Urinary adenosine excretion in patients receiving amphotericin B

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Background. *Intravenous amphotericin B (AMB) administration in animals causes renal vasoconstriction, ischemia, and oliguria that may result in irreversible renal injury; the mechanism of AMB nephrotoxicity may be similar in human beings. Adenosine is excreted in urine by the ischemic kidney. We hypothesized that adenosine excretion and oliguria would be a marker for patients who later would manifest AMB-associated renal insufficiency and that pre-AMB saline administration (which ameliorates AMB nephrotoxicity) would negate the change in adenosine excretion and urine output.*
Methods. *Twenty hospitalized patients being treated at the direction of their attending physician and who were receiving AMB (15 to 75 mg intravenously) had urine collected for 1 hour before and for 2 hours during AMB infusion. Eleven patients received normal saline solution (500 ml intravenously) before the AMB infusion; the other nine formed the comparator group. An aliquot of each urine collection was precipitated with perchloric acid to remove protein and cellular elements and centrifuged, and the supernatant was assayed for adenosine by using high-pressure liquid chromatography.*
Results. *Infusion of AMB was associated with a decrease in mean urine output both in patients who received saline solution (245 before versus 149 ml/hr during AMB infusion, $p = 0.04$) and in patients in comparator group (139 versus 89 ml/hr, $p = 0.027$). The mean urinary adenosine excretion was unchanged in the saline-loaded group (0.1354 before versus 0.1255 mmol/hr during drug infusion, $p = 0.25$) and was decreased in the comparator group (0.2276 versus 0.1127 mmol/hr, $p = 0.01$). Development of renal insufficiency did not correlate with the change in urine output or adenosine excretion.*
Conclusions. *AMB infusion in human beings results in decreased urine output and decreased adenosine excretion. The latter effect is prevented by a pre-AMB saline load. The changes in urine output and adenosine excretion are not predictive of the development of renal insufficiency. (Surgery 1997;121:190-3.)*

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SHORTLY AFTER THE INTRODUCTION of amphotericin B (AMB) in 1956, nephrotoxicity was observed.[1] The mechanism of AMB nephrotoxicity in human beings is unknown.[2] Infusion of AMB into the canine renal artery produces renal vasoconstriction and decreased glomerular filtration rate and urine output.[3] AMB may cause similar renal vasoconstriction in human beings that could result in ischemia and, ultimately, in irreversible renal injury. Adenosine is released by an ischemic kidney and is detectable in urine.[4] Administration of a saline bolus before AMB can ameliorate nephrotoxicity in human beings.[5, 6]

Assuming that a major mechanism of AMB nephrotoxicity in human beings is renal vasoconstriction with ischemia, we hypothesized that adenosine excretion and oliguria would be a marker for patients who later would manifest AMB-associated renal insufficiency. Because a pre-AMB saline bolus can decrease AMB nephrotoxicity, we also hypothesized that adenosine excretion and urine output would not be affected in the patient receiving a saline bolus.

PATIENTS AND METHODS

Experiments were conducted in 20 hospitalized patients (12 men and 8 women; mean age, 48 years; range, 17 to 83 years) who had Foley catheters in place and who were receiving AMB (15 to 75 mg intravenously during a period of 0.5 to 6 hours every day or every other day) for presumed or proven fungal infection. The study was approved by our institutional review board, and all patients signed an informed consent. None of the patients had a serum creatinine level greater than 2.0 mg/dl at the time of study, and none had greater than 10 red blood cells per high power field on urinalysis.

The data represent urine collections associated with

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Table I. Selected patient data

Patient		AMB	Infusion	Serum creatinine (mg/dl)		
No.	Age (yr)	dose (mg)	time (hr)	Baseline	Peak	Saline
1	50	50	6	1.5	1.5	+
2	46	20	0.5	1.1	2.2	+
3	53	50	4	2.0	2.0	+
4	50	60	4	2.0	2.4	+
5	42	20	0.5	0.6	1.9	+
6	24	20	0.5	0.8	2.1	+
7	68	60	4	1.4	1.4	+
8	20	20	0.5	0.6	6.1	+
9	68	40	4	1.7	4.5	+
10	35	40	4	0.5	0.7	+
11	73	30	3	1.9	2.6	+
12	37	60	4	1.1	1.1	—
13	49	50	4	0.8	0.9	—
14	17	35	6	0.7	0.7	—
15	28	35	4	0.4	0.5	—
16	83	15	4	1.0	1.2	—
17	36	40	4	0.9	2.4	—
18	26	35	4	0.9	1.0	—
19	49	50	5	1.9	1.9	—
20	41	75	4	1.8	2.8	—

Baseline, Value on day of study; *peak*, highest level subsequently recorded during AMB therapy; +, normal saline solution (500 ml intravenously during period of 30 minutes) given before AMB infusion.

a single dose of AMB in each patient. The urine collection was obtained immediately before and during either the second, third, or fourth dose of AMB given to that patient. Eleven patients received a bolus of normal saline solution (500 ml intravenously during a period of 30 minutes) immediately before AMB administration (Table I); the other nine patients received no saline solution. The managing clinical team made the choice of whether a patient would receive saline solution.

Urine was collected through a Foley catheter into a graduated container for 60 minutes immediately before AMB administration (baseline collection). Urine then was collected for 120 minutes beginning with AMB administration (infusion collection). An aliquot was taken from the baseline and infusion collections and acid-precipitated with concentrated perchloric acid. Processing with perchloric acid does not affect recovery of adenosine.[7] Precipitated aliquots were centrifuged, and an aliquot of the supernatant was adjusted to neutral pH. The samples were recentrifuged, and the final supernatant was stored at -70° C for future batch analysis.

The frozen samples were assayed for adenosine concentration by using high-pressure liquid chromatography.[7] The amount of adenosine excreted per hour was calculated from the adenosine concentration and urine output, adjusting for the reagent volume added during sample processing.

The Wilcoxon rank sum test was used to compare data between the saline and comparator groups in a specific period (unpaired samples), the Wilcoxon signed rank test was used to compare data between the baseline and infusion periods in a specific group (paired samples), and the Spearman rank correlation test was used to determine correlation between variables.

RESULTS

Adenosine level was stable in urine for 2 hours at room temperature (data not shown). The urine output and adenosine excretion during the baseline period were not different between the saline and the comparator groups ($p = 0.15$ and 0.30, respectively). Patient age, AMB dose, AMB infusion time, and baseline serum creatinine level also were not different between groups ($p > 0.05$, Table I). None of the patients had a mean arterial pressure less than 65 mm Hg before or during the AMB infusion. One patient (number 8, Table I) subsequently had kidney failure attributed to underlying disease and to AMB therapy.

Intravenous infusion of AMB was associated with a decrease in urine output in both the comparator and saline groups (Figure). Subsequent elevation of serum creatinine level in either group did not correlate with the baseline urine output, the infusion urine output, or the decrease in urine output from baseline to infusion period (Table II). Data of the patient with the 900% increase in serum creatinine level (patient 8, Table I) are omitted from the calculations in Table II, because that

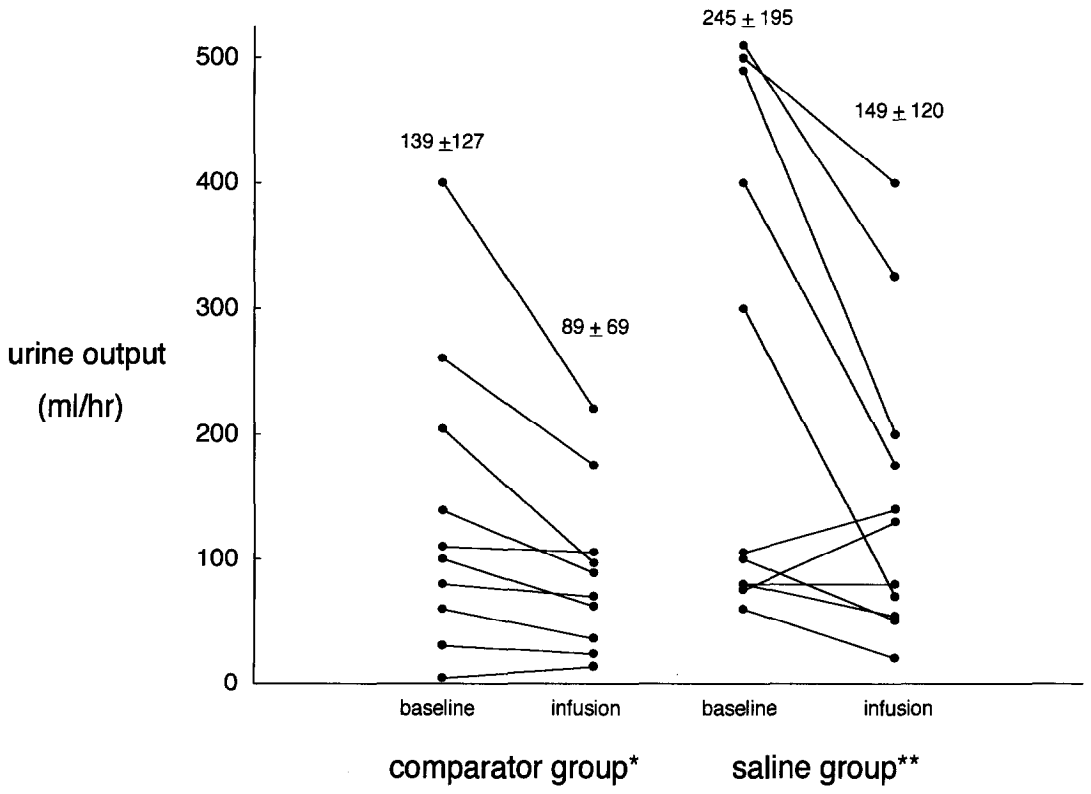

Figure. Infusion of AMB is associated with decrease in urine output in both comparator ($n = 9$) and saline groups ($n = 11$). Value above each set of data is mean ± standard deviation for that set. *$p = 0.027$; **$p = 0.04$, baseline versus infusion period, Wilcoxon signed rank test.

Table II. Spearman correlation coefficients

	Correlation coefficient	
Variable	*Comparator group*	*Saline group*
Baseline urine output	-0.0958 (0.5)	0.5242 (0.07)
Infusion urine output	-0.1125 (0.5)	0.3636 (0.14)
Change in urine output, baseline to infusion	0.3625 (0.17)	-0.3576 (0.14)
Baseline adenosine excretion	0.1375 (0.4)	0.0303 (>0.5)
Infusion adenosine excretion	0.0208 (>0.5)	-0.1454 (0.37)
Change in excretion, baseline to infusion	0.2125 (0.31)	0.4545 (0.09)

Correlation coefficients were derived from maximal creatinine (expressed as % increase in serum creatinine from value on study day to maximal subsequent value during AMB therapy) versus given variable. The *p* value of each coefficient is recorded in *parentheses*.

patient's rise in creatinine level is more than three standard deviations from the group mean.

Intravenous infusion of AMB was associated with a decrease in urinary excretion of adenosine in the comparator group (from 0.2276 mmol/hr in the baseline period to 0.1127 mmol/hr during infusion, $p = 0.01$) and did not change adenosine excretion in the saline group (from 0.1354 mmol/hr in the baseline period to 0.1255 mmol/hr during infusion, $p = 0.25$). Subsequent elevation of serum creatinine level in either group did not correlate with baseline adenosine excretion, infusion adenosine excretion, or the decrease in adenosine excretion from baseline to infusion period (Table II).

Administration of a saline bolus before AMB did not prevent development of renal insufficiency. The maximal degree of creatinine elevation observed at any time after the study was not different between the saline and comparator groups ($p = 0.15$, Table I).

DISCUSSION

We hypothesized that AMB infusion would result in oliguria and renal adenosine excretion in patients. This hypothesis was based on observations that AMB causes renal vasoconstriction and ischemia in animals[2, 3]; ischemia would be manifested by a release of adenosine by the ischemic kidneys and decreased urine output. We also hypothesized that administration of a pre-AMB saline bolus would abrogate the adenosine release and oliguria. Finally, we wanted to determine whether adenosine release or oliguria would be predictive of future renal insufficiency while on AMB therapy (an attempt to find a marker for AMB nephrotoxicity).

We found that AMB infusion in our patients resulted in decreased urine output that was not prevented by a

saline bolus. Urine output has multiple determinants; the decrease we observed may reflect a change in tubule function rather than in glomerular filtration rate. Although the mean urine output in the baseline period was greater in the saline (245 ml/hr) versus the comparator group (139 ml/hr), this difference did not reach significance ($p = 0.15$). The trend toward higher baseline urine output in the saline group may be an effect of the saline infusion.

We also found that AMB infusion resulted in a decrease in urinary adenosine excretion (opposite to our hypothesis) and that this decrease was prevented by a saline bolus. We are unsure how to interpret this last observation, but it does not support our hypothesis. Neither urine output nor adenosine excretion was predictive of subsequent renal insufficiency. In addition, a saline bolus before AMB infusion did not prevent the development of renal insufficiency.

One interpretation of our study results is that AMB infusion in human beings, within the dose and infusion parameters used, does not result in renal vasoconstriction and ischemia and that these factors are not the cause of AMB-associated nephrotoxicity. This interpretation would suggest another cause for AMB nephrotoxicity, such as autooxidation with free radical formation[8, 9] or membrane pore formation[10] with cytotoxicity in the medullary thick ascending limb.[11, 12] In the dog study[3] that described the AMB vasoconstrictive effect on the kidney, 5 mg/kg of drug was infused directly into the renal artery during a period of 30 minutes (compared with less than 1 mg/kg during a period of 30 to 360 minutes intravenously in the present study). It may be possible that at a higher dose or quicker infusion time the renal hemodynamic effect observed in the dog may be seen in human beings. With a clinically relevant dose and administration technique, however, this renal hemodynamic effect is not apparent in human beings.

It is also possible that human kidney ischemia occurs with AMB administration but that oliguria and adenosine release may be inadequate markers of the effect. It is well established that adenosine is released by ischemic tissue, including the kidney, and is detectable in tissue, blood, and urine.[4, 13, 14] We chose to assay urinary adenosine because adenosine level is unstable in blood and tissue[15] but is stable in urine. It has been shown in patients that urinary adenosine is a marker for nephrotoxicity caused by administration of hypertonic intravenous contrast, another nephrotoxic agent that is believed to cause renal vasoconstriction, ischemia, and oliguria.[16] We believe that oliguria in combination with renal adenosine release is indicative that renal ischemia has occurred. Because we did not observe these two phenomena together in our study, it is doubtful that renal ischemia was present in our patients.

We were not able to show that oliguria and urinary adenosine excretion are markers for the development of AMB nephrotoxicity in patients because, we believe, renal vasoconstriction with ischemia is not the mechanism of AMB nephrotoxicity in usual clinical circumstances. Other mechanisms, such as oxidative injury or membrane pore formation, may be responsible for AMB nephrotoxicity.

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