COMPARATIVE STUDY OF THE EFFECTS OF SAGAMICIN, 1 N-HABA-SAGAMICIN, UK-4, Sch 21420, GENTAMICIN AND AMIKACIN ON THE RESPIRATION ACTIVITY OF ISOLATED RAT KIDNEY MITOCHONDRIA

J. P. BENDIRDJIAN, B. FOUCHER and J. P. FILLASTRE

Groupe de Physiopathologie Tissulaire
Université de Rouen, France

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In the present state of our knowledge the mitochondrial system appears to be one of the possible sites of aminoglycoside activity[1]. For several years, we have been investigating the effect of these antibiotics on mitochondrial respiration. This enables us to establish a classification of aminoglycosides according to their effects on mitochondria. This classification correlates closely with the results of clinical studies in the human concerning the potential nephrotoxic effect of these antibiotics[2]. Recently we have suggested that the toxic effect of 3,6-disubstituted derivatives of 2-deoxystreptamine (2 DOS) against mitochondria is directly linked to the number of free amino-groups of the molecules (of which the lone pair of electrons on nitrogen is accessible)[3]. In order to extend this concept, we report on some others 4,6-disubstituted 2 DOS derivatives: Sagamicin, 1 N-HABA-sagamicin, UK-4 and Sch 21420 (Fig. 1). We compared the effects of these four new antibiotics to those of amikacin and gentamicin.

Renal mitochondria were isolated from Sherman rats according to the method of PACKER *et al*[4]. Oxygen consumption was measured at 25°C under state 3 conditions in the presence of succinate+rotenone as substrate.

Similarly to the other aminoglycosides we have tested, sagamicin, 1 N-HABA-sagamicin, UK-4 and Sch 21420 induced inhibition of state 3 mitochondrial respiration. As shown in Table 1, for all concentrations sagamicin appeared to be the most toxic and both amikacin and Sch 21420 the least toxic. However, the present study showed that differences in toxicity between sagamicin, 1 N-HABA-sagamicin, UK-4 and gentamicin were relatively slight. Only the effects of amikacin and Sch 21420 appeared clearly lower. Based on our previous results, we have established a relationship between the concentration of the antibiotic in the experimental physiological medium and the percentage of inhibition of state 3 mitochondrial respiration reference.[2,3] Using this relationship: $\Delta\% = a \log_{10}[C] + K$ with $[C]$ expressed in 10^{-5} M, two characteristic values can be assigned to each antibiotic. One of these: a, indicates the effect of variations in concentration on the toxicity of the molecule; the other: K expresses intrinsic *in vitro* toxicity in arbitrary values. The theoretical concentration at which toxicity appears can also be determined using the relationship as follow: $C_{min} = e^{-2.3(K/a)}$. The values of a, K and C_{min} found for the six antibiotics tested are shown on Table 2. These

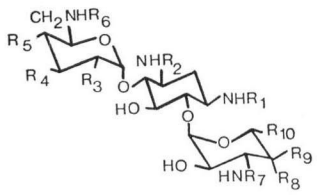
Table 1. Effect of six aminoglycosides on coupled state 3 respiration of rat kidney mitochondria.

Mitochondria (1 mg/ml) were incubated at 25°C in 1.6 ml of a medium containing 80 mM KCl, 10 mM phosphate-buffer, 20 mM Tris-HCl pH 7.2 and the antibiotic. Succinate (10 mM) was added 1 minute after mitochondria, and ADP (0.25 mM) 1 minute after succinate.

Results are expressed as a percentage of inhibition from control values (respiration without antibiotic). Each value is the mean±s.e.m. of seven experiments (three rats per experiment).

	Antibiotic concentration in 10^{-5} M					
	8	16	32	64	128	196
Gentamicin C_1	14.5±4.0	23.5±3.5	30.5±5.0	37.5±6.0	45 ±6.5	48±6.0
Sagamicin	15 ±2.0	25 ±3.5	38 ±5.0	46 ±4.0	51 ±4.5	53±6.5
1 N-HABA-Saga.	10.5±2.5	16 ±3.5	26.5±3.5	33 ±4.0	41 ±6.0	44±5.5
UK-4	12 ±3.0	21 ±5.0	34 ±4.0	41 ±8.0	45.5±5.0	52±4.0
Amikacin	—	3.5±1.5	9 ±2.0	16 ±1.5	24 ±2.0	31±3.0
Sch 21420	—	2 ±1.0	7.5±1.5	15 ±2.0	27.5±2.5	34±2.0

Fig. 1. Structure of some derivatives of 2-deoxystreptamine 4,6-disubstituted.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Gentamicin C_1	-H	-H	$-NH_2$	-H	-H	$-CH_3$	$-CH_3$	-OH	$-CH_3$	-H
Sagamicin	-H	-H	$-NH_2$	-H	-H	$-CH_3$	$-CH_3$	-OH	$-CH_3$	-H
1 N-HABA-Saga.	HABA	-H	$-NH_2$	-H	-H	$-CH_3$	$-CH_3$	-OH	$-CH_3$	-H
UK-4	-H	$-CH_3$	$-NH_2$	-H	-H	-H	$-CH_3$	-OH	$-CH_3$	-H
Amikacin	HABA	-H	-OH	-OH	-OH	-H	-H	-H	-OH	$-CH_2OH$
Sch 21420	HAPA	-H	-OH	-OH	-OH	-H	$-CH_3$	-OH	$-CH_3$	-H

HABA=$-$CO$-$CHOH$-(CH_2)_2-NH_2$, HAPA=$-$CO$-$CHOH$-CH_2NH_2$

values indicate for amikacin and Sch 21420 a clearly lower intrinsic toxicity than these of the other four antibiotics. However, as soon as the toxic threshold is reached, the effect of logarithmic variations in concentrations on the toxicity is approximately the same for all six drugs. Among the six aminoglycosides tested in this study, 1 N-HABA-sagamicin has six nitrogenous groups while sagamicin, UK-4, Sch 21420, amikacin and gentamicin C_1 have five. However, if one considers the substituents of these nitrogenous groups, it appears the proton affinity of these groups is quite different. In the case of methyl-amino-group, the donor inductive effect of CH_3 tends to increase the proton affinity of nitrogen but, simultaneously, it increases the steric hindrance making access to the lone pair of electrons more difficult. Our previous studies showed that the results obtained with gentamicin C_1 and sisomicin which have four NH_2 and one $NHCH_3$ group were close to those obtained with tobramycin, kanamycin B and dibekacin which have five NH_2-group[3]. It was thus concluded that the steric factor is not strong enough to counteract the donor-inductive effect of CH_3 and that, as a result, the proton affinity of both NH_2 and $NHCH_3$-group would be closely similar. On the other hand, for both the 1 N-HABA and 1 N-HAPA derivative, because of the strongly electron withdrawing effect of the carbonyl-group and of the important steric hindrance of the substituent, the C 1 amino-group is strongly deactivated. Thus the C 1 nitrogen no longer possesses proton affinity. However, since the N-substituent carries a free amino-group itself, the parent and the final molecules have, in final analysis, the same number of nitrogen having proton affinity. Hence, 1 N-HABA-sagamicin, sagamicin, gentamicin C_1 and UK-4 appear as having five nitrogenous-groups with proton affinity and both, Sch 21420 and amikacin as having only four. From these considerations, it is particularly interesting to compare sagamicin and 1 N-HABA-sagamicin. The effect of the original molecule and that of its derivative are relatively close. In an earlier study, an identical observation was made concerning kanamycin A and 1 N-HABA-kanamycin

Table 2. Values of a and K in the relationship $\Delta\%=a \log_{10}[C]+K$ relating the percent of inhibition of state 3 respiration to the concentration of antibiotic.

$C_{min}=e^{-2.3(K/a)}$ is the theoretical minimum concentration expressed in 10^{-5} M at which the effect of antibiotic appears. r^2=coefficient of correlation, n=number of experimental points.

	a	K	C_{min}	r^2	n
Gentamicin C	24	− 6	1.8	0.99	6
Sagamicin	28	− 8	1.9	0.96	6
1 N-HABA-sagamicin	25	−12.5	3.2	0.99	6
UK-4	28	−12	2.7	0.98	6
Amikacin	25	−28	13.1	0.99	5
Sch 21420	30	−36	15.8	0.98	5

A (amikacin). Both drugs acted similarly. Likewise, Sch 21420 is 1 N-HAPA-gentamicin B and the action of gentamicin B was found similar to those of amikacin and kanamycin A. For all of these 1 N-substituted derivatives, the fact that the amino-groups is carried by a side chain does not seem to modify in an important way the toxicity of the molecule on mitochondria. Since the parent and final molecules act similarly, our previous studies showed that no precise relationship could be established between the mitochondrial effect of 4,6-disubstituted 2 DOS derivatives and the number or the site of the hydroxyl groups of their molecule[8].

Thus, the present study seems to confirm the previously established relationship between number of nitrogenous-groups having proton affinity and toxicity of 4,6-disubstituted 2 DOS derivatives.

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