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Development of generic immunoassay for the detection of a series of aminoglycosides with 6′-OH group for the treatment of genetic diseases in biological samples

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ABSTRACT

Over the last two decades, a growing number of scientific evidences highlighted the potential therapeutic value of several structures of aminoglycoside antibiotics (including gentamicin and G418) for the treatment of various genetic diseases caused by nonsense mutations. These findings resulted in a fast evolvement of synthetic derivatives of aminoglycosides which were shown to be more target specific and less toxic than the clinically used antibiotics. The emerging progress in drug design and development has necessitated the urge to develop a fast, easy and accurate procedure for the determination of these potential therapeutic agents in various biologically derived matrices. Here we describe the preparation of a generic polyclonal antibody that was used for the development of homologous and heterologous immunoassays for the detection of a wide range of natural and synthetic aminoglycoside derivatives, highlighted today as potential therapeutic agents for the treatment of various genetic diseases. A common two-ring scaffold, NB82, present in the majority of compounds exhibiting potent biological activity, was used as a generic immunization hapten for the immunization of two rabbits. By using a series of chemical steps, NB82 was selectively conjugated *via* the N-1 position through glutaric acid linker to a carrier protein. Sensitivity (I_{50}) values for the recognition of three representative compounds NB82, NB84 and NB124 were determined to be 10 ± 3 ng mL^{-1}, 0.5 ± 0.04 μg mL^{-1} and 1 ± 0.12 μg mL^{-1}, respectively. Limits of detection were determined to be 1 ± 0.3 ng mL^{-1} for NB82, 20 ± 7 ng mL^{-1} for NB84 and 15 ± 8 ng mL^{-1} for NB124. The developed assays were further exploited for the *in vivo* monitoring of the therapeutic compounds in mice serum. Serum experimentations exhibited similar detection limits as observed for the PBS calibration experiments, demonstrating no interference with assays sensitivity, with rather high recovery ratios ranging from 92 to 107% in whole blood samples.

1. Introduction

Aminoglycosides are highly potent antibacterial agents, which are known to exert their deleterious effects on bacterial cells by interfering with the translation process [1,2]. Over the last 20 years, a few natural aminoglycosides have been highlighted as promising therapeutic agents for the treatment of several genetic disorders caused by nonsense mutations [3–5]. These mutations generally lead to the production of truncated, non-functional proteins. In human, these mutations have been linked to nearly 2000 genetic disorders such as cystic fibrosis (CF), Duchenne muscular dystrophy (DMD), ataxia-telangiectasia, Hurler syndrome, hemophilia A, hemophilia B and Tay-Sachs [6,7]. For many of these diseases there is no effective treatment. Aminoglycoside antibiotics including gentamicin, paromomycin and G418 (Fig. 1) have been shown to suppress premature termination codons and partially restore

Abbreviations: 2-DOS, 2-deoxy-streptamine; AHB, (S)-4-amino-2-hydroxybutanoyl; CB, carbonate buffer; CF, cystic fibrosis; CE, capillary electrophoresis; DCC, N′,N′-dicyclohexylcarbodiimide; DDW, double distilled water; DMD, Duchenne muscular dystrophy; DMF, dimethylformamide; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; FIA, fluoroimmunoassays; GAG, glycosaminoglycan; GC, gas chromatography; GTA, glutaraldehyde; HPLC, high-performance liquid chromatography; HRP, horseradish peroxidase; TLC, thin layer chromatography; KLH, Keyhole Limpet Hemocyanin; MPS I-H, mucopolysaccharidosis type-I-Hurler; NHS, Nhydroxysuccinimide; OVA, ovalbumin; PBS, phosphate-buffered saline; PBST, PBS containing Tween-20; PMe_3, trimethylphosphine; RIA, radioimmunochemical assays; S.E.M., standard error mean; THF, tetrahydrofuran; TMB, 3,3′,5,5′-tetramethyl benzidine.

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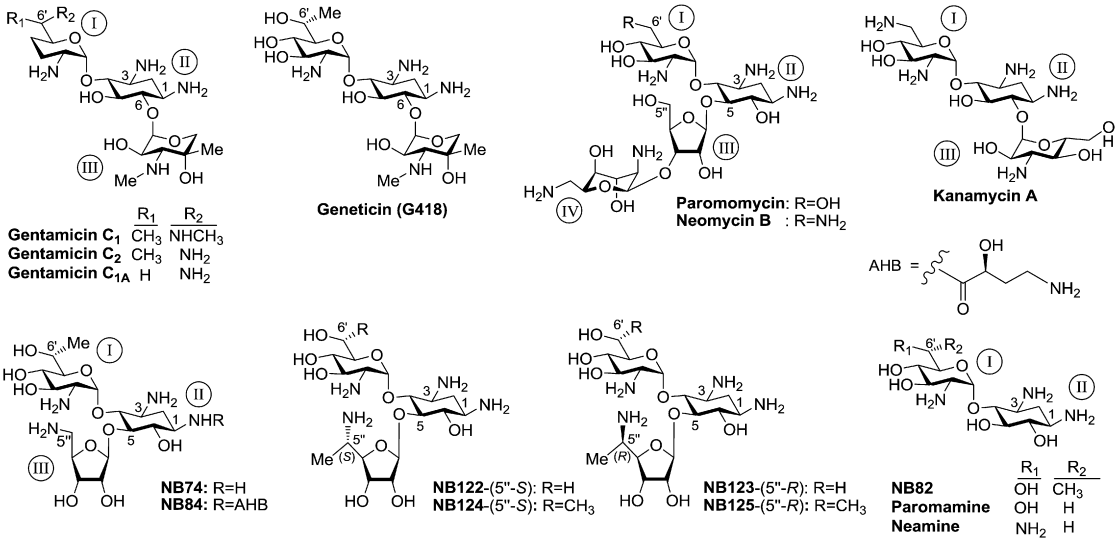

Fig. 1. Chemical structures of standard and semi-synthetic aminoglycoside derivatives used in this study.

functional protein production for more than twenty genetic diseases [8]. However, the potential toxicity associated with aminoglycosides has limited their clinical applications in suppression therapy. Over the last few years several synthetic derivatives of aminoglycosides were developed to overcome some of the enhanced toxicity issues. Many of these derivatives have been designed and synthesized in our lab [9–12]. Our most recent lead compounds of NB-series, including NB74, NB84, and NB122–NB125 (Fig. 1) demonstrated a remarkable biological activity altogether with reduced toxicity values in various *in vitro*, *ex vivo* and *in vivo* systems [10–14].

Further progress in drug development necessitated a fast and effective methodology for the detection and quantification of the developed lead NB-compounds in various biological derived matrices. Over the last few decades many analytical and bioanalytical assays have been suggested for the qualitative and quantitative analysis of aminoglycosides that are in clinical, veterinary and agricultural use to treat bacterial infections. These methods were applied for the detection of drug residues in a wide range of biological derived matrices such as cell tissues [15], serum [16], milk [17], eggs [15,18], and honey [18]. Chemical methodologies included the application of gas chromatography (GC) [19], thin layer chromatography (TLC) [20], high-performance liquid chromatography (HPLC) [21] and capillary electrophoresis (CE) [22]. Biological methodologies included the development of microbiological assays [23], radiochemical and radioimmunochemical assays (RIA) [24], enzyme linked- and fluoro-immunoassays (ELISA, FIA) [16,25,26], nano-sensors and nano-particle based immunoassays [27,28].

Immuno-based methodologies such as ELISA, FIA and RIA have shown to be as sensitive and accurate as the chemical methods [29]. These methods could be easily applied on a wide variety of biologically derived matrices, are simple to perform and analyze, and do not require the use of high cost instrumentations. Immuno-based methodologies are commercially available for the detection of some widely used natural aminoglycosides such as gentamicin, streptomycin, dihydrostreptomycin, neomycin and kanamycins (*e.g.* MaxSignal® gentamicin ELISA Test Kit, Aminoglycosides enzyme immune-assay-EIA kit for the detection of gentamicin, neomycin, streptomycin and dihydrostreptomycin – Europroxima, and kanamycins ELISA kit – Wanger). These commercially available kits can be exploited for highly sensitive detection of aminoglycosides in various matrices, and some of them are used in hospitals to monitor the serum levels of clinically used aminoglycosides such as gentamicin and amikacin. However, these assays are mostly used for the detection of aminoglycoside antibiotics that are based on a neamine core, containing an amino group at their 6′ position (ring I, Fig. 1). The use of the derived antibodies for the detection of therapeutic derivatives containing other substituents on the neamine core is quite limited due to low cross-reactivity values. Recent documentations demonstrated the superiority of 6′-hydroxyl containing aminoglycosides such as paromomycin and G418 over the 6′-amino derivatives in the treatment of various genetic disorders [4]. These derivatives are mostly based on either a paromamine or a 6′-(R)-methyl-paromamine (NB82) 2-ring core (Fig. 1) and are extensively explored for the treatment of various genetic diseases [3,9–14]. To our knowledge, no immunoassays exist for the detection of aminoglycosides containing a 6′-hydroxyl group.

The aim of the present study was to generate a generic antibody that will be able to recognize a wide range of potential therapeutic members containing a 6′-hydroxyl group. For this purpose, the 2-ring scaffold NB82 designed in our lab, was chosen to serve as an immunogen for the development of a generic antibody. The resulting antibody was shown to cross-react with a series of standard and synthetic derivatives of aminoglycosides that shared structural similarity with the immunization scaffold using a homologous ELISA. The antibody was further used for the development of 3 highly sensitive heterologous ELISAs for the detection of selected potential therapeutic agents. The assays were shown to have high recovery from spiked blood and serum samples and have been applied for *in vivo* monitoring of the synthetic derivatives in mice serum. The approach of a generic immunoassay developed in this study can be applied for the development of similar assays for the detection of other classes of drug candidates.

2. Materials and methods

2.1. Materials

Paromomycin, neomycin, geneticin (G418), gentamicin and kanamycin were purchased from Sigma as sulfate salts. All the synthetic derivatives including neamine, paromamine, NB82, NB74, NB84, NB122, NB123, NB124 and NB125 were prepared as described previously by Baasov and coworkers [9–12] and were used as their sulfate salts. All other chemicals and biochemicals, unless otherwise stated, were obtained from Merck or Sigma.