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MESTRADO INTEGRADO EM MEDICINA

2019/2020

Joana Oliveira e Sousa Ferraz Brandão

Streptococcus pyogenes in tumor treatment: the past, present and future

Fevereiro, 2020

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Streptococcus pyogenes in tumor treatment: the past, present and future

Mestrado Integrado em Medicina

Área: Doenças Infecciosas

Tipologia: Monografia

Trabalho efetuado sob a Orientação de:

Doutor António Carlos Megre Eugénio Sarmento

Trabalho organizado de acordo com as normas da revista:

Nature reviews journals

Fevereiro, 2020

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Streptococcus pyogenes in tumor treatment: the past, present and future

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À minha Família e ao meu desporto, a Ginástica Acrobática, que me ensinaram a aceitar todas as oportunidades que a vida propõe com empenho e dedicação.

Aos meus Amigos, que me orgulho de ver crescer e tornarem-se todos os dias pessoas mais completas, sem nunca perderem o seu singular sentido de humor e vontade de entrega ao outro.

À Tuna Feminina de Medicina do Porto e à Família que construí na *mui nobre* Faculdade de Medicina por me terem ensinado a importância da abstração das convicções pessoais pelo bem de uma causa maior e que, independentemente dos percalços que surgirem, no final tudo se concretizará.

STREPTOCOCCUS PYOGENES IN TUMOR TREATMENT: THE PAST, PRESENT AND FUTURE

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4200-319

Abstract

In light of the recent emergence of immunotherapy as a possible treatment in Oncology, and considering the concept of spontaneous tumor regression following an infectious episode, the present work aimed to understand the role of infectious diseases in tumor treatment, specifically concerning the pathogen *Streptococcus pyogenes*. The pertinence of this bacteria in cancer treatment has been investigated throughout the last century and has achieved promising results, but it still isn't a reliable tool.

This work reviews the most relevant aspects of *Streptococcus pyogenes* usage in cancer treatment, including: *Streptococcus pyogenes*-based formulations, administration protocols used, possible mechanisms of action for the evidenced tumor regression, main side effects observed and possibilities for clinical assessment of responders and non-responders, as well as the most relevant differences in the outcomes observed in different clinical studies.

The current evidence suggests that *Streptococcus pyogenes* has established itself as a reliable inducer of the immune system, which can positively contribute to cancer treatment. However, further clinical evaluations are still needed to validate the present findings and allow for a conclusion to be drawn.

Key words: *streptococcus pyogenes*; OK432; immunotherapy; bacterial infection; tumoral regression

Resumo

Aliando a recente expansão da Imunoterapia, uma opção promissora no tratamento de tumores e cancro, ao conceito antigo de regressão espontânea de massas tumorais pós-infeção, surge a pertinência de avaliar o papel das doenças infecciosas no tratamento tumoral.

No âmbito da presente revisão, abordou-se com especial enfoque o *Streptococcus pyogenes.* A relevância desta bactéria advém de ainda não se ter consagrado como uma ferramenta de tratamento, apesar dos resultados promissores que têm vindo a ser obtidos no último século.

Assim, nesta revisão estão reunidos os aspetos mais relevantes do uso desta bactéria no tratamento de massas tumorais, nomeadamente: preparações obtidas com base no Streptococcus pyogenes, protocolos de administração, possíveis mecanismos de ação para a regressão tumoral evidenciada, principais efeitos colaterais observados e possibilidades de monitorização clínica, assim como as diferenças mais relevantes nos resultados observados em diferentes estudos clínicos.

As evidências atuais sugerem que o *Streptococcus pyogenes* é um indutor do sistema imunológico, o que poderá ser uma importante contribuição para a abordagem dos doentes com tumores e cancro. No entanto, para se ter uma noção concreta do seu papel, constatamos que são ainda necessários mais estudos clínicos.

Palavras-chave: *streptococcus pyogenes* tumor treatment; OK432; imunoterapia; infeção bacteriana; regressão tumoral

Introduction

The idea of inducing infection as a tumor treatment has already been studied for many years. The current approaches to cancer treatment are frequently associated with high morbidity and mortality, since they often rely on radio- and chemotherapy and extensive surgical procedures. Thus, there is an imperative need to find new, less harmful, therapeutic approaches. Such options may include the stimulation of the immune system, namely by using bacteria based treatments — a well-established and widely accepted example can be found in the use of *Bacillus Calmette-Guérin* (BCG), an immunotherapy approach for bladder cancer.[1,2]

Currently, the several immunotherapeutic approaches available in Oncology focus on the stimulation of the host's immune system to attack cancer cells. This enhanced capability is thought to be in the basis of an old concept, the spontaneous tumor regression. This phenomenon is characterized by the reduction of the tumor's size and/or proliferation rate, and is frequently associated with prior bacterial, fungal, viral or protozoan infection or vaccination therapy before the cancer diagnosis, specifically in the absence of prior cancer treatments. Being the presence of an acute febrile state the key event proposed to anticipate the tumor's regression.[2]

The rationale for exploring bacteria to possible applications in immunotherapy treatment is built on the fact that these unicellular organisms thrive in unfavorable environments — such as tumor's — while having several mechanisms which incapacitate other cells, thus potentially controlling neoplastic disease.[3] Additionally, they activate the host's immune system, making it more capable of attacking cancer cells.[4]

Streptococcus pyogenes is a known human pathogen, that belongs to group A streptococci. This bacteria was the base of Coley's Vaccine in the late 18th century.[1,5] Accordingly, *Streptococcus pyogenes* is a bacteria that fulfills the aforementioned criteria, as it exhibits various mechanisms which may affect tumor progression, such as: direct tumoral cell lysis; humoral activation linked to infectious insult; and stimulation of immune response by presenting tumoral antigens to dendritic cells for the activation of cellular immunity through the detection of tumoral components by dendritic cells.[6-9] For these reasons and with the increasing knowledge of *Streptococcus pyogenes*' pathogenicity, some researchers believe it can prove itself as a valuable anti-tumor agent.

Thus, the goal of this review is to examine the literature regarding bacteria based treatments in tumor treatment, specifically with *Streptococcus pyogenes*, and its possible role in forward-looking therapies.

Methods

This review comprises information of 113 articles, obtained through a search on PubMed®. This search was conducted in June 2019 using the query "*Streptococcus pyogenes* tumor treatment", resulting in 326 articles found. After a review of the titles, 105 articles reporting infections as precipitants of cancer regression were selected, with the remainder of papers being excluded for reporting infections as complications in cancer patients. Twenty-three additional articles were excluded after a careful evaluation of the abstracts – 18 due to language (articles in Japanese, Chinese and German) and 5 due to duplication of articles.

A second more specific search was performed on PubMed® in December 2019 in order to assemble the most recent findings and clarify specific topics. So, in addition to the 82 articles collected in the original search, 31 new articles were added, given their adequacy to the topics included in the present review. For the clinical evaluation of the role of *Streptococcus pyogenes,* we included only controlled randomized clinical trials and excluded most case reports, since no meta-analysis was being applied in the present paper to understand the statistic effect of these cases.

I. Historic Review

Infection and tumor regression have been connected for centuries. The oldest report available dates to 2600 bC in ancient Egypt, where poultice and incision were used to cause infection, which would then stimulate tumor regression.[1,2] Later, there have been worldwide reports concerning immunotherapy applied to cancer, with the work of Doctor William Colley (1862-1936) who introduced the Coley's Vaccine — a mixed bacterial vaccine obtained from *Streptococcus pyogenes* and *Serratia marcescens* used for the treatment of Sarcomas —, being one of the more remarkable ones.[2,10]

Before Doctor Colley's work was published, two other doctors, Karl David Wilhelm Busch (1826-1881) and Friedrich Fehleisen (1854–1924) had also reported that tumors regressed after the patient contracted an infection.[1,11]

Doctor Busch was one of the first to link regression of head and neck sarcomas with a prior episode of erysipela. In a 19 year-old patient with a neck sarcoma, he applied a bandage removed from a patient with acute erisipela, to a small burnt lesion in the young adult's tumor. After 14 days of 40ºC fever, the tumor shrank from the size of a small child's head to the size of a small apple, during this time, her condition deteriorated extensively but the patient survived and the tumor regressed after the infection was resolved. Nonetheless, since no follow up was conducted, the final outcome was not reported.[1,2]

During Busch's experiments, the agent that induced erysipelas was unknown. Some years later, Doctor Fehleisen isolated the agent and named it as *Streptococcus erisipelas,* now known as *Streptococcus pyogenes.* Subsequently, since he was able to culture this bacteria, he tried to apply cultured cells to cancer patients, and achieved some remissions.[1]

Posteriorly, other doctors tried to induce infections to treat their patients' cancer having achieved promising results, but none pursued this matter as extensively as Coley did. However, while this therapeutic option was arising, other, more technological and advanced techniques were applied in cancer investigation and treatment, relegating this field.[12]

I-a) Coley's Vaccine

In the late 18th century, Coley treated 10 patients with terminal osteosarcoma with a vaccine that comprised *Streptococcus pyogenes* and *Serratia marcescens*, and proposed a connection between the development of fever and a positive response to the vaccine with tumor regression.[2] He

did so based on multiple cases where he observed cancer patients going into remission, after infection or fever induction.[13]

According to Coley, a good result depended mostly on the magnitude of the fever. Later, Coley-Nauts, his daughter, described that the injection of bacterial serum, its characteristics and protocol of administration, as well as its proximity to the tumor, were also key factors in predicting the success of the treatment.[14] Since then, different streptococcal serums were used with no specific dosages having been found concerning the most effective vaccines.[1,5]

One of the most peculiar findings was that concomitantly to the fever, there was a decrease of the patient's pain, allowing for the reduction of analgesics.[2,15]

Since the emergence of this therapy, there have been several reported cases of good outcomes of the application of Coley's Vaccine. Highlighting Coley-Nauts' research, among the 1200 cases she collected, 270 were inoperable tumors who achieved full remission after the administration of Coley's Vaccine Protocol.[14]

II. Formulations with Streptococcus pyogenes for anticancer therapy

As previously mentioned, *Streptococcus pyogenes* was initially used in mixed bacterial vaccines. Nonetheless, many other formulations were later tried: Isolated formulas[8], and Streptococcal lysates[4], namely OK432 (Picibanyl, NSC-B116209, Sapylin, Polyssaccharide K)[16-18], OKPSA[19-21], 60F fraction[22-27] and SAGP[28-30]. More recently, it was reported the insertion of enzymes such as Streptococcal arginine deaminase[31,32], and genetic therapy with insertion of streptolysin's gene[33,34] and streptococcal gene Em55[35-37] in tumoral cells. Scientific research was conducted *in vitro* and *in vivo* pursuing clinical applicability. From all the serums, OK432 was found to be the more frequently used, so we targeted it extensively in our review.

II-a) Proposed mechanisms of action of Streptococcus pyogenes derived products

Several studies were reviewed, and a possible mechanism of action was gathered. Globally, there was a consensual belief that both innate and adaptive immune system have a role in streptoccocal anticancer therapy.[2,5,15] The unspecific cytotoxicity combined with specific immune reactions[4] seems to lead to tumoral cell' death by necrosis and apoptosis.[5,15]

The local inflammation caused by streptococcal components induces a cytokine and chemokine storm with special increments in TNF, INF and various interleukins.[7,10,38-40] Contrary to other causes of increase of interleukins, with streptococci stimulation it occurs independently of

CD18 action, probably by a Toll-like receptor pathway, possibly Toll-Like receptor 4.[20,39] The increase in interleukins is sensed by dendritic cells and interpreted as an attack on the host, activating them.

Furthermore, *Streptococcus pyogenes* is recognized as a pathogen by the humoral system of the majority of the population. It was observed that streptococcal antigens linked to Pathogen-associated molecular pattern (PAMPs) — pattern recognizing receptors[7,10,40] in dendritic cells (DC) could induce the subsequent activation of tumor specific T-cells.[13] Moreover, there is also a heat-shock response further activating DC and antigenic recognition up-regulating immunogenicity.[10,14]

A recent study proposed that OK432 induced a specific type of dendritic cell, the IFN-DC, that produces type I interferon (IFN) and Interleukin-12 (IFN-γ) to a further extent and has the capability to increase natural killer action over targeted cells. This IFN-DC, when induced by OK432, showed an ability to specifically kill tumoral cells *in vitro*, by increasing its antigen' presenting ability and promoting the tumor necrosis factor-related apoptosis, thus inducing TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand-mediated killing activity.[41]

Streptococcal components could particularly induce monocytes to secrete a chemoattractant that leads to differentiation and proliferation of macrophages, which are effectors of streptococci anti-tumor activity.[29,42,43] Additionally, they increased natural killer activation factor (NFA) to directly stimulate NK cell activity[44] and the antitumor effects were also thought to comprise Nitric Oxide (NO) related actions, since the rise of inducible NO synthase and NO production was reported .[45-47]

Furthermore, when a fibrin compound was added to a streptococcal preparation, there was an increment in plasminogen's activity in tumor stroma and further macrophage activation, leading to a delayed type-like hypersensitive reaction.[48]

Additionally, it was hypothesized that the binding of streptococci formulas to IAP-Specific 6-protein coupled receptor, activates PTPases that dephosphorylate epidermal growth factor (EGFR) and subsequently block p42/44 MAPK, inhibiting tumoral cell proliferation.[49]

Summarizing, there are two major events occurring, namely cell adhesion and chemokine response towards tumoral cells that promote apoptosis and necrosis of tumor cells.[22,39] Without these no anticancer activity was observed.[22,39]

Some authors successfully proceeded to the insertion of streptolysin gene in cancer cells causing cell membrane damage, loss of extracellular macromolecules and severe ATP depletion that lead to necrosis due to the inability to activate caspases[33,34]; and the insertion of Em55 gene,

resulting in the expression of Protein M (a streptococcal virulence factor) recognizable by the host's immune system, that could enhance cell mediated immunity and leading to specific tumoral cell death.[35,36]

Finally, after the inflammatory reaction ends, streptococcal formulas also contributed to the stimulation of fibroblasts, collagen fibers deposition, promotion of secretion of growth factors and incremental angiogenic ability, leading to faster wound healing.[50,51]

II-b) Streptococcal formulas

As explained before, OK432 is the most frequently implemented and studied streptococcal lysate, having been used in several case reports, nonrandomized clinical trials, and recently, randomized clinical trials. So, its action and immunologic effect have been collected extensively, as well as its side effects and treatment protocols.

Nevertheless, we believe that special attention should be given to the later developments with the arginine deaminase gene[32] and streptolysin gene[33,34], since the experimental reports seemed promising.

II-c) Arginine deaminase

It is known that disturbances in arginine metabolism are a common feature in several tumor cell lines. This resides on the fact that in the absence of arginine, the cells are forced to stop at G1, over regulating heat-shock protein’s genes which leads to an autophagocytic response and increases the tumoral cells sensibility to other insults.[31,32]

Arginine deaminase (ADI) is an enzyme that is present on *Streptococcus pyogenes* and has the ability to metabolize arginine in ammonia and citrulline. Recently, it has been successfully inserted in Glioblastoma multiforme tumoral cell lineage, and it has been reported that in both *in vitro* and *in vivo* experiments it provoked the degeneration of the tumor.[31,32]

II-d) Genetic therapies

Streptolysin is a known streptococcal virulence factor that causes direct extensive cell membrane damage, therefore the injection of this activated protein in tissues can cause extensive injury. However, the attempts to make it transportable through the human body and only active on site have been ineffective.[33] An attempt has been made to insert the streptolysin gene on tumoral

cells. First, an adenovirus prototype was used as vector; afterwards a spliceosome-mediated RNA-transplicing technology was attempted.[33,34]

Both experiments were conducted *in vitro* and showed successful outcomes. In both cases, it was reported that tumoral cells produced streptolysin and this led to membrane disruption and tumor cell death. This matter introduced *Streptococcus pyogenes* as a promising new agent in suicide gene cancer therapy.[33,34]

Before the use of streptolysin, the use of streptococcal gene Em55 was also attempted, given that it encodes M protein, a surface virulence factor. This M protein binds to glycosaminoglycans (GAGs) and enhances antiphagocytic and antiopsonization activity of *Streptococcus pyogenes* that further increased the immune system's response.[35,36] Then it was attempted to insert a DNA plasmid containing Em55 gene in tumoral cells, that were subsequently used to create a vaccine. [37] Although it seemed promising, no further studies were found regarding this matter.

III. Clinical studies of Coley Vaccine and OK432

From the reports we reviewed concerning Coley's Vaccine and OK432's *in vivo* application, we were able to assemble the most important and consensual features.

III-a) Route of administration

The following methods were explored: intramuscularly, locally intra- or peritumoral and intravenously.[5,14] Although in the early experiments, much concern about first applications as intratumoral injection was given, thinking that it could precipitate severe complications such as tumor lysis syndrome or circulatory collapse[14], in the later studies intratumoral injection was indicated as the most effective method[52-68]. In fact, in cases of malignant ascites intraperitoneal was the best choice[22,69-71]. In the most recent experiments, the systemic approach didn't seem as powerful in reducing tumor burden specially concerning pancreatic cancer.[15]

III-b) Administration protocol

Both single and multiple administrations of the streptococcal formulas were attempted. Single administration seems to have the fewest effects[58], however it had a significant positive result in intrapleural administration in intrapleural malignancies.[72-77]

Multiple exposure, with a 24 to 48 hours interval between injections, showed the greatest efficacy[14], having results ranging from lower recurrence rate, enhancement of disease stability and better survival rate, to complete tumor regression.[6,43,58,78-87]

Concerning the dosage of streptococcal content in the vaccines, it has been customized through different studies, and no standardization was found in the articles reviewed. However the first experiments mention a gradual increase of the vaccine's bacterial content until a fever of >39ºC was present.[12,14]

III-c) Combination therapy

Regarding the reports on the usage of streptococcal derivates to treat cancer patients, several reported that this procedure was conducted after the patient had undergone other treatments or was used as a complement to other therapies. More specifically, it was used as a complement to pleurodesis[72-77], in combination with chemotherapy, after mastectomy with axillary dissection in breast cancer patients[51,88], as an adjuvant agent to chemo- or/and radiotherapy and as an immune maturation agent [16,89-92]. These promising reports indicate that streptococcal formulations could enhance tumor treatment of other antineoplastic drugs[7,15], allowing the use of lower radiation doses, even below those proposed as tumoricidal.[93] Finally, as indicated before, its application also allowed for the decrease of analgesic dosage.[2]

III-d) Clinical assessment of response to application of *Streptococcus pyogenes* derivates in cancer patients

From the reports that regarded a clinical approach to the use of streptococcal derivates to treat cancer patients, especially through OK432 and mixed bacterial vaccines, the most relevant observations were gathered concerning the changes in physical examination and possible laboratorial criteria to assess a positive response.

Good outcomes were often reported, including diminished growth tumor, significantly reduced healing time[51,88], reduction of recurrences, controlled dissemination[52-54,56,57,63] and prolonged survival time.[16,22,26,43,55,59-62,64-71,89-92,94,95]

Concerning the assessment of the clinical signs and symptoms, a shift in the characteristics of the tumoral mass was observed macroscopically — first by becoming gradually less hard and immobile, and afterwards paler, softer and more mobile, until it discharged a caseous secretion or

simply regressed.[5] One important aspect reported was that no apparent effect on healthy tissues was observed, not even when it was used in combination with radiation therapy.[93]

Moreover, in closed cavities, the sclerosing effect of the injection with OK432 was able to enhance adhesion and obliterate the newly created space, with the most successful cases being those where the injection was applied in macrocystic lesions (>1cm), with concomitant aspiration of the cavity's content before the injection of OK432.[12,75] Nonetheless, there were reports indicating that streptococcal formulas were unable to produce a significant impact if curative surgery had already been performed.[56,95]

Concerning the laboratorial complementary assessment of response to the injection of *Streptococcus pyogenes* components, both blood and cutaneous tests were reported to have promising results. Hanaue et al. observed that the presence of a normal or higher peripheral leucocyte count and the levels of lymphocyte and the percentage of T cells correlated significantly with survival and anticipated a better outcome.[96] Later, Chirigos et al. reported that the T cell role in survival was more specifically due to an increment on NK cytotoxicity observed with just 0,001µg/mL of OK432, and with peak activity at 1µg/mL.[97] Moreover, Aoyagi et al. noted the reduction of CEA to normal values within 6 months of OK432 injection.[54]

Regarding the effects of *Streptococcus pyogenes* exposure on skin tests, Hananue et al. reported a significant correlation with survival concerning phytohemagglutinin skin test (PHA skin test) and serum titer glutinine. The first test, PHA skin test, consists of phytohemagglutinin application in the skin, that nonspecifically stimulates normal lymphocytes, thus indicating the efficiency of a healthy cell-mediated immunity system. In the second test, a significant correlation with life-span rate ($p<0,01$) was observed when the serum antibody titer rose 64 times higher, after stimulation with *Streptococcus pyogenes* components.[96]

The most widely used skin tests were PPD and SU-PS skin test, and both showed good correlation with life span rate.[98] The PPD is a test that uses a purified protein derivate of tuberculin and was also linked to a better survival rate[96], specially when it was observed the conversion from negative to positive 2 to 6 weeks after OK432 peritumoral injection.[16,98] Nonetheless, there was one study which reported no change in intratumoral injection 89 and another describing no changes when the injection was systemic.[16]

Concerning the SU-PS skin test, in healthy patients the reaction is usually positive and changes to negative as the cancer progresses.[94] Its mechanism of action is still not well understood[98], but there are several reports arguing that in most cancer patients it re-shifted from

negative to positive 2 to 6 weeks after the OK432 injection.[89,94,98] Further analysis showed that whilst SU-PS changed reactivity, it was also detected an increase in lymphocytes, T-helper cells and leucocytes (due to neutrophils enhancement).[98] This confirmed that a delayed hypersensitive reaction is in the basis of the response to *Streptococcus pyogenes* anti-tumor activity, and therefore is able to recall an antigen and anticipate a response to OK432 injection.[94]

Although, the mechanisms behind *Streptococcus pyogenes* formulas usage in cancer treatment have been studied extensively, there is a lack of solid clinical evidence approaching its effects on patients with tumors. Nonetheless, we gathered some informations concerning the tumors in which OK432 had been used. In prospective studies, the biggest flaw was the small number of subjects, but they still reported the possibility of a reliable efficacy of OK432 in the treatment of recurrent cervical cysts and lymphoceles[99-101], metastatic and/or recurrent head and neck tumor[102], sialoceles post-parotidectomy[103], juvenile nasopharyngeal angiofibroma[104], malignant brain tumors[16,92], oral melanoma[105], gastric carcinoma[52,57], hepatocelular carcinoma[65,68], colorectal carcinoma[72], malignant ascites[68] and malignant pleural effusions.[75] Accordingly, the observations collected retrospectively concerning OK432 in treatment of refractory/recurrent ovarian cancer[86,87] and malignant pleural effusions[73,76,77] were also promising, having reported an increase in disease stability and better outcomes with survival rate free of disease being significant after OK432 administration.

III-e) Randomized controlled trials of OK432 in tumor treatment

Two multi-center randomized trials conducted in Japan reported that intratumoral injection of OK432 before curative surgery in gastric cancer had a positive impact in the 5- and 10-years survival rate in stage III cancers, as well as it was effective in reducing the regional lymph node metastasis. Nonetheless, both reported that overall there were no differences in outcomes between experimental and control groups.[53,56] This matter was later clarified by Oba et al. in a meta-analysis with 8009 patients that further concluded that adjuvant OK432 injection followed by curative surgery improved survival rate.[63] More recently, in an individual patient meta-analysis, this improvement was considered as borderline significant in stage III and IV gastric cancers, leaving the most significant response in earlier cancer stages.[95]

Recently, in the last 10 years (2010-2019) only three randomized controlled trials were found to have been conducted with streptococcal lysates, namely OK432. The three randomized controlled trials found concerned approaches towards seroma formation after axillary

lymphadenectomy in breast cancer. In all there was a significant correlation between OK432 use and reduced incidence of seroma after axillary lymphadenectomy and better outcomes.[51,88,106] Thus, it was reported significantly less volume and duration of drainage after surgery, fewer and smaller seroma occurrences and shorter duration of healing time.[51,84,88] Nevertheless, the impact of its use on survival time wasn't obtained due to inadequate follow up time.[106] This vouches for OK432 as a potent, effective and safe sclerosing agent that can complement axillary lymphadenectomy surgical protocols.[84,88]

III-f) Main side effects of streptococcal components use

Most of the adverse reactions were described as mild, ranging from constitutional symptoms like fatigue[38] or anorexia[38,53], to more localized manifestations on the site of injection, or local inflammation, hyperemia[50,75], chest pain[17,75,107] and abdominal pain.[53]

The most frequently reported event was fever, often controllable with antipyretic therapy, only a few studies reported to have stoped treatment as soon as fever developed.[17,38,50,52,55,75,98,102,107,108]

Intraorbital or intracranial injections require close monitoring, since rapid elevations of intracranial pressure can happen, and preventive measures must be taken.[16,109] Six patients were reported to have fatal adverse events due to embolism, acute nephritis, hemorrhage and inadequately dosed first injections.[5]

Discussion

Contrary to older misconceptions, some authors concluded that infection or fever doesn't always have to be an unwanted and frightening circumstance in cancer patients and it doesn't necessarily represent the organism's failure to defend itself.

We acknowledge the existence of a dichotomy between infection and cancer since it has been established that a chronic infection enhances the malignancy's risk.[14] However, there are some evidences that an infection can also stimulate the immune system and trigger a reaction that will lead to tumor regression.

Additionally, current methods for cancer treatment (chemotherapy, radiation and often invasive surgeries) have many disadvantages, such as high morbidity rates.[2,7,9] Thus, the adequate therapy for tumors should have a good anticancer activity with nearly no toxicity, the ability to recognize and selectively act in neoplastic cells without damaging healthy tissues, and the capability to overcome neoplastic cells constant alterations.[2]

Fehleisen, Busch, Coley and Coley-Nauts believed that infection could treat tumors. Their work can contribute to a change of paradigm in cancer treatment.[13,14,110,111] In our opinion, their work is promising and also alerts for new conceptions that can contribute for scientific advances.[1,12,14]

Thus, from this review, we think that immunotherapy with streptococcal formulas can be a valuable contribution for the treatment of cancer. However, the lack of further research in this area is a possible explanation for its limited use.[14,15,98] In the future, we propose that further investigation should be carried out regarding some more unclear subjects such as optimal administration condition, which tumors will be the most susceptible, the best timing to initiate treatment, vaccine dosage, interval time between re-injections, injection site, indicators of good responders, and unwanted events and how to avoid them.[6,10,15,62,112,113]

One of the limitations of this paper, was the inability to review some papers as we did not master the language. The lack of double blinded randomized studies is also a reality.

Therefore, it is the authors belief that this matter is worth further investigation and investment. We acknowledge that in the recent years many breakthroughs were presented in the immunotherapy field, but there still is a vast amount of solutions and strategies that are yet to be uncovered, as well as older methods that might have been forgotten, but may still prove revolutionary.

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