AMINOGLYCOSIDE ANTIBIOTICS. VII ACUTE TOXICITY OF AMINOGLYCOSIDE ANTIBIOTICS

KEI-ICHI FUJISAWA, TOSHIO HOSHIYA and HIROSHI KAWAGUCHI

Bristol-Banyu Research Institute, Ltd. Meguro, Tokyo, Japan

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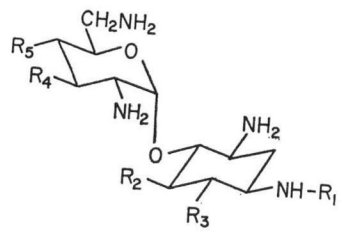
The aminoglycoside antibiotics are important chemotherapeutic agents for treatment of serious gram-positive and gram-negative infections. However they are limited in clinical use primarily because of their oto- and nephrotoxic potential, though there are marked quantitative differences among the antibiotics with respect to such toxicity. The oto- and nephro-toxicity of antibiotics are not necessarily reflected in their acute lethal toxicity (LD_{50}). However, it has generally been recognized that the aminoglycoside antibiotics with greater acute toxicity are prone to have more toxic potential on the renal, auditory and/or vestibular functions.[1,2,3,4]

It has been a common practice in laboratories to determine the acute LD_{50} of any new antibiotic or derivative at an early stage of works to estimate the toxic potential of the material under study. The LD_{50} value reported with an aminoglycoside antibiotic often differs considerably between laboratories. Such variation may be explained by several factors such as the strain, sex and age of the animals, the volume and rate of injection, and the pH of the antibiotic solution administered to the animals. A significant pH effect on the acute intravenous toxicity has been reported with kanamycins A and B.[5]

The present paper reports the comparative acute intravenous toxicity of various aminoglycoside antibiotics determined under the same experimental conditions. Male albino mice of *dd* strain weighing 18~20 g were injected via the tail vein with graded doses of antibiotic solution. Each antibiotic was dissolved in distilled water and the pH of the solution was adjusted to 6.0±0.1 by N/10 sulfuric acid. The injection volume was standardized at 0.2 ml per 10 g of body weight and the injection speed was 0.4 ml/7 seconds. The animals were given food and water *ad libitum* before and during the experiment, and observed for 4 days following treatment to record the mortality. Results of multiple tests were combined, and the LD_{50} and ranges were determined according to the method of MILLER and TAINTER.[6]

The antibiotics examined in the present study include natural and semisynthetic aminoglycoside antibiotics,* all having a 2-deoxystreptamine (DOS) moiety in the molecule. They are classified into three groups, I, II and III, according to the types of glycosidic substitution on the DOS ring. Group I consists of the pseudodisaccharides which have a pyranose sugar attached to the C-4 hydroxyl group of DOS, and includes neamine (**1**), 4′-deoxyneamine[8] (**2**), 3′, 4′, 5, 6-tetradeoxyneamine (**3**), l-(L-γ-amino-α-hydroxybutyryl) neamine[9] (l-AHB-neamine, (**4**) and 4′-deoxy-l-AHB-neamine[8] (**5**). Structures of these compounds are shown in Fig. 1.

Fig. 1. Structure of Group I antibiotics



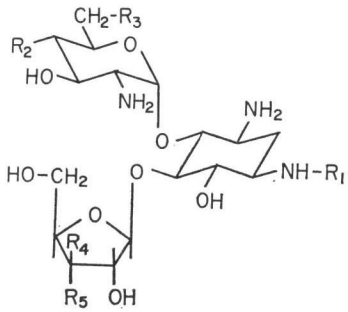
	R_1	R_2	R_3	R_4	R_5
1	H	OH	OH	OH	OH
2	H	OH	OH	OH	H
3	H	H	H	H	H
4	AHB*	OH	OH	OH	OH
5	AHB	OH	OH	OH	H

* L(−)-γ-amino-α-hydroxybutyryl

* The antibiotic samples employed in the present study were mostly isolated in our laboratories except for kanamycin B and 3′,4′-dideoxykanamycin B (Meiji Seika Co.), lividomycins A and B (Kowa Co.), paromomycin I (Sankyo Co.), tobramycin (Eli Lilly & Co.) and gentamicin C complex (Schering Corp.). Separation of gentamicin C complex into its individual components, C_1, C_2 and C_{1a}, was performed by the published method.[10]. 3′, 4′, 5, 6-Tetradeoxyneamine (**3**) was prepared by a method similar to that published by S. UMEZAWA *et al.*[10] for the preparation of 3′, 4′-dideoxyneamine.

Group II antibiotics are those having a O-pyranosyl and O-furanosyl substitutions at C-4 and C-5, respectively, of the DOS ring. This group is further classified into 3 subgroups according to the number of cyclic moieties in the antibiotics. Thus, group II_a (3 rings, Fig. 2) includes ribostamycin[9,11] (**6**), 5-xylosylneamine[9] (**7**), 4′-deoxy-xylosylneamine[8] (**8**), butirosin A[9,12] (**9**), 4′-deoxybutirosin A[7] (**10**) and Bu-1709E_1[13] (**11**). Antibiotics of

Fig. 2. Structures of Group IIa antibiotics



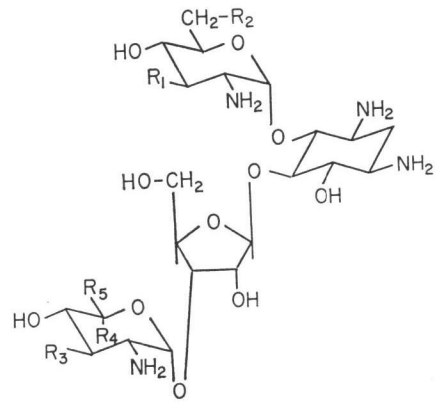
	R_1	R_2	R_3	R_4	R_5
6	H	OH	NH_2	H	OH
7	H	OH	NH_2	OH	H
8	H	H	NH_2	OH	H
9	AHB	OH	NH_2	OH	H
10	AHB	H	NH_2	OH	H
11	AHB	OH	OH	OH	H

group II_b (4 rings, Fig. 3) have an additional pyranose sugar attached to the C-3″ position of the D-ribose portion, and include neomycins B and C (**12**, **13**), paromomycin I (**14**) and lividomycin B[14] (**15**). Lividomycin A (**16**), the 4‴-mannosyl derivative of lividomycin B, provides the sole example for group II_c (5 rings, Fig. 3).

Group III antibiotics, which have two pyranosyl substitutions at the C-4 and C-6 hydroxyl groups of the DOS moiety, constitute an important series of aminoglycoside antibiotics including kanamycins A, B and C (**17**, **18**, **19**), tobramycin[15] (**20**), 3′, 4′-dideoxy-kanamycin B[16] (DKB, **21**), gentamicins C_1, C_2, C_{1a}[17] (**22**, **23**, **24**) and amikacin[18] (BB-K8, **25**).

The acute intravenous toxicities of the above 25 antibiotics are shown in Table 1.

Fig. 3. Structures of Group IIb and IIc antibiotics



	R_1	R_2	R_3	R_4	R_5
12	OH	NH_2	OH	CH_2NH_2	H
13	OH	NH_2	OH	H	CH_2NH_2
14	OH	OH	OH	CH_2NH_2	H
15	H	OH	OH	CH_2NH_2	H
16	H	OH	MAN*	CH_2NH_2	H

* D-mannosyl

These data suggest a definite relationship between the toxicity and structure of these antibiotics.

The acute toxicities of group I antibiotics were compared with that of neamine (**1**), a principal component of most aminoglycoside antibiotics. AHB acylation at the C-1 amino group reduced the toxicity to nearly one-half as seen in the comparison of **1** *vs* **4** and **2** *vs* **5**. Deoxygenation of the C-4′ hydroxyl group also lowered the toxicity to an appreciable extent (**1** *vs* **2**, **4** *vs* **5**), while 3′, 4′, 5, 6-tetradeoxyneamine (**3**) was twice as toxic as **1**.

A pentosyl substitution of neamine at the C-5 hydroxyl of the DOS ring caused a marked reduction in toxicity, and ribostamycin (**6**) and its xylosyl congener (**7**) were less than half as toxic as neamine. The toxicity-decreasing effect of pentose addition can also be recognized by comparing **4** *vs* **9** and **5** *vs* **10**. No significant difference is found between ribosyl and xylosyl substitution as can be seen in the toxicity of compounds **6** and **7**. It has been reported[9] that these two compounds are also similar with respect to their antimicrobial activity. The toxicities of other group II

Table 1. Acute intravenous toxicity of aminoglycoside antibiotics

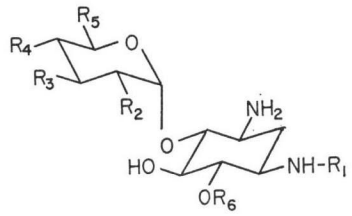
Group	Compound	Antibiotic name	No. of test	LD_{50} in mg/kg (range)
I	**1**	Neamine	4	125 (121~129)
	2	4′-Deoxyneamine	3	195 (181~209)
	3	3′, 4′, 5, 6-Tetradeoxyneamine	1	60 (44~ 76)
	4	1-AHB-neamine	2	260 (247~273)
	5	4′-Deoxy-l-AHB-neamine	3	330 (318~342)
II_a	**6**	Ribostamycin	5	260 (250~270)
	7	5-Xylosylneamine	3	280 (265~295)
	8	4′-Deoxy-5-xylosylneamine	2	280 (260~300)
	9	Butirosin A	5	520 (502~538)
	10	4′-Deoxybutirosin A (Bu-1975C_1)	5	520 (515~526)
	11	Bu-1709E_1	1	890 (656~944)
II_b	**12**	Neomycin B	3	24 (21~ 27)
	13	Neomycin C	1	44 (37~ 51)
	14	Paromomycin I	2	160 (145~175)
	15	Lividomycin B	2	140 (130~150)
II_c	**16**	Lividomycin A	2	280 (252~308)
III	**17**	Kanamycin A	7	280 (269~291)
	18	Kanamycin B	4	132 (124~140)
	19	Kanamycin C	2	225 (198~252)
	20	Tobramycin	6	79 (74~ 84)
	21	3′, 4′-Dideoxykanamycin B (DKB)	3	71 (67~ 75)
	22	Gentamicin C_1	3	88 (78~ 98)
	23	Gentamicin C_2	2	70 (65~ 75)
	24	Gentamicin C_{1a}	4	70 (59~ 81)
	25	Amikacin (BB-K8)	5	300 (285~315)

antibiotics are compared with that of **6** or **7**. AHB acylation at C-1 of the DOS ring reduced the toxicity to about one-half (**7** *vs* **9**, **8** *vs* **10**) as in the case of group I antibiotics. On the other hand C-4′ deoxygenation was not effective in lowering the toxicity of group II_a antibiotics (**7** *vs* **8**, **9** *vs* **10**) in contrast to its effect observed with group I. Replacement of the C-6′ amino group by a hydroxyl group considerably reduced the toxicity (**9** *vs* **11**). The antibiotic activity of **11** has been reported to be much lower than that of **9**.[18]

Substitution of the C-3″ hydroxyl group of ribostamycin (**6**) with a 2, 6-diaminohexose gives rise to neomycins B(**12**) and C(**13**), which were 6~10 times more toxic than **6**. Paromomycin I (**14**) is the 6′-deamino-6′-hydroxy derivative of **12** and showed markedly lower toxicity than **12** indicating a significant contribution of the 6′-amino group to the toxicity of these antibiotics. Lividomycin B (**15**) is synonymous with 3′-deoxyparomomycin I, and the fact that **15** showed about the same level of toxicity as compared with **14** suggested a minimal effect of 3′-deoxygenation on the toxicity of group II antibiotics. Lividomycin A(**16**) (4‴-mannosyl-lividomycin B) was only one-half as toxic as **15**, indicating an apparent toxicity-reducing effect of the mannosyl substitution.

The acute LD_{50}'s of group III antibiotics were compared with that of kanamycin B(**18**) because it includes the basal moiety neamine in the molecule. Kanamycin A(**17**), which is the 2′-deamino-2′-hydroxy derivative of **18**, was much less toxic than **18** (greater than 50 % reduction). Similar toxicity-reducing effect of deamination was also seen with

Fig. 4. Structures of Group III antibiotics



	R_1	R_2	R_3	R_4	R_5	R_6
17	H	OH	OH	OH	CH_2NH_2	3AG*
18	H	NH_2	OH	OH	CH_2NH_2	3AG
19	H	NH_2	OH	OH	CH_2OH	3AG
20	H	NH_2	H	OH	CH_2NH_2	3AG
21	H	NH_2	H	H	CH_2NH_2	3AG
22	H	NH_2	H	H	CH_3 | $CH-NH-CH_3$	GAR**
23	H	NH_2	H	H	CH_3 | $CH-NH_2$	GAR
24	H	NH_2	H	H	CH_2NH_2	GAR
25	AHB	OH	OH	OH	CH_2NH_2	3AG

* 3-Amino-3-deoxy-D-glucosyl residue
** Garosaminyl residue

kanamycin C(**19**), the 6′-deamino-6′-hydroxy derivative of **18**, though the effect was greater with the C-2′ amino group. In contrast to the group II antibiotics, deoxygenation at the C-3′ hydroxyl group showed a significant enhancement of toxicity in the group III antibiotics which have a 2′-amino function as was seen with tobramycin (3′-deoxykanamycin B, **20**) and 3′, 4′-dideoxykanamycin B(**21**). On the other hand 4′-deoxygenation is likely to have little effect on the toxicity judging from the nearly equivalent toxicity of **20** and **21**. Toxicities of the gentamicin C components (**22**, **23**, and **24**), all of which lack the 3′-hydroxyl group, were similar to those of **20** and **21**. Toxicity-reducing effect of AHB acylation in group III antibiotics (**17** *vs* **25**) was not significant as compared with that observed with the group II antibiotics.

It seemed rather unusual that the 3′-deoxygenation of paromomycin did not intensify the toxicity to an appreciable extent (**14** *vs* **15**), since deoxygenation vicinal to an amino function generally resulted in the enhancement of acute toxicity as exemplified in several of the group III antibiotics (**20** through **24**). Recently UMEZAWA *et al.*[20] suggested, in their studies on the enzymatic phosphorylation of lividomycin and ribostamycin, that the 3′-hydroxyl group of ribostamycin might be located close to the 5″-hydroxyl group of the ribose moiety and hence a single enzyme phosphorylates the 3′-hydroxyl of ribostamycin as well as the 5″-hydroxyl of lividomycin which lacks the 3′-hydroxyl group. The minimal toxic effect of 3′-deoxygenation found in lividomycin might also be explained by the suggested conformation of the antibiotic where the 5″-hydroxyl group would be performing the function of the 3′-hydroxyl group which is missing.

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