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Original article

Characterising vancomycin's immunotoxic profile using Swiss and CFW mice as an experimental model

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Abstract

Immunotoxicology can lead to determining the adverse effects of different compounds on the immune system. Sometimes, many drugs (especially antibiotics) induce immune alterations, mainly auto-immunity. This study was aimed at determining vancomycin's immunotoxic effect by comparing the original molecule to two of the most used copies. Thirty-two mice from two murine strains (Swiss and CFW) were treated with three antibiotic formulations for studying its effect on splenic lymphoid and peripheral blood cell populations by using haemograms, flow cytometry and blastogenesis assays. The results indicated that vancomycin produces neutropenia and lymphocytosis in peripheral populations and that it induces a selective immunomodulatory effect on splenocyte sub-populations, depending on formulation and the strain so treated.

Keywords: Vancomycin; Immunotoxicology; Murine model; Swiss strain; CFW strain; Flow cytometry

1. Introduction

Most drugs have contributed towards resolving different pathologies (controlling symptomatology, correcting metabolic damage or eliminating a pathogen); however, they have also induced alterations in the organism which can only be shown by implementing new assessment techniques [1]. Immunotoxicology's contribution has been justified by to the point of being indispensable in analysing whether a compound has an effect on the immune system for cataloguing it as being a truly safe compound [2,3].

Effects on the immune system can be classified into four main groups: (i) immunosuppression, which can be defined as a lowering of immune system effector activity at the level of cell numbers or soluble mediators (cytokines, anti-microbial peptides, etc.) [4]; (ii) immunostimulation, which can be catalogued as overexpression of cell populations or soluble mediators associated with a defined immune system activity [5,6]; (iii) hypersensitivity, which can be defined as being an overstated immune system reaction against compounds which are normally tolerated [7]; and (iv) autoimmunity, an immune system response against own tissue structures [8–10].

The knowledge gained with these studies has led to verifying that some drugs are associated with producing adverse effects on the immune system, particularly in terms of triggering autoimmunity. It was reported almost 30 years ago that antibiotics in particular are able to induce autoimmune reactions [11] which can be transitory (i.e. ranging from disappearing when therapy with a drug is suspended to producing an irreversible disease). Certain antibiotics such as penicillamine, penicillin [12,13] or minocycline [14] have induced autoimmune effects, thereby questioning their safety

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in experimental models or in clinical assays in humans. Such findings have promoted the study of adverse reactions on the immune system, even for molecules which are being sold and have provoked toxic effects on other systems [3].

Even though the incidence of toxic reactions due to vancomycin has become reduced as its purification has improved and better dosing schemes have been defined, specific adverse effects continue to be reported such as ototoxicity, nephrotoxicity and "transitory" neutropenia [15]. Nephrotoxicity becomes pronounced with the simultaneous use of aminoglucosides, diuretics and amphotericin B. They also produce thrombocytopenia and lack of local tolerance [16,17]. Another adverse reaction associated with administering vancomycin is the reddening of the face, neck and the upper part of the trunk, accompanied by pruritus and erythema (Red Man Syndrome) [18–20].

It has also been stated that drug manufacture protocols should be associated with the vancomycin's toxic events (i.e. variable outcome may be expected in terms of safety depending directly on production factors and the type of formulation and not on the antibiotic efficacy). Vancomycin's immunotoxic potential in its alternative formulation was thus studied bearing this situation in mind and the debate regarding "generic drugs" together with two generic molecules selected according to the Hospital Militar's administration indices (Bogotá, Colombia) as used in treating *Staphylococcus aureus* infections over a 1-year period.

Two strains of mice were used in this study. Swiss mice have been used in a wide range of tests such as determining the immunotoxic potential of immunomodulatory substances extracted from plants [21,22], associated with the metabolism of timerosal (specifically, of mercury) [23] and related with hypersensitivity to allergens [24] and CFW strain mice which, though being less commonly used in pre-clinical immunotoxicology assays, have also been used with good results in detecting the smoke effects and some of its components on the immune system [25].

In this study, it was found that independently of their *in vivo* efficacy, the three formulations differed in their *ex vivo* and *in vitro* activity at the experimental level in splenocytes from these two mice strains, revealing differing effects on the immune system depending on formulation. Even though an effect called "transitory neutropenia" has been reported by the manufacturers, it is evident that the term "transitory" is not well defined since low levels for the polymorphonuclear subpopulation have been found in CFW strain animals receiving the antibiotic scheme even 2 weeks after the last dose (subacute neutropenia), as well as a selective immunomodulatory effect on splenocyte sub-populations, depending on the formulation and strain treated.

2. Methods

2.1. Selecting animals and administering vancomycin

A therapeutic dose of vancomycin was administered to 32 4-week-old female mice (16 Swiss strain and 16 CFW strain). The 16 animals from each strain were distributed into four groups. Each group was treated with a different antibiotic molecule (Baxter original molecule (O)) and two generic ones (Abbott generic 1, and Vanaurus generic 2) and control group inoculated with the excipient. The animals were weighed and intravenous dose adjusted to 15 mg/kg weight, corresponding to the established paediatric dose. The mice were injected in their tail dorsal vein every 24 h for 3 days, according to the protocol recommended by the Bioethics Committee. During the experimentation, mice were fed with standard pellet diet (Rodentina, Purina S.A., Bogotá, Colombia) and water *ad libitum*. All experiments were approved by the ethical committee of the Fundación Instituto de Inmunologia de Colombia. All mice were handled according to the procedures required by the Colombian Ministerio de la Protección Social (Resolution No. 008430 of 1993).

Generic molecule trademarks were selected according to a report issued by the Hospital Militar (2005) concerning vancomycin formulation chosen by the medical committee during the previous year. Baxter's vancomycin (O) accounted for 30% of the drug treated cases, Abbott's vancomycin for 25% and Vanaurus' vancomycin for 8%.

2.2. Ex vivo and in vitro assays

Twenty-four hours after the third administered vancomycin dose, two animals from each group were bled by ocular puncture for the respective haemograms. They were then euthanised by cervical dislocation for extracting the spleens which were perfused with sterile RPMI 1640 medium the splenocytes obtained by density gradient separation (1.077 density, Ficoll-Hypaque). Splenocytes were divided into two fractions; one part was used for evaluating the *in vitro* immunomodulatory potential of the drugs by blastogenesis assay where the cells were exposed to the formulations placed in triplicate in 96-well dishes at 2×10^5 splenocyte concentration in the presence of the three different vancomycin formulations (Baxter, Abbott and Vanaurus laboratories). All wells contained 10 μg/ml of concanavalin A (ConA), except for the negative (background) control, for evaluating the formulations' suppressor potential on the cell proliferation as induced by this mitogen. Such cell proliferation was quantified by staining with resazurin (Sigma–Aldrich Corp, St. Louis, USA) which enters cell mitochondria and interacts as electron transport chain's intermediate acceptor.

Once reduced, resazurin formed a compound (resorufin) which absorbs the light at a different wavelength. According to cell activity, the stain (which is initially blue) changed to different tones of purple, pink and finally white and transparent. A fluorescence reader (Tecan, Genios) was used to measure the metabolic activity in relative fluorescence units (RFU). Inverse stimulation indices (ISI) were calculated from the relative fluorescence data, indicating when a treatment induces reduction of splenocyte proliferation (effector immune activity suppression). ConA RFU were divided into each treatment RFU (original, generic 1, generic 2). If the ISI was