PROTECTIVE EFFECT OF D-GLUCARO-δ-LACTAM AGAINST AMINOGLYCOSIDE-INDUCED NEPHROTOXICITY IN RATS

TETSUTARO NIIZATO, TAKEMI KOEDA, TAKASHI TSURUOKA, SHIGEHARU INOUYE and TARO NIIDA

Central Research Laboratories, Meiji Seika Kaisha, Ltd.
Kohoku-ku, Yokohama, 222 Japan

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The nephrotoxicity of rats caused by dibekacin (3′,4′-dideoxykanamycin B) or kanamycin, with or without dextran was effectively reduced by D-glucaro-δ-lactam potassium salt, as evidenced by lower levels of blood urea nitrogen and kidney edema rate, better excretion of antibiotics, and less morphological damage. Protection was dosage related, and potentiated with increasing doses, but only when the two drugs were given simultaneously. Among three alkali-metal salts examined, the potassium salt was almost equal to the lithium salt, but surpassed the sodium salt in effectiveness.

Inorganic salts, in particular potassium chloride were found to be effective for the protection of normal rats, but their effect decreased for the dehydrated rats, especially in the presence of dextran.

Aminoglycosides such as dibekacin (3′,4′-dideoxykanamycin B), kanamycin and gentamicin are clinically useful broad spectrum antibiotics, but may occasionally cause renal damage. Protection of the renal function from aminoglycosides by use of other chemicals has been recently reported by several workers, using an animal model. FURUNO *et al.*[1] reported the protective effect of D-glucarates administered before injection of aminoglycosides and dextran. DELLINGER *et al.*[2] described the protective influence of cephalothin against gentamicin-induced renal failure, and LUFT *et al.*[3] the protection with high doses of cefazolin or cephaloridin from gentamicin nephrotoxicity.

We report here the protective effect of D-glucaro-δ-lactam, an oxidation product of an antibiotic nojirimycin[4], against dibekacin or kanamycin-induced nephrotoxicity in rats.

Materials

Dibekacin sulfate and kanamycin sulfate were products of Meiji Seika Kaisha, Ltd. D-Glucaro-δ-lactam alkali-metal salts were synthesized in this laboratory by the procedure similar to that previously reported[5]. The sodium salt was obtained as an amorphous powder, mp 185~190°C (decomp.). The potassium salt crystallized from water as monohydrate showed mp 216~218°C. The lithium salt was crystallized from water-ethanol with mp 273~274°C (decomp.). Dextran injectable (10%, w/v) was supplied by Daigo Eiyo Kagaku Ltd., Tokyo. Other inorganic salts were commercial products of reagent grade.

Methods

Experiment I

Male Wister SPF rats, 7~8 weeks old, were fed commercial rat chow without water for 48 hours. Unless otherwise stated, the dehydrated rats were injected intramuscularly with 100 mg base /kg of dibekacin or 300 mg base/kg of kanamycin dissolved in distilled water, immediately followed by an intraperitoneal injection of 2 g/kg of dextran (M. W. *ca* 40,000). D-Glucaro-δ-lactam in the form of alkali-metal salts was administered intramuscularly or intraperitoneally, 5~10 minutes before antibiotic

injection. Twenty-four hours after the last injection of drugs, during which time dehydration was continued, urine accumulated in the metabolic cage was recovered, blood was collected through the inferior vena carva, and bilateral nephrectomy was exercised. Concentrations of blood urea nitrogen (BUN), glutamic-oxalacetic-transaminase (GOT), lactic dehydrogenase (LDH) and inorganic phosphorous (InP) in serum were determined by use of a Technicon Autoanalyzer SMA 12/60, and expressed as mean±standard deviation.

After kidney edema rate (KER) (relative weight of a pair of kidneys to the whole body) was determined, right kidney was trisected, and was placed in buffered 10% formalin. The specimen sectioned at 4 μm were stained with hematoxylin and eosin or, in a few cases, with the AZAN-MALLORY reagent, and subjected to routine microscopic observation. Degree of histological abnormalities were scored as follows: normal, 0; minimal, 1; mild, 2; moderate, 3; severe, 4. Concentration of antibiotics in serum and left kidney homogenate were determined by the usual cup assay using *Bacillus subtilis* ATCC 6633 as a test organism.

Experiment II

Male Wister SPF rats weighing 185 g on an average were fed rat chow without water for 48 hours, and dehydration was continued during the experiment. The dehydrated rats were given intramuscularly 150 mg base/kg of dibekacin, with or without D-glucaro-δ-lactam potassium salt or potassium chloride that was administered intramuscularly or intraperitoneally, 5~10 minutes before antibiotic injection. Twenty-four hours later, rats were sacrificed, and analyzed by the procedures described in Experiment I.

Experiment III

Male Wister SPF rats, 7~8 weeks old, were housed with free access to water and rat chow. Dibekacin was given intramuscularly once daily in dosage of 300 mg base/kg/day for 3 days. Five to ten minutes before antibiotic injection, D-glucaro-δ-lactam potassium salt or inorganic salts were administered intramuscularly, intraperitoneally or orally. Twenty-four hours later, rats were sacrificed and analyzed by the procedures described in Experiment I.

Results and Discussion

1. Protective Effect of D-Glucaro-δ-lactam against Dibekacin (3′,4′-Dideoxykanamycin B, DKB) or Kanamycin (KM)-dextran-induced Renal Damage in the Dehydrated Rats (Experiment I)

It is well known that a plasma expander, dextran, potentiates the nephrotoxicity of aminoglycosides, and administration of DKB or KM coupled with dextran into the dehydrated rats produced most acute and severe renal failure among the three experiments I, II and III.

Animals receiving 300 mg base/kg of KM and 2 g/kg of dextran showed 40% increase of KER value compared to a control, and slower excretion of the antibiotic. Alteration of biochemical parameters was noted, especially elevation of BUN value due to renal failure. Histological abnormalities were recognized primarily around the proximal renal tubules and to a lesser degree the collecting ducts. These findings included dilatation of tubular lumina, vacuolation and necrosis of epithelial cells of proximal tubules, deposition of eosinophilic cast in tubules, and proacidification and pycnosis of epithelial cells of collecting ducts, accompanied by desquamation of epithelium, mucous stagnation and disordered arrangement.

When 284 mg/kg of D-glucaro-δ-lactam potassium salt (GL-K) was administered concurrently, all of these indices were noted to be closer to normal ones (Table 1): that is, KER decreased from 1.39 to 1.09, BUN from 125 to 39, and all of pathological scores lowered to below 1.0. The antibiotic was better excreted from blood and kidney. In accordance with improvement in serum and kidney,

Table 1. Protective effect of D-glucaro-δ-lactam potassium salt (GL-K) on kanamycin (KM) or dibekacin (DKB) dextran-induced renal damage in the dehydrated rats*[1].

Aminoglycoside	Compound	Dose (mg/kg)	Route	KER (%)	Urine		Antibiotic assay	
					Volume (ml)	pH	Serum (μg/ml)	Kidney (μg/g)
KM+dextran	GL-K	284	im	1.09±0.08	5.1	6.0	1	527
KM+dextran	—	—	—	1.39±0.12	4.8	5.8	31	1,073
Control (dextran)	—	—	—	0.99±0.09	3.4	6.0	0	0
DKB+dextran	GL-K	459	im	0.92±0.04	3.1	6.0	2	101
DKB+dextran	—	—	—	1.13±0.12	1.3	6.0	30	198

	BUN (mg/dl)	GOT (mU/ml)	InP (mg/dl)	LDH (mU/ml)	Mean score of morphological damage per rat*[2]					
					1	2	3	4	5	6
KM+dextran+GL-K	39±14	239±102	5.6±0.4	749±189	1.0		0	0.4	0	0
KM+dextran	125±33	267±100	7.4±2.5	696±119	1.9		0.8	2.0	0.6	0.5
Control (dextran)	22±4	185±47	5.1±1.0	805±216	1.1		0	0	0	0
DKB+dextran+GL-K	65±34	342±182	6.4±1.0	598±112	0.1	0.4	0	0.1		0
DKB+dextran	132±68	444±308	12.0±4.3	730±325	1.6	2.3	0.6	1.4		1.0

*[1] Each group of 8 dehydrated rats weighing 200 g on an average was given KM (300 mg base/kg) or DKB (100 mg base/kg) and dextran (2 g/kg), with or without prior administration of GL-K. Renal damage was determined 24 hours after antibiotic injection.

*[2] **1**: Vacuolation of tubular epithelial cells
2: Hyaline droplet degeneration of tubular epithelial cells
3: Necrosis of tubular epithelial cells
4: Dilatation of tubular lumina
5: Cast in tubules
6: Abnormalities of collecting ducts

urine parameters such as protein and glucose contents showed good recovery. However, pH of urine changed little by the addition of GL-K, differed from D-glucarates[1].

The same result was obtained from the DKB-dextran treated animals, and the effect of GL-K was comparable to that against KM-dextran-induced nephrotoxicity. Particularly noteworthy was the striking improvement of histological scores. Except for hyaline droplet degeneration that was stained red by the AZAN-MALLORY reagent, other items became almost normal with the addition of GL-K.

As shown in Table 2, the protective effect of GL-K was recognized at a dose of 2.5 mol per mol of DKB, and was potentiated progressively with increasing doses up to 10 mol per mol of DKB. In addition to the BUN, there was alteration of other biochemical parameters such as GOT, InP and LDH depending upon the dosages of GL-K*. Of these, the GOT value was most closely related with BUN, and may have been related to the renal dysfunction, since other parameters including GPT remained almost constant. In order to assess the role of an anion, we examined the effect of potassium chloride for comparison. The latter showed no significant protection at the doses given, demonstrating the important role of D-glucaro-δ-lactam anion for the protection.

Table 3 indicates the influence of administration route on the protective effect of GL-K. Either

* Independent study showed that GL-K did not cause any change in biochemical parameters mentioned above, and produced no morphological change at a dose of 4,000 mg/kg (po) (unpublished work).

Table 2. Dose-response of D-glucaro-δ-lactam potassium salt (GL-K) and potassium chloride (KCl) against dibekacin (DKB)-dextran-induced renal failure*.

Compound	Mol/mol of DKB	BUN (mg/dl)	GOT (mU/ml)	InP (mg/dl)	LDH (mU/ml)	KER (%)
GL-K	0.5	185±17	738±160	16.6±1.6	1077±360	1.23±0.09
GL-K	1.0	188±21	760±152	17.0±1.3	1035±323	1.30±0.16
GL-K	2.5	149±37	464±190	13.1±3.2	676±173	1.31±0.19
GL-K	5.0	120±52	423±209	10.4±4.0	542±437	1.15±0.07
GL-K	10.0	70±27	390±98	8.1±0.6	436±423	1.08±0.04
KCl	0.5	189±17	726±121	17.0±1.1	1077±302	1.27±0.12
KCl	1.0	182±26	668±185	16.9±2.8	962±308	1.32±0.11
KCl	2.5	194±21	836±84	17.6±1.6	1272±284	1.26±0.06
KCl	5.0	176±13	776±137	15.9±1.2	914±140	1.34±0.06
KCl	10.0	161±42	702±166	13.7±2.5	787±179	1.28±0.14
Control (dextran)	—	21±4	208±36	7.3±0.9	469±227	0.96±0.08
Control (DKB+dextran)	—	181±20	746±109	16.7±1.6	1193±238	1.30±0.12

* Each group of 8 dehydrated rats weighing 182 g on an average received test compounds intramuscularly, and 5~10 minutes later, DKB (100 mg base/kg) and dextran (2 g/kg). Biochemical parameters and KEG were determined 24 hours after antibiotic injection.

Table 3. Effect of injection route on the protection of D-glucaro-δ-lactam potassium salt (GL-K) against kanamycin (KM)-dextran-induced nephrotoxicity in the dehydrated rats*.

Compound	Dose (mg/kg)	Route	BUN (mg/dl)	KER (%)
GL-K	600	im	18±3	0.77±0.11
GL-K	600	ip	15±4	0.67±0.06
GL-K	600	sc	20±11	0.79±0.08
Control (KM +dextran)	—	—	263±19	0.88±0.08

* Each group of 8 dehydrated rats weighing 385 g on an average received GL-K, and 5~10 minutes later, KM (150 mg base/kg) and dextran (3 g/kg). After administration of drugs, rats were accessible to water, and 3 days later the extent of renal injury was examined.

Table 4. Effects of dose separation on the protection of D-glucaro-δ-lactam potassium salt (GL-K) against kanamycin(KM)-dextran-induced nephrotoxicity in the dehydrated rats*.

First injection	Second injection (6 hours after 1st)	Route	BUN (mg/dl)
KM+dextran	none	—	115±8
KM+dextran	GL-K	im	119±9
GL-K	KM+dextran	im	102±7
KM+dextran+ GL-K	none	im	34±7

* Each group of 8 dehydrated rats weighing 185 g on an average was given KM (150 mg base/kg) and dextran (2 g/kg), with or without administration of GL-K (600 mg/kg). BUN values were obtained one day after the final injection of drugs.

intramuscular or intraperitoneal or subcutaneous injection was effective, and there was observed no significant difference among them.

Effect of spacing of doses on protection by GL-K was evaluated by comparing the BUN of animals administered with KM and GL-K simultaneously or separated by a 6-hour interval. Regardless of the order, administration of the two drugs 6 hours apart abolished the protective effect of GL-K, and the resultant BUN levels were similar to that due to KM-dextran alone (Table 4). This suggested that concurrent presence of the two drugs at kidney was essential for the protection.

Table 5 shows the comparative effect of three alkali-metal salts (lithium, potassium and sodium) of D-glucaro-δ-lactam. As far as the BUN and KER values were concerned, the potassium salt appeared

Table 5. Comparison of three alkali-metal salts of D-glucaro-δ-lactam (lithium, potassium and sodium salts) on the protective effect against dibekacin (DKB)-dextran-induced nephrotoxicity in the dehydrated rats*.

Salt	Dose (mg/kg)	BUN (mg/dl)	KER (%)
Lithium	522	65±14	0.84±0.07
Potassium	600	70±23	0.87±0.07
Sodium	561	94±14	0.94±0.07
Control (DKB+dextran)	—	155±10	1.01±0.12

* Each group of 8 dehydrated rats weighing 227 g on an average was given DKB (100 mg base/kg) and dextran (2 g/kg), with or without prior intramuscular injection of test salts. BUN and KER were obtained 24 hours after antibiotic injection.

Table 6. Effect of D-glucaro-δ-lactam potassium salt (GL-K) and potassium chloride (KCl) on dibekacin (DKB)-induced nephrotoxicity in the dehydrated rats*.

Compound	Dose (mg/kg)	Route	BUN (mg/dl)	KER (%)
GL-K	600	ip	39±7	1.06±0.08
GL-K	600	im	59±9	1.06±0.05
KCl	181	ip	84±23	1.09±0.07
KCl	181	im	109±39	1.09±0.05
Control (DKB)	—	—	130±20	1.14±0.07

* Each group of 8 dehydrated rats weighing 185 g on an average was given DKB (150 mg base/kg), with or without prior administration of compounds listed. Analytical data were obtained one day after antibiotic injection.

to be equally effective as the lithium salt, but surpassed the sodium salt, implying the importance of cations for protection. In the following experiments, the potassium salt of D-glucaro-δ-lactam was exclusively used.

2. Protective Effect against DKB-induced Renal Damage in the Dehydrated Rats (Experiment II)

A single injection of heavy dose of DKB (150 mg base/kg) to the dehydrated rats caused definite renal injury, though its extent was less than that due to DKB-dextran combination. Marked elevation in BUN level and KER percent occurred in all rats treated. In contrast, concurrent administration of GL-K intramuscularly or intraperitoneally, produced a significant diminution in BUN and KER values, compared to those of DKB alone (Table 6).

It is further noted in Table 6 that potassium chloride which was ineffective in experiment I, now became effective in experiment II, though its effect was weaker than GL-K, as judged from BUN and KER levels.

3. Protective Effect against DKB-induced Renal Damage in Rats Allowing Water *ad libitum* (Experiment III)

The kidneys of rats under normal condition were more resistant to aminoglycosides than those under dehydrated condition. At least three consecutive injections of larger doses of DKB were necessary for the clear-cut appearance of renal failure in normal rats.

Table 7 summarized the protection by GL-K from DKB nephrotoxicity in experiment III. All of rats treated with daily injection of DKB (300 mg base/kg/day) for 3 days had marked elevation in BUN and KER values, together with advanced morphological damage, primarily on the epithelial cells of proximal tubules*.

* With regard to biochemical parameters other than BUN, GOT in serum increased in parallel to BUN, but InP and LDH could be no more correlated with BUN in the experiment III. This was in sharp contrast to the good correlation of the four parameters in the experiment I, and suggested some biochemical differences in the manner of renal damage induced in I and III. In consistent with this, there were observed morphological differences between I and III. For instance, the renal damage in III was characterized by the marked symptoms of nuclear and cytoplasmic alteration, infiltration of round cells and cast in tubules (Table 7).

Table 7. Protective effect of D-glucaro-δ-lactam potassium salt (GL-K) against dibekacin (DKB)-induced nephrotoxicity in normal rats*1.

Compound	Dose (mg/kg /day)	Route	KER (%)	Urinary volume (ml)	DKB assay serum (μg/ml)	BUN (mg/dl)	GOT (mU/ml)	InP (mg/dl)
GL-K	600	im	0.87±0.09	8.1	4.2	42±18	315±112	6.6±0.7
GL-K	1,500	po	0.83±0.08	6.3	3.0	41±11	222±71	6.3±0.7
Control (DKB)	—	—	0.97±0.06	9.5	21	106±49	384±147	6.9±1.3
Control (none)	—	—	0.68±0.05	4.8	0	24±4	99±28	7.1±0.5

	LDH (mU/ml)	Mean score of morphological damage per rat*2					
		1a	**2**	**3**	**4a**	**5**	**6**
GL-K	906±286	0.1	1.4	0.4	0.1	0	0.3
GL-K	783±302	0	1.1	0	0.3	0	0.1
Control (DKB)	1088±409	0.7	2.4	1.6	1.3	0.6	1.0
Control (none)	933±333	0	0	0	0	0	0

*1 Each group of seventeen normal rats weighing 230 g on an average was given a daily injection of DKB (300 mg base/kg/day), with or without GL-K for 3 days. Renal damage was examined 24 hours after the final injection of drugs.

*2 **1a**: Nuclear and cytoplasmic alteration of tubular epithelium
2 : Hyaline droplet degeneration of tubular epithelial cells
3 : Necrosis of tubular epithelial cells
4a: Infiltration of round cells into cortex stroma
5 : Cast in tubules
6 : Abnormalities in collecting ducts

The increase in BUN and KER were considerably inhibited by simultaneous administration of GL-K at a dose of 600 mg/kg/day (im) or 1,500 mg/kg/day (po) for 3 days. Microscopic examination also revealed a marked reduction of morphological damage with the addition of GL-K, and DKB remaining in serum decreased from 5 to 7 times less than that with DKB alone. Slight increase in urinary volume was probably due to diuretic property of the potassium cation, but it was not significant compared to that of animals with DKB alone.

For a comparison, the effect of potassium chloride was determined in Table 8, and it was again effective, as in experiment II, as evidenced by a low BUN value. Since the effect of potassium chloride was detected, we have compared other inorganic salts with GL-K to estimate the roles of cations and anions for protective activity (Table 8). As far as the BUN was concerned, potassium chloride and probably potassium sulfate and potassium bicarbonate were almost equal to GL-K, but potassium phosphate remained ineffective. The corresponding sodium salts, that is sodium chloride, sodium sulfate and sodium bicarbonate were still effective, but seemed to be inferior to the corresponding potassium salts and GL-K. As judged from KER however, these results were not always supported. Apparently, further work will be necessary to confirm the protective activity of inorganic salts, but the results so far obtained appear to indicate the importance of both cations and anions for the appearance of the protective effect.

FURUNO *et al.*[1] and DELLINGER *et al.*[2] reported that the effects of D-glucarates and cephalothin were only protective, but not curative. The same seemed to be true for D-glucaro-δ-lactam, since it lost activity when administered 6 hours after injection of antibiotic.

Table 8. Comparison of D-glucaro-δ-lactam potassium salt (GL-K) with various inorganic potassium and sodium salts on the protective activity against dibekacin (DKB)-induced nephrotoxicity in normal rats*.

Compound	Dose (mg/kg/day)	Route	Lethal rate	BUN (mg/dl)	KER (%)
GL-K	600	ip	0/8	45±12	0.95±0.08
GL-K	600	im	0/15	45±9	0.92±0.06
GL-K	800	po	0/8	46±10	0.92±0.07
KCl	181	ip	0/8	44±5	1.00±0.11
KCl	181	im	0/8	36±8	0.98±0.05
KCl	241	po	0/8	46±11	0.93±0.08
K_2SO_4	212	im	0/7	32±4	0.83±0.06
$KHCO_3$	336	im	0/7	48±31	0.95±0.12
K_2HPO_4	212	im	1/7	116±62	0.95±0.11
NaCl	163	im	2/15	53±9	0.97±0.09
Na_2SO_4	173	im	0/7	83±25	0.92±0.10
$NaHCO_3$	280	im	1/7	57±19	0.95±0.10
Control (none)	—	—	0/7	23±3	0.76±0.13
Control (DKB)	—	—	0/37	104±32	0.98±0.06

* Each group of normal rats of average weight 231 g received once daily administration of test compounds, immediately followed by intramuscular injection of DKB (300 mg base/kg/day) for 3 days. One day after the final administration of drugs, damage in kidney function was examined.

As for the effective principle, these authors[1,2] stressed the importance of anions, but we have shown the important role of cations as well as anions. Although the precise mechanism of kidney injury by aminoglycosides was not clear, HARRISON *et al.*[6] suggested that their polycationic nature may directly act on the renal lysosomes, perhaps with acidic lipoproteins in the lysosomal matrix, and as a result, release the contents in the urine. If so, excess of cations like K^+ would effectively compete with aminoglycosidic cations in tubular lysosomes.

In addition to cations, the importance of an anion, D-glucaro-δ-lactam was demonstrated by the effective protection of the kidney from the coupled action of aminoglycosides and dextran, where inorganic salts and, as previously reported[1], common organic salts were ineffective. In this connection, it is interesting to note that aminoglycosides given to patients with chronic pyelonephritis caused prompt rise of β-glucuronidase activity in urine, which probably came from renal cells[6]. Since D-glucaro-δ-lactam and D-glucarates as well are strong β-glucuronidase inhibitors[8], further study on the interaction between aminoglycosides and D-glucaro-δ-lactam in renal tubules may be of interest.

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