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The Effect of Oral Activated Charcoal on the Systemic Clearance of Gentamicin in Rabbits with Acute Renal Failure

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Abstract—The effect of oral administration of activated charcoal on total body clearance of gentamicin administered intravenously (2 mg kg^{-1}) has been studied in normal rabbits and rabbits with induced renal failure. Gastric intubation of a single dose (10 g) of activated charcoal to normal rabbits produced a significant reduction in gentamicin serum concentrations compared to control. Significant differences between treated and control groups, compatible with enhancement of gentamicin elimination, were observed in the calculated pharmacokinetic parameters (Kel, $t_{\frac{1}{2}}$, CL and AUC). To examine whether renal failure could augment the effect of activated charcoal in enhancing the systemic clearance of gentamicin, uranyl nitrate was used (0·75 mg kg^{-1}, i.v.) to induce acute renal failure in rabbits. The derived pharmacokinetic parameters of gentamicin during the control phase in these animals were consistent with severe renal failure. The administration of activated charcoal, 2·5 h following gentamicin injection, produced a steeper decline in gentamicin concentration-time profiles and significant changes in Kel, $t_{\frac{1}{2}}$ and CL. The Kel and CL values increased to about 200%, while the $t_{\frac{1}{2}}$ value decreased to about 50%. The apparent changes in the pharmacokinetic parameters induced by charcoal administration were more marked in rabbits with renal failure than in normal rabbits; however, induction of renal failure did not augment the charcoal-induced clearance of gentamicin quantitatively.

Currently, attention is being given to the use of activated charcoal to enhance the systemic clearance of drugs after oral absorption or intravenous administration (Levy 1982; Editorial 1987). It has been demonstrated that activated charcoal given orally can enhance the systemic elimination of drugs such as dapsone (Neuvonen et al 1983), digoxin (Boldy et al 1985), disopyramide (Huang 1988), methotrexate (Gadgill et al 1982), phenobarbitone (Berg et al 1982), quinine (Lockey & Bateman 1989), salicylate (Hillman & Prescott 1985) and theophylline (Berlinger et al 1983).

The effect of activated charcoal on the systemic clearance of aminoglycosides, as a group of drugs with a narrow therapeutic index, deserves consideration. They are known to produce concentration-dependent nephrotoxicity and ototoxicity, and are eliminated largely unchanged, by the kidney, with a clearance that correlates well with creatinine clearance (Barza et al 1975).

Although in one study it has been observed that charcoal was not effective in enhancing the clearance of a therapeutic dose of the aminoglycoside, tobramycin, in normal individuals (Davis et al 1988), these results cannot be extrapolated to subjects with renal failure (RF) or toxic concentrations of aminoglycosides. A significant effect for charcoal in enhancing digoxin elimination has been demonstrated in patients with RF, but a relatively small effect was seen in normal subjects (Park et al 1985). So, in the presence of RF, activated charcoal may have a role in enhancing aminoglycoside clearance.

Whether induced-RF could augment the effect of activated charcoal in enhancing the systemic clearance of gentamicin, a widely used aminoglycoside, has been examined in rabbits.

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Materials and Methods

Chemicals

Gentamicin sulphate ampoules, 80 mg/2mL were purchased from Elkin-Sinn, Inc., Cherry Hill, NJ. Activated charcoal, AX-21 (with an internal surface area of 2800–3500 m^2 g^{-1}) generously provided by Anderson Development Co., Adrian, MI, was used without pretreatment. All chemicals and solvents were of analytical grade.

Animal studies

New Zealand adult male rabbits (3–5 kg) were fasted 15 h before and during the experiment, but water was freely available. In normal rabbits, gentamicin (2 mg kg^{-1}) was administered intravenously over 1 min through the marginal vein of the left ear. This was followed immediately, in a cross-over design, by gastric intubation of either activated charcoal (10 g in 40 mL of water) or water (40 mL). One week elapsed between the two treatments. The marginal vein of the right ear was cannulated with a polyethylene tube (Fr. no. 2) for blood sampling (1·5 mL) just before gentamicin administration and at 0·25, 0·50, 1·0, 1·5, 2·0, 3·0, 4·0 and 5 h post drug. After blood clotting, serum samples were taken following centrifugation and frozen until assayed.

As assessed by serum creatinine levels, a single dose of gentamicin (2 mg kg^{-1}, i.v.) administered to these animals was not associated with impairment of renal function one week later. The levels before and one week after gentamicin being within the range of 1·16–1·49 mg dL^{-1}.

RF was induced in the rabbits using uranyl nitrate (0·75

mg kg^{-1}, i.v.) as described by Øie (1985). Two days later RF was confirmed from serum creatinine levels. At least a 3-fold increase in serum creatinine levels was accepted as an indication of RF. On the day of the experiment (2 days after uranyl nitrate administration), these rabbits were subjected to the same procedure as described for the normal animals, except that they received charcoal 2·5 h following gentamicin, and blood samples were obtained at 0, 0·25, 0·5, 1, 1·5, 2·5, 3, 3·5, 4, 4·5 and 5 h following drug injection.

Assay procedures

Both homogeneous enzyme immunoassay (EMIT) and a microbiological assay were used to determine gentamicin concentrations in serum. The agreement between the two methods was within 10% in the concentration range 1 to 15 $\mu g\ mL^{-1}$ ($r = 0{\cdot}981$, $P < 0{\cdot}05$). All assays for each rabbit were run in duplicate on the same day. The intra-day coefficients of variation (CV) for the EMIT assay were 5·65% and 3·05% using concentrations of 3·5 and 7·0 $\mu g\ mL^{-1}$ ($n = 8$), respectively. The inter-day CVs were 7·37% and 3·41% over the same range of serum concentrations ($n = 8$).

For the microbiological assay of gentamicin, the agar diffusion technique was employed using sterilized plastic petri dishes (20 × 100 mm), phosphate buffer of pH 8 (to prepare the standard stock solution of gentamicin, 1 mg mL^{-1}) and *Staphylococcus epidermidis* ATCC 12228 as recommended by USP XXI (1985). Seeded tryptone soya agar (Oxoid) with 10^5 cfu^{-1} mL of *S. epidermidis* was poured into the plates and wells of 8 mm diameter were cut into the agar. The wells were filled with six dilutions of gentamicin standards (0·5, 1, 2, 4, 8 and 16 $\mu g\ mL^{-1}$) prepared in rabbit serum, with triplication of each of the samples. Agar plates were incubated at 35°C for 24 h and the resultant inhibition zones were measured and the inter-day CVs were 7·45% and 6·51%. The intra-day CVs were 7·26% and 3·58% for 3·5 and 7·0 $\mu g\ mL^{-1}$ ($n = 8$), respectively.

Creatinine serum levels were assayed colorimetrically using the Boehringer Mannheim GmbH test kit.

Data analysis

For the pharmacokinetic analysis, it was assumed that gentamicin kinetics were represented by a one-compartment open model. The elimination rate constant (Kel) was estimated by least square regression analysis, while the half-life of elimination ($t_{\frac{1}{2}}$) was calculated from the relationship $t_{\frac{1}{2}} = 0{\cdot}693/\text{Kel}$. An intercept of ordinate at time zero was used to estimate the initial serum concentration (Co) of gentamicin. The apparent volume of distribution (Vd = Dose/Co), the clearance (CL = Vd.Kel) and the area under the concentration-time curve (AUC) extrapolated to infinity (AUC = Co/Kel) were also calculated.

The data are presented as mean ± s.d. (standard deviation). The *t*-test for paired and unpaired data, according to the experimental design, was employed to assess the effect of charcoal treatment on the pharmacokinetic parameters. Differences between two related parameters were considered statistically significant for *P* values equal to or less than 0·05.

Results

Administration of 2 mg kg^{-1} of gentamicin intravenously to rabbits produced a mono- or biexponential decline in gentamicin serum levels over a concentration range of 1–15 $\mu g\ mL^{-1}$. The initial exponential decline phase was not considered in the pharmacokinetic analysis.

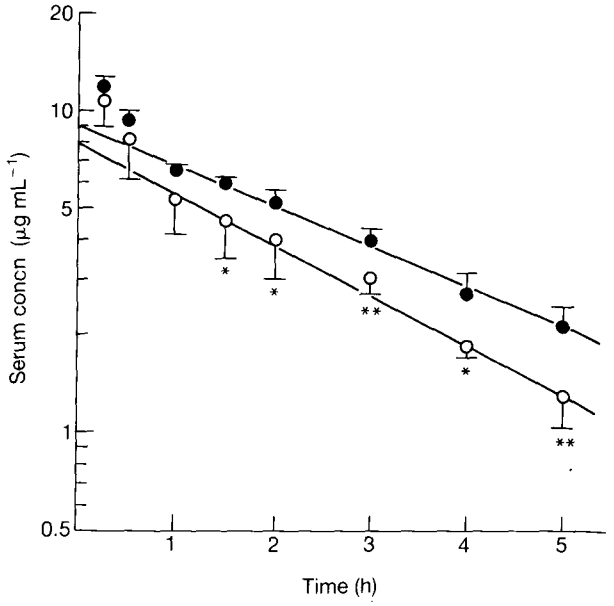

FIG. 1. The serum concentration profile of gentamicin administered intravenously (2 mg kg^{-1}) to rabbits with (○) or without (●) treatment with activated charcoal. Each point represents the mean ± s.d. of 6 rabbits. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

Effect of activated charcoal in normal rabbits

The serum-concentration profiles of gentamicin administered i.v. to normal rabbits with or without oral activated charcoal are shown in Fig. 1. Charcoal produced a significant reduction in gentamicin serum levels from 1·5 h onwards. Significant differences were observed in the parameters Kel, $t_{\frac{1}{2}}$, CL and AUC between treated and control groups (Table 1). Compared with the control group, the Kel and CL values in the treated animals increased to 120·0 ± 16·9% and 142·9 ± 25·1%, respectively. Charcoal decreased the $t_{\frac{1}{2}}$ and AUC to 84·5 ± 11·2% and 71·3 ± 11·5%, respectively. Although the Vd value was increased to 121·9 ± 34·6%, this change was not statistically significant. The apparent gastrointestinal clearance of gentamicin was calculated (CL with charcoal treatment—CL without charcoal treatment) and found to be 27·75 ± 16·30 mL kg^{-1} h^{-1}.

Table 1. Pharmacokinetic parameters of gentamicin administered intravenously (2 mg kg^{-1}) to rabbits with or without treatment with activated charcoal.

Parameter	Control	Charcoal	Change (% of control)
Kel (h^{-1})	0·289 ± 0·047	0·349 ± 0·067*	120·0 ± 16·9
$t_{\frac{1}{2}}$ (h)	2·40 ± 0·39	2·04 ± 0·37*	84·5 ± 11·2
Vd (L kg^{-1})	0·222 ± 0·032	0·272 ± 0·090	121·9 ± 34·6
CL (mL kg^{-1} h^{-1})	64·10 ± 6·44	91·37 ± 14·38*	142·9 ± 25·1
AUC (μg h mL^{-1})	31·23 ± 3·06	22·22 ± 3·21**	71·3 ± 11·5

Each value represents the mean ± s.d. of 6 rabbits
* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

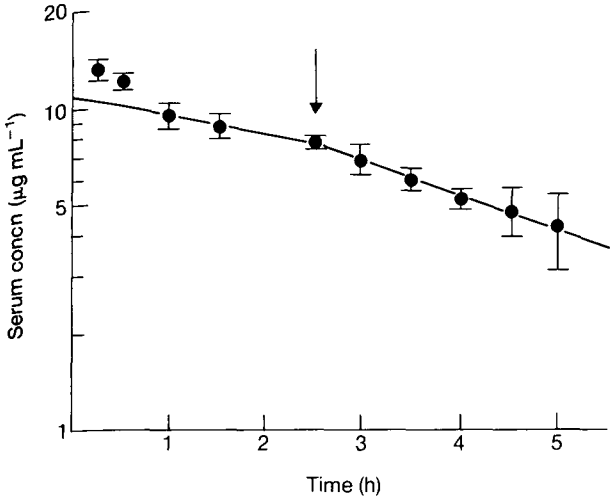

FIG. 2. The serum concentrations of gentamicin administered intravenously (2 mg kg^{-1}) to rabbits with induced renal failure. The arrow indicates the time of activated charcoal administration. Each point represents the mean ± s.d. of 6 rabbits.

Effect of activated charcoal in RF rabbits

Administration of uranyl nitrate (0·75 mg kg^{-1} i.v.) to rabbits produced marked and significant elevation of serum creatinine levels 48 h following injection (4·62 ± 1·37 mg dL^{-1}) compared with pretreatment values (1·29 ± 0·18 mg dL^{-1}). The effect of charcoal on gentamicin clearance was examined in a one-day experiment, because uranyl nitrate produces variable RF which is time-dependent (Øie 1985). The time course of serum gentamicin levels before and after charcoal administration is shown in Fig. 2. The derived pharmacokinetic parameters during the control phase (initial 2·5 h) were consistent with RF (Table 2). The dramatic decline of gentamicin clearance (22·81 ± 8·23 mL kg^{-1} h^{-1}) and the prolongation of $t_{\frac{1}{2}}$ of elimination (6·68 ± 4·05 h), compared with the values from normal rabbits (Table 1), confirmed the induction of renal failure. Charcoal administration, 2·5 h following gentamicin injection, produced a steeper decline in the concentration-time profile (Fig. 2) and significant changes in Kel, $t_{\frac{1}{2}}$ and CL parameters (Table 2). The apparent gastrointestinal clearance of gentamicin (CL during charcoal phase—CL during control phase) was 22·39 ± 14·69 mL kg^{-1} h^{-1} which was not significantly different from that of normal rabbits (27·75 ± 16·30 mL kg^{-1} h^{-1}).

Table 2. Pharmacokinetic parameters of gentamicin administered intravenously (2 mg kg^{-1}) to rabbits (n = 6) with induced renal failure before (control phase) and after (charcoal phase) activated charcoal administration.

Parameter	Control phase	Charcoal phase	Change (% of control)
Kel (h^{-1})	0·127 ± 0·057	0·247 ± 0·106*	212·6 ± 79·3
$t_{\frac{1}{2}}$ (h)	6·68 ± 4·05	3·13 ± 1·13*	52·9 ± 23·8
CL (mL kg^{-1} h^{-1})	22·81 ± 8·23	45·20 ± 15·59*	213·3 ± 80·1

Each value represents the mean ± s.d.
*$P < 0·05$.

Discussion

Activated charcoal administered orally to normal rabbits produced a significant increase in the elimination of gentamicin from the systemic circulation as demonstrated by the observed increase in the systemic clearance (about 40%) and decrease in AUC (about 30%). A modest but significant decrease (about 15%) was also noted in the half-life of elimination. These findings are in contrast with the results of tobramycin reported on human subjects (Davis et al 1988). Those authors found that neither the $t_{\frac{1}{2}}$ nor the CL of tobramycin were significantly altered by oral activated charcoal. Despite that, our result is not unexpected since the pharmacokinetic properties of gentamicin and other aminoglycosides (small Vd, negligible binding to plasma proteins and low hepatic extraction ratio) are favourable for removal by any dialysis process. It has been shown that haemodialysis (Danish et al 1974), as well as peritoneal dialysis (Jusko et al 1976), is effective in the removal of circulating gentamicin.

Although it has been shown in humans that small amounts of gentamicin are secreted in the bile (Pitt et al 1973), there is no evidence that gentamicin undergoes enterohepatic cycling. If the fate of gentamicin in man and rabbit is similar, adsorption of small amounts of gentamicin secreted in the small intestine via bile, cannot explain the observed 40% increase in gentamicin clearance. It is likely that activated charcoal's effect on the kinetics of intravenously administered gentamicin is by adsorbing drug that has crossed the bowel wall from the mesenteric vessels, a mechanism by which oral charcoal enhances the systemic elimination of phenobarbitone (Arimori & Nakano 1986) and theophylline (McKinnon et al 1987).

Uranyl nitrate produced predictable RF as indicated by the marked elevation of serum creatinine levels (about 3-fold) observed 48 h following injection. RF was further confirmed by the large decline in systemic clearance and prolongation of $t_{\frac{1}{2}}$ of elimination of gentamicin in RF compared with normal control animals (Tables 1, 2).

In acute RF, activated charcoal produced marked apparent changes in the pharmacokinetic parameters compared with those observed in normal rabbits treated with charcoal (Tables 1, 2). Activated charcoal was more effective in decreasing the half-life of elimination of theophylline in patients with a long serum half-life of the drug (Radomski et al 1984) and produced a significant effect in increasing digoxin elimination in patients with renal failure (Park et al 1985) but with only a small enhancement of elimination in normal subjects. As the amount of a drug exsorbed into the intestinal lumen increases in proportion to dose (Arimori & Nakano 1987, 1988), enhancement of drug clearance induced by oral charcoal would be more discernible in subjects with toxic drug concentrations, since the diffusion gradient into the intestinal lumen would be greater.

Our findings in rabbits are consistent with those observations; the relative changes in the pharmacokinetic parameters induced by charcoal were more pronounced in rabbits with RF than in normal rabbits (Tables 1, 2). In quantitative terms, however, the absolute differences in CL (apparent gastrointestinal clearance) were not significantly different in rabbits with RF and normal rabbits. Thus, RF does not

augment charcoal-induced clearance of gentamicin in quantitative terms.

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