CASE STUDY: POLYSPECIFIC IMMUNOGLOBULINE Y (IGY) THERAPY IN A PELVILINGUAL CARCINOMA DIAGNOSED PATIENT INFECTED WITH *STAPHYLOCOCCUS AUREUS* METHICILLIN-RESISTANT

STUDIU DE CAZ: TERAPIA CU IMUNOGLOBULINE Y (IGY) POLISPECIFICE LA UN PACIENT DIAGNOSTICAT CU CARCINOM SCUAMOS PELVILINGUAL, INFECTAT CU *STAPHYLOCOCCUS AUREUS* METICILINO-REZISTENT (MRSA)

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Abstract

Infections caused by multidrug-resistant microbial agents often complicate the treatment of patients with severe primary conditions such as neoplasms, organ failure or chronic diseases. The methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main global-spread nosocomial bacteria for which significant efforts are currently being made to develop new therapeutic options. Avian immunoglobulin (IgY) is used in various fields of human and veterinary medicine, with proven action against a wide range of microbial infections (bacterial, fungal and viral). In this study, IgY was successfully administered to a patient with MRSA-overloaded neoplasm, being incorporated into therapeutic products for general administration and local applications.

Rezumat

Infecţiile cauzate de agenţi microbieni multidrog-rezistenţi complică deseori abordarea terapeutică a pacienţilor cu afecţiuni primare grave, precum neoplasme, insuficienţe de organ sau boli cronice. Stafilococul auriu meticilino-rezistent (MRSA) reprezintă una dintre principalele bacterii nosocomiale cu răspândire globală pentru care în momentul de faţă se fac eforturi importante în elaborarea de noi opţiuni terapeutice. Imunoglobulina aviară (IgY) este utilizată în diverse domenii ale medicinei umane şi veterinare, având acţiune dovedită împotriva unei game largi de infecţii microbiene (bacteriene, micotice, respectiv virale). În studiul de faţă, IgY a fost administrată cu succes la un pacient cu neoplasm supraienfectat cu MRSA, fiind încorporată în produse terapeutice pentru administrare pe cale generală şi pentru aplicaţii locale.

Introduction

Immunoglobulins Y (IgY) represent an alternative to mammalian immunoglobulin; they are successfully applied for scientific, diagnostic, prophylactic and therapeutic purposes.

Since mammalian antibody technology has a number of disadvantages (the purification of mammalian serum antibodies is low, laborious and expensive, increased stress for the animals involved), it has increasingly been replaced by IgY-based technology.

A major advantage of poultry use is that avian immunoglobulin is harvested directly from egg yolk instead of serum, thus avoiding blood harvesting.

Nowadays, hens are recognized as a convenient and cheap source of antibodies.

It has been reported that from a hyper immune egg it is obtained a similar immunoglobulin quantity to that obtainable from 300 ml of rabbit blood (Michael et al., 2010).

Used as an alternative to antibiotic therapy, IgY does not induce antimicrobial resistance and does not exhibit remanence in the treared organism (Chiurciu et al., 2017).

Globally, localized head and neck squamous cell carcinoma is the 8th most common malignancy, with approximately 500,000 new cases reported each year.

These tumors have various clinical aspects, their management involving surgery, radiation therapy or chemotherapy, either individually or in combination (Li et al., 2003).

Staphylococcus aureus is frequently located at the cutaneous and intranasal level (Guimarães et al., 2009).

Humans and animals are a natural reservoir for staphylococci, including *Staphylococcus aureus.*

Thus, asymptomatic colonization is much more common than infection.

Transmission of staphylococci occurs via direct contact with the carrier, and colonization may be transient or persistent (Chambers, 2001).

Staphylococcus aureus include a large scale of strains with variable pathogenicity (Tobias et al., 2012).

Staphylococci are naturaly sensitive to most antibiotics, but excessive use of antimicrobials has led to the development of resistant multi-drug strains (Guimarães et al., 2009).

When reporting *Staphylococcus aureus* methicillin-resistant (MRSA) infection, a thorough investigation often reveals recent hospitalization, contact with a person who has been hospitalized, or recent antimicrobial therapy (Chambers, 2001).

Thus, MRSA has spread extensively throughout the world, becoming one of the major pathogens causing nosocomial infections.

As it creates important issues in the medical field but also in communities with no apparent association with hospitalization, its diagnosis as quickly as possible and the application of effective therapy has become priority objectives for its control (Yamada et al., 2013; Klevens et al., 2007).

As antibiotics have a smaller spectrum of action, alternative variants are being studied. Passive immunization of patients with MRSA infections using avian immunoglobulins (IgY) specific to this pathogen seems to be an increasingly approachable option in the near future.

In the **Romvac S.A. Imunoinstant Research and Development Department**, specific avian immunoglobulin with specificity for many infectious bacterial, fungal and viral agents is obtained, with pathogenicity both for humans and various animal species (Chiurciu și col., 2017; Topilescu și col., 2014).

In the present study, using hyper immune egg and products derived therefrom, a protocol for the complementary therapy of a patient with MRSA-infected squamous cell carcinoma was developed.

1. Materials and methods

The hyperimmune egg and products derived therefrom

All procedures applied within the Department are in line with EU Directive 2010 / 63 on the handling of animals used for scientific purposes.

All the studies performed are approved in advance by the Ethics Committee of Romvac S.A. Within the Romvac S.A.

Imunoinstant Research and Development Department, hyperimmune eggs and products derived therefrom are used in human patients as well as in the field of veterinary medicine.

Products for human patients are both for general administration (notified as dietary supplements) and for topical applications (notified as dermato-cosmetic products).

To obtain the hyperimmune egg, clinically healthy chickens of the Rhode Island breed, 18-19 weeks old, and body weight of about 2.6 kg are used.

Birds are housed in a battery-growing system in controlled microclimate factories. Birds are fed by a standardized, ecological diet, on the principle of *ad libitum*.

Antigens used

For the immunization of laying hens, an inoculum prepared from 18 pathogenic, bacterial and fungal strains, was used: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Salmonella spp.,Salmonella enteritidis, Salmonella typhimurium, Streptococcus mutans, Streptococcus grup B, Proteus mirabilis, Acinetobacter baumannii, Clostridium difficile-corpi bacterieni, Clostridium difficile-anatoxina, Helicobacter pylori,Candida albicans, Candida glabrata, Candida krusei.*

Hens immunization and eggs collecting

After preparation of the inoculum, it is administered intramuscularly to laying hens according to an immunization schedule: three inoculations with 0.5 ml of antigen in two separate points in the chest muscles.

Eggs are harvested from the 14th day after the third immunization, when the yolk antibody titer reaches a maximum level and their concentration is between 100-250 mg of IgY / egg.

The collected eggs are stored at 2 – 8 ℃.

Elaboration of polyspecific immunoglobulin Y products

From the hyper immune eggs, a wide range of human products is prepared, which includes polyvalent and monovalent solutions, lyophilized powders, sprays, aerosol solutions, tablets, gels, creams, suspensions, shampoos.

Other product categories are being investigated.

Qualitative and quantitative testing of immunoglobulin Y from eggs and derived products

The harvested hyper immune eggs are tested for the specificity of immunoglobulin contained in inoculated antigens by the ELISA *in-house* indirect method.

For derived products, the total immunoglobulin Y concentration is tested by the *in-house* ELISA test, direct method.

The microbiological control follows the sterility for the oral solution with IgY, respectively the microbiological contamination for lyophilized powder, spray and cream, according to the Romanian Pharmacopoeia, 10th edition.

The patient included in the study

M.B., 39-year-old male, suffers from pelvic floor squamous cell carcinoma, with first clinical signs being observed in January 2018.

Prior to the consultation in the Imunoinstant Complementary Medicine Cabinet, he was hospitalized twice.

During the first hospitalization, which occurred shortly after the patient had observed the neoplasm, he underwent surgery at that level, radiotherapy and chemotherapy.

In May 2018, the patient was readmitted accusing solid dysphagia, the appearance of a suppressed, ulcerated, retro-auricular tumor formation of a previous cervical tumor, both about 1 month old and with progressive worsening.

Clinical consultation revealed postoperative bilateral lateral-cervical scarring, a supine, retro-auricular tumor, ulcerated, enlarged lateral-cervical and parotidian right (Figure 1.), an anterior cervical tumor with super elevation, semi-rigid consistency, fixed to deep, spontaneously painful and palpitating, with overdosed inflammatory phenomena, post-pelviglosectomy posture, free nasal vestibule, free nasal passage, free cavity, normal laryngeal crown, moving vocal cords and sufficient glottis space.

The second hospitalization took place over 5 months. In September 2018, a radiological pulmonary examination was performed, its conclusions being: no imaging nodular imaging for radiographic visible secondary pulmonary determinations, cord with dimensions within normal limits.

Fig. 1. Enlarged, suppurated and ulcerated, latero-cervical and parotidian right retro-auricular tumoral formation (September 2018)

Laboratory analyzes performed in the same month revealed the following pathological changes:

- HCT ↘ (34.1%);
- HGB ↘ (11.2 g/dl);
- RBC ↘ (3.97x10^6/µl);
- Neutrophiles ↗ (8.31x10^3/µl);
- Lymphocytes ↘ (1.14x10^3/µl);
- PLT ↗ (446x10^3/µl);
- VSH ↗ (111 mm/hour);
- Fibrinogen ↗ (750 mg/dl);
- Sodium ↗ (146.4 mmol/l).

In September 2018, the patient underwent the following medical procedures:

- Incision and drainage of anterior cervical tumor formation;
- Tracheotomy;
- Anchoring trachea to the skin;
- Bubble cannula mounting.

A bacteriological examination of the ulcerative tumor was performed, which identified the infection with *Staphylococcus aureus*.

The postoperative progression is favorable under treatment and it is decided to discharge the patient in September 2018, with the following recommendations:

- hygienic-dietary regimen, respectively changing the cannula once / day, changing and washing the mandrel 5x / day;
- Continuing oncological treatment.

Avian immunoglobulin therapy

In October 2018, the patient is consulted within the Imunoinstant Complementary Medicine Cabinet.

At the time, the ulcerated right retro-auricular tumor suppressed and had no healing tendency despite antibiotic therapy prescribed at the time of discharge. Samples of purulent secretion are collected to identify pathogens that prevent lesion healing. *Staphylococcus aureus* methicillin-resistant (MRSA) is identified.

Next, ELISA assays are performed to determine the specificity of the immunoglobulins contained in products derived from the hyperimmune egg to the identified bacterial strain (Figure 2.).

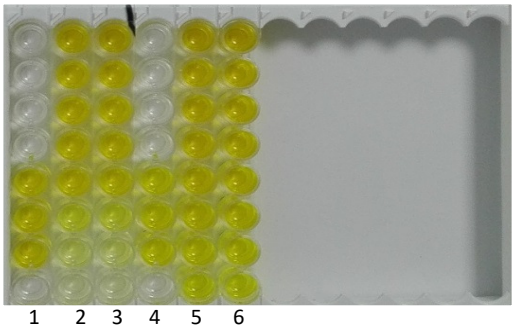

Figure 2. Testing for the specificity of the immunoglobulin Y contained by the lyophilized powder (lanes 1-3) and the sterile stackable solution (lanes 4-6) administered to the patient versus the staphylococcus identified in the plague

Therapist establishes the following therapeutic protocol

For oral administration:

- Lyophilized powder obtained from an integral hyperimmune egg containing 100 mg IgY / dose, 2 doses / day given in the morning after breakfast, for 4 months. After ingestion of the product, the patient does not consume anything for 2 hours;
- Purified IgY, aqueous extract, 80 mL sterile solution with a total IgY content of 150 mg, one bottle administered in the evening, before bedtime, for 4 months. After ingesting the solution, the patient does not consume anything until the morning.

Locally:

- purified IgY spray, aqueous extract, sterile; sprays 3 times / day for 4 months. Applies after the local toilet. The area is not wiped (drying is expected). After applying the spray, apply a membrane-based cream, made from a hyper immune egg with a healing role.

2. Results and discussions

The patient has strictly respected the therapist's established protocol. He did not report adverse effects following the

administration of avian immunoglobulin products. IgY therapy was complementary to oncology treatment, did not interfere with it, but, on the contrary, constituted an immune support.

The patient experienced a relief of the side effects of oncology treatment. In February 2019, the plague is completely healed (Figure 3.).

Fig. 3. The patient healed after 4 months of complementary therapy with avian immunoglobulin (January 2019)

The patient performs a computer tomography (CT) examination, after which the following aspects are identified: certain dimensional regression of the cervical tumor masses, with the maintenance of the right supra tracheotomy nodular lesions, respectively with the right supraclavicular infiltration aspect; small cervical bilateral and mediastinal adenopathy with global dimensional and numerical stability compared to previous examinations; without secondary bilateral pulmonary nodular certain CT detectable; otherwise, the CT examination did not show any noticeable particularities.

The patient repeats microbiological analyzes. It is harvested crust from the cutaneous area where previously the ulcer tumor was located.

The result is negative for pathogenic microbial flora.

3. Conclusions

Avian immunoglobulin (IgY) therapy is an effective and safe alternative to classic treatment with synthetic antimicrobial agents, especially for strains resistant to already established molecules, as well as for patients with various conditions (neoplasm, extensive post-traumatic lesions, organ failure, other chronic diseases) complicated by bacterial and / or fungal infections.

IgY therapy has no adverse effects, but has only one contraindication, that it is not administrated to patients with known allergy to the egg.

In the present case, avian immunoglobulin formed immune support ameliorating the adverse effects of oncological treatment.

In addition, MRSA infection was cured, fact confirmed by microbiological analyzes performed after the end of the therapy.

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