THE EFFECT OF FRUSEMIDE AND PIRETANIDE ON THE RENAL CLEARANCE OF GENTAMICIN IN MAN

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- 1 Gentamicin alone (dose 1 mg/kg) or in combination with frusemide (dose 0.25 mg/kg) or piretanide (dose 0.1 mg/kg) was administered intravenously to six healthy male volunteers.
- 2 Blood samples were collected at various times for 24 h and urine for up to 48 h.
- 3 Both diuretics increased the renal clearance of gentamicin during the period of the diuresis, without influencing either the glomerular filtration rate or the distribution of the antibiotic.
- 4 The results are discussed in relation to gentamicin-induced nephrotoxicity.

Introduction

The aminoglycoside antibiotic gentamicin is a low molecular weight compound, weakly basic, which is excreted, essentially unchanged, by glomerular filtration (Pechere & Dugal, 1979). It accumulates in the renal proximal tubular cells of both animals and man suggesting a causal relationship in the development of gentamicin-induced nephrotoxicity (Kosek, Mazze & Cousins, 1974; Vera-Roman, Krishnakantha & Cuppage, 1975; Wellwood *et al.*, 1976; Houghton *et al.*, 1978).

Enhancement of gentamicin-induced nephrotoxicity by frusemide has been demonstrated in animals (Smith, Bryant & Balazs, 1975; Adelman *et al.*, 1979) and implicated in man (Noel & Levy, 1978). However a recent study has demonstrated that diuretics may reduce gentamicin-induced nephrotoxicity in the mouse (Whiting, Petersen & Simpson, 1981).

The present study examines the effect of frusemide and piretanide, a chemically related loop diuretic, on the renal clearance of gentamicin in man, in an attempt to provide some insight into the mechanism of this nephrotoxicity.

A preliminary account of this work was presented to the British Pharmacological Society Meeting, Aberdeen, September, 1980.

Methods

Subjects

The subjects were six healthy males (24–31 years of age, body weight 74 ± 11 kg). All had normal blood

biochemical and haematological profiles at the beginning of the study.

Following an overnight fast an indwelling cannula was inserted into a forearm vein for injection of the study drugs and for venous sampling. Food was withheld for the initial 4 h of each of the three experimental periods during which time water was taken in volumes equal to that of the urine voided.

After an initial urine voiding, a rapid intravenous injection of either gentamicin alone (dose 1 mg/kg) or in combination with frusemide (dose 0.25 mg/kg) or piretanide (dose 0.1 mg/kg) was administered. Urine was collected for approximately 48 h whilst venous blood was sampled at various times during the first 24 h. There was a 6 week gap between each of the three experimental periods.

Analysis

Gentamicin concentrations in plasma and urine were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California, U.S.A.). Glomerular filtration rate (GFR) was assessed after a single intravenous injection of ^{51}Cr -EDTA, given at the beginning of the experiment. (Chantler *et al.*, 1969) and urinary sodium was measured by flame emission spectroscopy.

The results are expressed as the arithmetic mean $\pm$ s.d. unless otherwise stated. Gentamicin alone groups were compared to the diuretic combination groups by a paired Student's *t*-test, $P < 0.05$ being considered significant.

Frusemide (Lasix) and piretanide were supplied by Hoechst UK Ltd, Hounslow, Middlesex, U.K. Gentamicin sulphate (Genticin) was obtained from Nicholas Laboratories, Slough, Berkshire, U.K.

Pharmacokinetic analysis

When the gentamicin sulphate was administered the plasma concentration of the antibiotic declined in a bi-exponential manner. The plasma concentration (C) at any time (t) was given by the following relationship:

$$C = A \exp(-\alpha t) + B \exp(-\beta t)$$

where α and β are both hybrid rate constants and A and B are the extrapolated zero time intercepts of each exponential. The values of A, α , B and β were obtained for each subject by computer, non-linear, least-square regression using the AUTOAN programme (Sedman & Wagner, 1977). A two-compartment open model, with drug distribution occurring between the central compartment and peripheral compartment and elimination occurring out of the central compartment was used in the further analysis of the data. The volumes of distribution of the central compartment (V_c) and β phase (V_β), the fraction of drug in the central compartment (F_c) and the half-life of the β phase ($T_{1/2\beta}$) were also calculated.

The average renal clearance of gentamicin (C_R) in any one time period was calculated from:

$$C_R = Ae/AUC$$

where Ae is the amount of gentamicin excreted in the urine during the time period and AUC is the area

under the plasma concentration v time curve for the same period expressed as $\text{mg ml}^{-1} \text{ min}$.

The proportion of gentamicin excreted in the urine (P) for any time period being given by:

$$P = \frac{Ae}{D}$$

Results

The urinary volumes, sodium concentrations and GFR following a single intravenous dose of gentamicin alone or in combination with frusemide or piretanide are shown in Table 1. The diuretics appear equipotent at these doses, judged by urinary volume and natriuresis. GFR was not significantly altered by diuretic combination therapy.

A bi-exponential decline in plasma gentamicin concentration following intravenous administration of gentamicin alone or in combination with diuretic was observed. This is shown for one subject after gentamicin alone, in Figure 1. The pharmacokinetic parameters are listed in Table 2. There are no significant differences in these derived parameters between the three experimental groups. Hence, from this analysis, the distribution and elimination of gentamicin are apparently not influenced by the concomitant administration of the diuretics.

The administration of frusemide or piretanide produced an immediate brisk diuresis which persisted over approximately 180 min. During this period the amount of gentamicin excreted in the urine was significantly higher than when gentamicin was

Table 1 The glomerular filtration rate, urine volumes and sodium excretion following intravenous gentamicin alone (dose 1 mg/kg) or in combination with frusemide (dose 0.25 mg/kg) or piretanide (dose 0.1 mg/kg).

		Gentamicin	Gentamicin/ frusemide	Gentamicin/ piretanide
Glomerular filtration rate (ml min^{-1})				
	$^{51}\text{Cr-EDTA}$	139 $\pm$ 19.3	122 $\pm$ 38.0	109 $\pm$ 15.8
1-3 h	Volume (ml)	324 $\pm$ 165	1682 $\pm$ 497	1984 $\pm$ 292
	Sodium (mmol)	42 $\pm$ 20.1	82 $\pm$ 40.7	107 $\pm$ 72.0
3-7 h	Volume (ml)	323 $\pm$ 191	251 $\pm$ 125	489 $\pm$ 306
	Sodium (mmol)	33 $\pm$ 12.1	20 $\pm$ 9.4	36 $\pm$ 19.5
7-24 h	Volume (ml)	1284 $\pm$ 798	1029 $\pm$ 557	960 $\pm$ 335
	Sodium (mmol)	54 $\pm$ 15.4	54 $\pm$ 27.5	64 $\pm$ 22.2
24-48 h	Volume (ml)	1988 $\pm$ 823	2061 $\pm$ 764	1714 $\pm$ 477
	Sodium (mmol)	126 $\pm$ 14.4	135 $\pm$ 16.2	118 $\pm$ 61.0

All results expressed as mean $\pm$ s.d. from six patients.

Figure 1 Plasma concentrations of gentamicin following intravenous administration of gentamicin alone (dose 1 mg/kg) to one subject.

administered alone (Table 3). During this period of diuresis the average renal clearance (C_R) of gentamicin was significantly higher and since AUC was not affected by the diuretic administration, the increase in C_R is due to the increased rate of gentamicin excretion in the urine. A similar effect, though less marked, is observed if the experimental period from 0–420 min post-injection is considered (Table 4).

The concomitant administration of diuretics effectively doubles the proportion of gentamicin excretion in the urine (P) during the first 180 min post-injection (Table 5). During the first 420 min a similar effect is also observed, although the increase is less significant.

Discussion

The results of the present study confirm the bi-exponential decline in plasma gentamicin concentrations with time described by Schentag & Jusko (1977). The pharmacokinetic parameters derived in this study are similar to those obtained previously (Walker, Rise & Mitchard, 1979) and in anephric patients on haemodialysis (Letourneau-Sehab *et al.*, 1977). The total average clearance and plasma half life are also similar to those found by Walker and his colleagues (1979). Higher plasma concentrations of gentamicin administered with frusemide and measured over a shorter study period, have been described (Tilstone *et al.*, 1977). These results were attributed to a reduced GFR and total clearance of gentamicin. However, these effects of diuretics on renal function and gentamicin excretion were not confirmed in this study. In contrast both diuretics increased the amount of gentamicin eliminated in the urine during the diuretic period. Chiu & Long (1978) failed to demonstrate any effects of frusemide on gentamicin excretion in the rat but concluded that the diuretic, in the absence of volume repletion adequate to maintain renal function, caused accumulation of gentamicin. In the present study all subjects received a lower dose of diuretic and maintained their state of hydration, which allowed GFR to remain unaltered. This may account for the discrepancy between our findings and those of Chiu & Long (1978).

Gentamicin is excreted primarily by glomerular filtration although some tubular reabsorption has been inferred in some studies by the finding that the clearance of gentamicin is significantly less than the GFR (Chiu *et al.*, 1976; Schentag & Jusko, 1977). Although our findings also infer tubular reabsorption, the plasma protein binding of gentamicin was not determined and whether free gentamicin is reabsorbed remains controversial (Pastoriza-Munoz, Bowman & Kaloyanides, 1979). However, gentamicin does accumulate in the kidney and the uptake into the proximal tubular cell is predominantly from the

Table 2 The effect of frusemide or piretanide on the pharmacokinetic parameters of gentamicin

	A (mg l^{-1})	α (h^{-1})	B (mg l^{-1})	β (h^{-1})	V_C (l)	$V\beta$ (l)	F_C	$T_{1/2}\beta$ (h)
Gentamicin	7.3 ± 2.1	2.8 ± 0.4	3.6 ± 0.5	0.3 ± 0.1	7.7 ± 0.8	17.4 ± 1.0	0.5 ± 0.1	2.2 ± 0.19
Gentamicin/ piretanide	12.7 ± 5.0	4.3 ± 1.0	3.8 ± 0.6	0.3 ± 0.1	7.7 ± 1.9	15.0 ± 2.0	0.5 ± 0.1	2.0 ± 0.3
Gentamicin/ frusemide	10.3 ± 5.2	2.9 ± 1.3	4.0 ± 0.6	0.4 ± 0.1	5.7 ± 1.8	16.1 ± 2.7	0.3 ± 0.1	2.5 ± 0.5

All results expressed as mean $\pm$ s.e. mean from six patients.

Table 3 The effect of diuretics on the area under the plasma concentration-time curve, the amount excreted in the urine and renal clearance of gentamicin from 0–180 min after administration.

	Gentamicin	Gentamicin/ frusemide	Gentamicin/ piretanide
AUC _{0–180} (mg ml ⁻¹ min)	0.58 ± 0.14	0.65 ± 0.17	0.64 ± 0.09
Gentamicin excreted (mg) (Ae)	19.0 ± 4.5	39.9 ± 13.7**	40.0 ± 9.1**
C _R (ml min ⁻¹)	35 ± 9	61 ± 12**	71 ± 20**

All results expressed as mean ± s.d. from six patients

** $P < 0.01$ **Table 4** The effect of diuretics on the area under the plasma concentration-time curve, the amount excreted in the urine and renal clearance of gentamicin from 0–420 min after administration.

	Gentamicin	Gentamicin/ frusemide	Gentamicin/ piretanide
AUC _{0–420} (mg ml ⁻¹ min)	0.77 ± 0.20	0.88 ± 0.25	0.86 ± 0.24
Gentamicin excreted (mg) (Ae)	29.3 ± 5.9	48.2 ± 16.6*	54.7 ± 14.1**
C _R (ml min ⁻¹)	40 ± 11	56 ± 16*	71 ± 14**

All results expressed as mean ± s.d. from six patients

* $P < 0.05$, ** $P < 0.01$

luminal surface by a specific luminal carrier, although some peritubular uptake has been demonstrated (Pastoriza-Munoz *et al.*, 1979). This uptake may be consistent with the prolonged β -phase of elimination found after multiple dosing, which is implicated in the nephrotoxicity associated with gentamicin therapy. Frusemide is more highly bound than gentamicin in healthy subjects (Prandota & Pruitt, 1975) and is secreted into the proximal tubular cell. Odland & Beermann (1980) have confirmed that this secretion involves peritubular uptake via an anion transport system. The renal handling of piretanide has yet to be comprehensively described.

The finding of increased elimination of gentamicin with both diuretics suggests several possible levels of interaction. Altered plasma protein binding of gentamicin could lead to an increased filtered load although since gentamicin is poorly bound in the plasma this is unlikely. Within the kidney a diuresis would result in increased medullary washout whilst mutual interactions may occur within the tubular

Table 5 The effect of diuretic combination therapy on the percentage of a gentamicin dose excreted in the urine

	Time after administration (min)	
	180	420
Gentamicin	22 ± 7.1	40.3 ± 10.1
Gentamicin/ Frusemide	52.7 ± 18**	63.9 ± 22.3
Gentamicin/ Piretanide	58.6 ± 5.0**	79.9 ± 11.1*

All results are expressed as mean ± s.d. from six patients

** $P < 0.01$, * $P < 0.05$.

lumen decreasing tubular uptake. Displacement of gentamicin from carriers on the proximal tubular cells may also occur. Therefore, with normal renal function accumulation may be decreased by diuretic administration with less potential nephrotoxicity.

However, with impaired renal function, medullary washout would be decreased with a decreased filtered load of gentamicin, whilst peritubular uptake may increase. Despite blood concentrations usually associated with therapy, accumulation may occur and it is possible that this may account for the reported

increased nephrotoxicity with combined gentamicin and frusemide therapy, in the presence of renal impairment.

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