DNA abasic lesion is cleaved by Antibiotic Drugs Aminoglycosides: A promising effect that could potentiate antitumor genotoxic agents?

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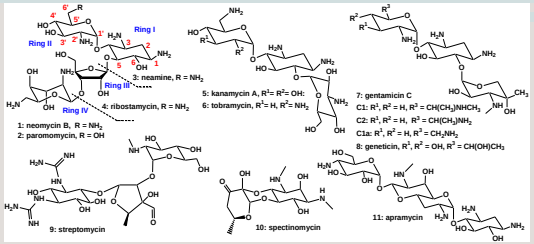
Introduction

We have investigated two subfamilies of natural aminoglycoside drugs: the neomycin-class (compounds 1-4) and the kanamycin-class (compounds 5-8) and the synthetic derivatives 9-11. Most of those compounds appeared to cleave efficiently DNA at abasic site lesions. If this effect could be partly related to their toxicity, it could also find promising applications in the potentiation of the anticancer effects of genotoxic agents (ionizing radiations and alkylating agents).



Aim

Aliphatic diamines and polyamines are known to catalyze the cleavage of DNA at abasic sites. Since aminoglycosides contain amino groups, we became interested in examining whether these drugs could also catalyze abasic sites cleavage.

Materials & Methods

✓ The cleavage activity was analyzed by measuring the ability to induce single strand breaks in depurinated pBR322 plasmid DNA inducing conversion of circular covalently closed form (supercoiled or form I) into the open circular form (relaxed or form II). Form I and form II were separated by agarose gel electrophoresis. The gel previously stained with ethidium bromide was scanned ith a Typhoon imager for analysis.

✓ For the most active aminoglycosides we determined their half maximal effective concentration (EC50) was at pH 7.2 in the presence of 100 mM KCl.

✓ Since aminoglycosides have a polycationic character, their binding to DNA and their cleaving properties at abasic sites should be influenced by the ionic strength. Experiments were then performed using 50, 100, 150 and 200 mM ionic strength concentrations (KCl).

Results & Discussion

✓ Aminoglycosides 1-11 do not cleave undamaged plasmid DNA.

✓ Antimicrobial aminoglycoside drugs showed significant cleavage activity at AP sites (at 5.10^{-4}, 5.10^{-5} and 5.10^{-6} M, Table 1).

✓ The most efficient natural aminoglycoside drug is neomycin with 0.1 µM EC50 (Table 2 and Figure 1).

✓ The cleavage activity of all the compounds is strongly dependent on the ionic strength. The lower ionic strength the higher is the cleavage activity (Figures 2 and 3).

Conclusion

Clearly, natural and synthetic aminoglycosides appeared to be able to cleave very efficiently DNA at abasic sites. Such an effect could participate to some of the observed toxic effects of aminoglycosides used as antibiotic drugs. On the other hand, this effect could become an advantage in antitumour therapy by potentiating the action of the many genotoxic agents known to produce large amounts of abasic sites in their DNA target.

Acknowledgements

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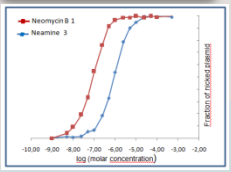
Tables

	Aminoglycosides										
	1	2	3	4	5	6	7	8	9	10	11
5 µM	0.73	0.11	0.48	0.28	0.26	0.58	0.56	0.08	0.05	0.08	0.06
50 µM	0.83	0.26	0.80	0.64	0.64	0.80	0.76	0.26	0.11	0.13	0.34
500 µM	0.90	0.58	0.85	0.76	0.80	0.84	0.80	0.58	0.17	0.18	0.63

Table 1. Fraction of the AP-site cleavage at pH 7.2, 37°C, 100 mM KCl by aminoglycosides antibiotics at 5, 50 and 500 µM. Aminoglycosides: **1**: neamine; **2**: ribostamycin; **3**: neomycin; **4**: paromomycin; **5**: kanamycin; **6**: tobramycin; **7**: gentamicin; **8**: geneticin; **9**: apramycin; **10**: streptomycin; **11**: spectinomycin.

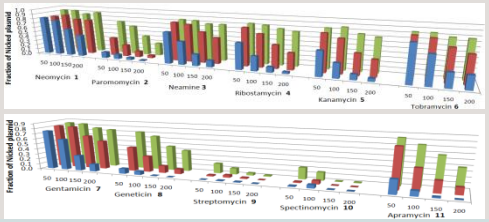
Figures



Compounds	EC_{50} µM, pH 7.2, 100 mM KCl
Neomycin B 1	0.094
Paromomycin 2	42
Neamine 3	0.89
Ribostamycin 4	2.2
Tobramycin 6	0.63
Gentamicin C 7	0.79
Geneticin 8	32

Table 2. Aminoglycoside concentrations for which 50% of the maximal abasic cleavage (EC50) is observed.

Figure 1. Fraction of nicked plasmid DNA as a function of molar concentration of neomycin B 1 and neamine 3. Fractions of cleavage of pBR322 plasmid DNA containing an average of two abasic sites per molecule were measured after 20 min incubation at 37 ° C and pH 7.2 in the presence of 100 mM KCl.



Figures 2 and 3. Cleavage of pBR322 plasmid DNA at pH 7.2 and 37°C in the presence of aminoglycosides tested at 5.10^{-6} M (blue), 5.10^{-5}M (red) and 5.10^{-4} M (green)and 50, 100, 150 and 200 mM KCl solutions.