Transplantation of rat kidneys with acute tubular necrosis into salt-loaded and normal recipients

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CHRONIC salt loading is known to confer remarkable protection against experimental acute tubular necrosis. For example, severe myohemoglobinuric acute renal failure is produced in normal water-drinking rats by injection of 50 percent glycerol intramuscularly (10 c.c. per kilogram), but in rats that are only allowed normal saline to drink, such renal failure is not produced. This protection is not conferred by an acute saline load, blood volume replacement, or osmotic diuresis.

Micropuncture studies have shown that in the rat acute renal failure is at least in part an angioplastic phenomenon of the afferent renal arterioles resulting in cessation of glomerular filtration. Hollenberg and associates found circumstantial evidence for this in patients with xenon washout studies, showing decreased renal cortical perfusion in "acute tubular necrosis." It has been suggested that chronic salt loading (as opposed to an acute salt load) protects the kidney over a period of time by depleting it of renin. However, it has not been possible in most laboratories to protect against acute tubular necrosis by immunizing against renin or angiotensin II. Still intrarenal rather than plasma changes in the renin-angiotensin system may be operative.

We have approached this question by performing renal transplantation between salt-protected and nonprotected rats to determine if salt protects by an extrinsic effect on the host or an intrinsic effect on the kidney itself. Original studies were hampered by the fact that relatively long ischemia times for transplantation in the rat (45 minutes) potentiated the effect of glycerol injection. Although 45 minutes of ischemia alone produced negligible elevation of blood urea in salt-drinking rats, the combination consistently caused severe renal failure, and controls were thus impossible. Furthermore, though controversial, it appears that ischemic renal failure, as opposed to other causes, is partly a tubular defect and the vasospasm associated with it may indeed be ameliorated by an acute plasma volume expansion.

With renal ischemia times under 20 minutes, we have shown recently that an unprotected kidney from a water-drinking rat is fully protected against acute tubular necrosis when placed into a glycerol-injected, salt-drinking recipient.

It seemed very unlikely then that even intra-renal renin depletion was the mechanism of protection from chronic salt loading against renal failure. In fact the condition of the host rather than the kidney would have appeared to be the important factor.

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Table I. Results of transplanting kidney from glycerol-injected donor into a noninjected recipient

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Donor group</th>
<th>Recipient group</th>
<th>Time between glycerol injection and removal of kidney from donor (hr.)</th>
<th>Blood urea of recipient 24 hours later* (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H₂O</td>
<td>H₂O</td>
<td>8</td>
<td>253.8 ± 27.2</td>
</tr>
<tr>
<td>2</td>
<td>H₂O</td>
<td>H₂O</td>
<td>2.5</td>
<td>176.1 ± 21.3†</td>
</tr>
<tr>
<td>3</td>
<td>NaCl</td>
<td>NaCl</td>
<td>2.5</td>
<td>68.5 ± 9.4‡</td>
</tr>
<tr>
<td>4</td>
<td>NaCl</td>
<td>H₂O</td>
<td>2.5</td>
<td>159.2 ± 17.7</td>
</tr>
<tr>
<td>5</td>
<td>H₂O</td>
<td>NaCl</td>
<td>2.5</td>
<td>152.4 ± 16.1</td>
</tr>
</tbody>
</table>

*All values are ± S.E.
†Compared to Experiment 1, P < 0.05.
‡Compared to Experiments 2, 4, and 5, P < 0.005.

This conclusion has very practical significance for the transplant surgeon.

The purpose of the present study was to determine the relative importance of salt loading in the donor or recipient when glycerol was given to the donor to induce renal failure prior to transplantation, rather than to the recipient after renal transplantation.

METHODS

Forty adult, male, DA rats, weighing 200 to 250 grams, were divided into two groups. One group of 24 was provided normal tap water to drink ad libitum, and the other 16 were allowed only 0.9 percent (normal) NaCl solution to drink ad libitum, for a period of 2 months prior to transplantation. The first group was labelled as "water drinkers" and the second group as "salt drinkers."

Five experimental groups were then established. In all groups acute myohemoglobinuric renal failure was induced by injection of the donor rat with 50 percent glycerol intramuscularly (10 ml. per kilogram) prior to transplantation. Bilateral nephrectomy was performed in the recipient animal simultaneously with all transplants. The method of transplantation was that of Fabre, Lim, and Morris, and ischemia times averaged 20 minutes. All animals were followed after transplantation with daily blood urea determinations by means of a standard microtechnique.

Experiment 1. Four water-drinking rats were injected with glycerol and 8 hours later one of their kidneys was transplanted into a nonglycerol-injected, water-drinking recipient.

Experiment 2. Four water-drinking rats were injected with glycerol and 2½ hours later one of their kidneys was transplanted into a nonglycerol-injected, water-drinking recipient.

Experiment 3. Four salt-drinking rats were injected with glycerol and 2½ hours later one of their kidneys was transplanted into a nonglycerol-injected, salt-drinking recipient.

Experiment 4. Four salt-drinking rats were injected with glycerol and 2½ hours later one of their kidneys was transplanted into a nonglycerol-injected, water-drinking recipient.

Experiment 5. Four water-drinking rats were injected with glycerol and 2½ hours later one of their kidneys was transplanted into a nonglycerol-injected, salt-drinking recipient.

RESULTS

The results of the five experiments are summarized in Table I. Blood urea at day one was considered as the measure of acute renal impairment.

Experiment 1. When the kidney was allowed to stay in a water-drinking donor for 8 hours after injection of glycerol, before being removed and transplanted into another
Experiment 2. When the kidney was removed from a water-drinking donor 2½ hours after glycerol injection and transplanted into another water-drinking rat (which had not been injected), a moderately severe renal failure still occurred. However, it was significantly less than that which occurred if the kidney remained in the donor for 8 hours (as in Experiment 1) prior to transplantation. Thus 2½ hours were adequate for a moderately severe renal insult to occur as a result of glycerol-induced myoglobinuria.

Experiment 3. When a chronic salt-drinking rat was injected with glycerol and its kidney was transplanted 2½ hours later to another chronic salt-drinking rat, there was no significant renal failure. This is similar to what occurs when any chronic salt-drinking rat is injected with glycerol, i.e., a remarkable protection is conferred against acute tubular necrosis that cannot be achieved with an acute salt load or blood volume expansion.

Experiment 4. When glycerol was injected into a chronic salt-drinking donor, protected against acute tubular necrosis as in Experiment 3, and its kidney was transplanted 2½ hours later into a water-drinking recipient, acute renal failure occurred. Despite the fact that the donor kidney came from a “protected” animal, the transference of this kidney to an “unprotected” host resulted in loss of salt protection. The elevation of urea at one day was not significantly different from Experiment 2 controls but was quite different from those in Experiment 5, in which the recipient was also salt “protected.”

Experiment 5. When the donor was a water-drinking rat and the recipient a salt drinker (vice versa from Experiment 4), a similar degree of renal failure occurred. A kidney from an “unprotected” water-drinking donor, injected with glycerol 2½ hours earlier, thus developed renal failure even though placed into a “salt-protected” recipient.

DISCUSSION

We have noted frequently the odd experience of transplanting each kidney from the same cadaveric donor into two separate recipients, with one functioning immediately and the other exhibiting “acute tubular necrosis.” Often the recipient in whom the kidney functioned immediately was either poorly dialyzed, relatively fluid overloaded, and/or uncooperative about restricting his salt intake. The recent experimental evidence that acute tubular necrosis is a renal vasospastic phenomenon that can be modified by chronic salt balance may help to explain this clinical curiosity.

An attractive explanation for the protection afforded the kidney by chronic salt loading has been that such a program depletes the kidney of renin. It has been thought that intrarenal renin release is responsible for the afferent arteriolar constriction that causes “acute tubular necrosis.” However, in our previous experiments, when a salt-protected, renin-depleted kidney was transplanted into a water-drinking recipient that was then given glycerol, acute myoglobinuric renal failure still occurred. When a nonrenin-depleted kidney from a water-drinking rat was transplanted into a salt-protected recipient that was then given glycerol, no functional renal impairment was produced. Therefore it seemed that the condition of the recipient was the determining factor, and renin depletion of the kidney was not the mechanism of protection.

In the present experiments, myoglobinuric renal failure was induced in the donor first to see if transplantation of the
kidney to a different recipient could improve or worsen its function by radically changing its environment.

In water-drinking animals, waiting only 2½ hours after glycerol injection before transplanting the donor kidney into a non-glycerol-injected recipient results in a much milder degree of renal failure than that which occurs when a full 8 hours pass before the kidney is transplanted (Experiments 1 and 2). Thus prolonged delay in removal of a donor kidney from the deteriorated environment of a hypotensive host may result in a severe post-transplant renal failure that could be averted by earlier harvesting of the kidney.

Glycerol injection causes only the mildest renal failure in chronically salt-loaded rats and, as one might expect, transplantation of a kidney from a glycerol-injected, salt-loaded donor into a salt-loaded recipient results in no significant renal failure when the ischemia time from the transplant itself is sufficiently low (Experiment 3). Experiments 4 and 5 show that the sodium balance of the donor and recipient is equally important when the renal insult originally occurs in the donor. Salt protection of the donor kidney is not enough when the insulted kidney is transferred to an unprotected recipient. On the other hand, if the donor is not salt protected when myohemoglobinuria is induced, transferring it to a salt-protected recipient will not reverse the renal failure already induced.

The clinical implications are that the chronic sodium balance of both the donor and the recipient are important in preventing acute tubular necrosis in renal transplantation. Last minute maneuvers, such as acute salt loading and diuretics, have been disappointing.

The theoretical implications are that (1) renin depletion of the kidney is at best not the sole mechanism of protection against acute renal failure in the salt-loaded host, and (2) once acute renal failure is established in an unprotected, nonsalt-loaded host, it cannot be reversed by transferring it to a protected, chronically salt-loaded environment. This would mean that the angiospasm which seems to account for acute renal failure either cannot be broken easily once it is induced, or that it causes a further damage which then can only recover with time even after the angiospasm is relieved. To work out these latter possibilities will require detailed micropuncture studies on this transplant model.

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REFERENCES
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