli (ETEC): ETEC is a frequent cause of
diarrhea for children in developing countries as well as for travelers to
these countries. It accounts for around one million deaths yearly. Oral
administration of IgY-ETEC is proven to be successful for the
treatment of gastrointestinal infections in animals (see: “fodder”). This
indicates a good hope that it will be of value also for humans.

Salmonella species: The passive protective efficac of avian IgY to
tcontrol experimental salmonellois in mice [15] and calves [16] has
been examined. The animals treated with specific IgY showed
increased survival. IgY inhibits the adhesion of Salmonella enteritidis
to human intestinal cells in vitro [17] indicating that it has potential for
salmonellosis in humans.

Chlostridium difficile: A test of the passage through the human
intestine for bovine IgG for chlostridium difficile was done by Chelly
et al. They found that stool samples neutralized the cytotoxicity of c.
difficile-toxins A and B. A low dose (1.6%-3.8 %) of given fecal bovine
IgG was found in regular stools. The dose was increased to 8.8 % after
having given omeprazol, and was still more increased after having
given the IgG in enteric capsules (32.7%) [18]. I have not found these
experiments for IgY, but they would hopefully give similar results.

Oral infections

Streptococcus mutans causes caries. A mouth rinse containing IgY-
mutans reduces the establishment of these bacteria in dental plaques of
humans [19]. The antibodies inhibit S. mutans adherence to saliva-coated hydroxyapatite discs in vitro and decrease the percentage of S. mutans per total streptococci in vivo [20]. Chewing gums with anti-streptomutans IgY against caries are sold commercially in Japan.

**Hemolytic streptococcus A IgY** would be a perfect prophylaxis and treatment for tonsillitis, but I have not seen any such studies.

**Gingivitis:** Several studies on specific IgY have been studied in mice on bacteria causing gingivitis and halitosis (bad breath) such as *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* [21]. All results were convincing with less gingival infections, less plaques and less gingival bleeding. For fusobacterium the histopathological slides of the gums were improved after IgY treatment for 15 days.

**Pharyngitis:** In a double blind study from China, patients were treated with IgY from eggs of hens vaccinated with different non-defined bacteria from pharynx. Either pharynx-specific IgY or placebo was sprayed six times daily into mouth and pharynx. The group treated with specific IgY had significantly less symptoms of pharyngitis than the placebo group (p<0.01) [22].

**Multiresistant bacteria; ESBL, MRSA and VRE**

The emergence of multiresistant bacteria such as MRSA (multiresistant *Staphylococcus aureus*), ESBL (extended-spectrum β-lactamase)-producing bacteria and VRE (vancomycin resistant enterococci) is a serious dilemma. The number of reports on infections with these dreadful bacteria has dramatically increased over the last decades. Multidrug-resistant *E. coli* strains disseminate worldwide for which there are no new effective antibiotics. Potentially a “super bug,” resistant to all antibiotics may emerge in the near future. Constant and careful worldwide surveillance for multidrug-resistant bacteria is urgently warranted [23].

Specific IgYs for multiresistant bacteria have potential to be a new effective way to treat them. IgY-ESBL has been produced from hens vaccinated with bacteria carrying ESBL genes and found to have specific binding capacity. Despite the tremendous need to find an effective treatment for ESBL, MRSA and VRE infections, there is very little done for possibilities to treat these bacteria with IgY. Clinical studies on this prospect are urgently needed.

**Viruses**

**Influenza**

Influenza viruses remain a major threat to global health due to their ability to undergo changes through antigenic drift and antigenic shift. IgY antibodies against influenza can be administered passively in humans (orally and probably intranasally) and can be used quickly and safely to help in the fight against a pandemic influenza. In a study using inactivated H1N1, H3N2, and H5N1 influenza viruses to immunize hens a high level of anti-influenza virus IgY was induced in sera and eggs, which lasted for at least 2 months after two immunizations [24]. In vitro IgY inhibited homologous as well as heterologous strains of viruses. In a mouse model, IgY to H5N1 protected 100% of the mice against lethal challenge with H5N1, when administered intranasally 1 h prior to infection. Of particular interest was the finding that IgY to H5N1 cross-protected against A/Puerto Rico/8/34 H1N1. Based on these animal results, it is very plausible that IgY-Influenza can be used to prevent and control influenza viral infections.

Many countries including Vietnam introduced mass vaccination of poultry with H5N1 vaccines. In a study from Vietnam eggs were bought directly on the supermarket. Specific IgY-H5N1 was found in these eggs. When administered intranasally in mice before and after lethal infection with H5N1 and related H5N2, IgY could prevent infection resulting in complete recovery [25].

**Human rotavirus (HRV)** is the major contributing agent of acute infantile gastroenteritis, resulting in more than one million deaths annually. HRV causes shortening and atrophy of the villi of the small intestine, followed by decreased water absorption leading to severe diarrhea and vomiting. Oral administration of IgY from eggs from hens immunized with three different serotypes of rotavirus (mouse, human and monkey) prevented diarrhea in mice infected with murine rotavirus [20,25]. A small clinical human study in Bangladesh gave promising results, but was never followed up [26].

**Fungi**

*Candida albicans* is a tremendous burden for oncologic patients treated with cytotoxic drugs and antibiotics. More or less severe candidiasis in mouth and throat will occur in more than 75% of leukemic patients during the period, when cytotoxic drugs are given intensively. In a study IgY candida was given prophylactically as a mouthwash every day to four children with leukemia during intensive treatment with cytotoxic drugs. None of them got any candidiasis, but three of four controls [27].

**IgY in Fodder and in Fish Cultivations**

**In fodder**

*Enteric colibacillosis* by ETEC encountered in neonatal calves [28] and piglets [29] is a major cause of death in these animals. The passive protective effect of IgY against induced diarrhea by ETEC has been studied in these animals. In both it gave a good prophylactic and therapeutic result.

**In fish—cultivations**

*Yersinia:* IgY against *Y. ruckeri* protects rainbow trout from infection [30].

*Edwardsiella:* Anti-Edwardsiella IgY prevents Japanese eels from Edwardsielliosis infected with *Edwardsiella tarda* [31] Table 1.

<table>
<thead>
<tr>
<th>Microbe(s)</th>
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<td>In vitro tests: Promising results</td>
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Table 1: Excerpt of studies on IgY.

<table>
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<tr>
<td>Coccidiosis</td>
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<td>Study on protection of chickens, fodder.</td>
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**Conclusion**

An abundance of studies on specific IgY antibodies from immunized hens conclusively suggest that they are very strong weapons against a series of common infections and can be used both as a complement and/or an alternative to antibiotics. Since IgY is not absorbed from the gastro-intestinal canal, it is especially attractive for peroral or local immunotherapy for a variety of bacterial, viral and fungal infections in the gastro-intestinal canal, including mouth, throat and airways, as well as for treatment on the skin and other local infections. In addition, specific IgY can be of tremendous help in fodder and for fish-cultivation.

It is high time for all involved in fighting infectious diseases-politicians, health authorities, pharmaceutical companies, physicians, researchers etc., to join in a strong pull for avian antibodies-IgY.

Use of IgY will diminish the development of antibiotic resistant microbes.

**References**