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On the presence of antibodies against bovine, equine and poultry immunoglobulins in human IgG preparations, and its implications on antivenom production ☆

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Abstract

Specific immunoassays were developed to detect anti-horse, anti-chicken and anti-bovine immunoglobulins in human IgG preparations. Three samples of 5% human IgG for intravenous use ("Inmunoglobulina G Endovenosa al 5%"™, Quimbiotec CA), were studied. All samples were produced from pools of >2500 plasma units from different donors. One sample was produced from an only Venezuelan plasma pool (2660 donors) and the other two were produced from a 1:1 blend of Venezuelan and Canadian plasma pools. The amounts of human IgG detected were 0.017 (0.015, 0.020) mg/ml ($n = 18$) against horse IgG, 0.37 (0.28, 0.48) mg/ml ($n = 18$) against cattle IgG and 1.27 (1.15, 1.40) mg/ml ($n = 15$) against chicken IgY. Similar results were obtained on individual Venezuelan plasma samples. The differences probably reflect the consumption and antigenicity of meat. Poultry and bovine meat are widely consumed in Venezuela and Canada, while equine meat is not consumed; also chicken is more heterologous to man and may be more antigenic than bovine meat. This suggests that when IgY immunotherapeutics are used in populations with an important dietary component of poultry meat and eggs, there is a risk of producing untoward reactions and less efficient antivenoms.

Keywords: Immunoglobulins; Antivenoms; Immunotherapy; γ-Globulin

1. Introduction

The production of antivenoms is of crucial importance to treat spider, snake and scorpion envenomings all over the world. Klemperer (1893) showed that egg yolk extracts laid by hens hyperimmunised against tetanus toxin were able to protect mice against this toxin. The first antivenom for human use was prepared by Calmette (1894a, b) based on earlier studies by Von Behring and Kitasato (1890) relating to the treatment of diphtheria and tetanus. Ever since Calmette's pioneering work, antivenoms are mostly produced in hyperimmunised mammals. Recently, a growing number of researchers are providing evidence on the medical usefulness of poultry (hens and quails) IgY antibodies (Kuhlmann et al., 1988; Gassmann et al., 1990; Polson, 1990; Carroll et al., 1992; Ermeling

☆ *Ethical statement*: This study did not require any animal or human subject, all immunoglobulines used were from commercial sources.

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et al., 1992; Landon et al., 1995; Erhard et al., 1996; Schade and Hlinak, 1996). In favour of the use of IgY are the easiness and low cost of handling poultry, and that IgY reactivity with human FC receptors is low which should result in a lesser chance of severe adverse reactions.

Still, IgY are very heterologous proteins, phylogenetically very remote from mammals. Thus far, their use has been as oral therapeutics or prophylactic agents against infectious agents or orally effective toxins, specially in veterinary medicine (de Roodt et al., 2004).

In this work we explore the presence of human IgGs against bovine and equine IgGs and poultry IgY in three batches of pharmaceutical human IgGs preparations, commonly named γ-globulins. γ-Globulins are batches of IgG from a large number of donors (>2500 in the case of the preparations we studied). This study is thus a sample of >7500 individuals. The sources of IgG in the γ-globulins studied are Venezuelan and Canadian donors, that is, people with a high likelihood of feeding on cattle and chicken, but from countries where consuming horse meat is unlikely or nil.

2. Materials and methods

2.1. Source of human IgG

Three samples of 5% human intravenous IgG (''Inmunoglobulina G Endovenosa al 5%''™, Quimbiotec C.A., Caracas, Venezuela; Lots IVL10-060502, IVL5-060404 and IVL2.5-06050) referred in the text as IVL10, IVL5 and IVL2.5 were studied. Each of the samples was produced from pools of >2500 plasma units from different donors. IVL5 was produced from an only Venezuelan plasma pool (2660 donors), and IVL10 and IVL2.5 were produced from a 1:1 blend of Venezuelan and Canadian plasma pools.

2.2. High-performance liquid chromatography (HPLC) methods

The human IgG preparations were analysed with a Shimadzu LC 6B (Shimadzu Corp., Kyoto, Japan) HPLC system using molecular exclusion ProteinPak 300SW HPLC (Millipore Corporation, Waters Chromatography Division, Milford, MA, USA) column eluting a rate of 1 ml/min with 100 mM sodium phosphate buffer at pH 7. The eluate was monitored measuring its absorbance at 280 nm (Shimadzu SPD-6AV, ultraviolet-visible absorbance detector). To estimate the molecular weight of the fractions eluted, the column was calibrated with a standard containing: thyroglobulin, 669 kDa; IgG, 160 kDa; egg albumin, 43 kDa; ribonuclease A, 12.8 kDa.

2.3. Antivenom measurement in plasma and IgG preparations

Three specific sandwich immunoassays were developed to detect anti-horse, anti-chicken and anti-bovine IgG's in human plasma and human IgG preparations. The immunoassays use specific horse, chicken and bovine commercial antibodies and commercial conjugates. Polystyrene microplates (Maxisorp, Nunc Inc., USA) were coated overnight at 4 °C with 100 µl/well of 10 µg/ml equine IgG (SIGMA, USA), bovine IgG (Santa Cruz Biotechnology Inc., California, USA) or poultry IgY (Santa Cruz Biotechnology Inc., California, USA) in 100 mM carbonate/bicarbonate buffer, pH 9.5. The plates were then washed six times with washing buffer (50 mM Tris/HCL pH 8, 150 mM NaCl and 0.05% Tween 20). The remaining binding sites were blocked with Tris/HCL pH 8, containing 0.5% gelatin and 0.05% Tween 20 for at least 2 h at 37 °C (100 µl/well). The plates were then washed six times with washing buffer. The human plasmas and human IgG were diluted 1/50, 1/100 and 1/500 in vehicle buffer (50 mM Tris/HCL pH 8, 500 mM NaCl, 0,1% gelatin and 0.05% Tween 20); 100 µl/well of samples were added to the plates and incubated for 1 h at 37 °C. Plates were washed six times with washing buffer and then 100 µl/well of goat anti-horse conjugated to peroxidase (SIGMA, USA), goat anti-chicken conjugated to peroxidase (SIGMA, USA), goat anti-bovine conjugated to peroxidase (SIGMA, USA) were diluted in vehicle buffer and used as positive controls of the assay. One hundred µl/well of goat anti-human conjugated to peroxidase (Santa Cruz Biotechnology Inc., California, USA) was added to the plates, diluted to 1/1500 and incubated for 1 h at 37 °C. After washing, 100 µl/well OPD (Pierce) diluted in phosphate–citrate buffer with sodium perborate (SIGMA, USA) were added and incubated for 10 min at 25 °C protected from light, the reaction was then stopped with 100 µl/well of 1 M sulphuric acid.Absorbances were read later at 492 nm in a microplate reader (Multiskan Labsystem).