Metabolic Fate of $^{14}$C-Acetate During Dialysis

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Summary

Acetate-$1-^{14}$C was infused into uraemic dogs during a 4-hour haemodialysis. Acetate levels in the arterial and venous lines achieved a steady state by the end of dialysis suggesting that the maximum rate of acetate metabolism had not been exceeded. Acetate disappearance after dialysis followed first order kinetics; the mean blood half-life of acetate was 3.3 minutes. Most (83.7%) of the infused acetate-$1-^{14}$C was metabolised to $^{14}$CO$_2$ within 8 hours after dialysis. Small, but significant amounts of acetate-$1-^{14}$C were incorporated into lipids of plasma, liver and aorta. Serum lipid concentrations were not significantly altered during or after dialysis.

Introduction

Acetate is the metabolisable anion most frequently used in peritoneal and haemodialysis solutions. Acetate is rapidly metabolised via the citric acid cycle to generate bicarbonate according to the following equation:

$$H^+ + CH_3COO^- + 2O_2 \rightarrow 2H_2O + 2CO_2$$

The consumption of a hydrogen ion during the metabolism of acetate is equivalent to generating OH$^-$ or HCO$_3^-$, resulting in the correction of uraemic acidosis. However, the effect of uraemia on the metabolism of acetate is unknown and it has not been determined whether acetate is rapidly metabolised to CO$_2$ in all uraemic patients, or whether significant quantities of acetate enter other metabolic pathways, e.g. lipid biosynthesis. The latter pathway is of considerable interest since haemodialysis patients have a high incidence of hyperlipidaemia.
(Bagdade et al, 1968), which perhaps contributes to the increased morbidity and mortality from cardiovascular disease seen in this group of patients (Burton et al, 1971; Lindner et al, 1974).

This study was undertaken to determine (1) the rate of removal of acetate from the circulation following haemodialysis; (2) the metabolic fate of acetate during and after dialysis, and (3) the extent of incorporation of acetate into plasma and tissue lipids.

METHODS

Six male dogs weighing from 19 to 35 kg were subjected to bilateral nephrectomy under sodium thiopental anaesthesia. Sixty hours later the dogs were re-anaesthetised, intubated and attached to a respirator cycling at 7 l/min. The mean serum creatinine was 11.9 mg/dl at this time. An arteriovenous shunt was placed between the femoral artery and vein and haemodialysis was carried out for 4 hours using a hollow fibre artificial kidney (Cordis Dow Model 4, 1.5 m² surface area). Dialysis solution flow rate was 500 ml/min and the blood flow rate was 4 ml/min/kg but never less than 100 ml/min. Acetate concentration in the dialysis solution was 39.5 mM. The dogs were heparinised with an intravenous bolus of 3000 units of heparin followed by a constant infusion of 1000 units per hour. Acetate-1-14C was infused into the venous line at a rate of 50 μCi/kg/4 hours. Specimens of blood and dialysate were obtained before the initiation of dialysis and heparinisation. Blood was obtained from the arterial and venous lines during dialysis and arterial blood was obtained from the femoral shunt following dialysis. During dialysis, specimens of expended dialysate were collected in NaOH in order to trap dissolved 14CO2 as NaH14CO3. Aliquots were then acidified and the released 14CO2 measured by an ionisation chamber method (Davidson et al, 1969). This radioactive volatile component of the dialysate represents acetate-1-14C converted to 14CO2 and H14CO3− which have been removed by haemodialysis. The non-volatile component of radioactivity remaining in the dialysate was then measured by scintillation counting. This non-volatile component was assumed to be primarily unmetabolised acetate-1-14C, but other products of acetate metabolism, such as pyruvate, may also have been present.

The concentration of 14CO2 in expired air during and after dialysis was measured continuously by means of a vibrating reed electrometer-ionisation chamber method (Davidson et al, 1969). The quantitative collection of expired air was accomplished by placing the head of the dog in a Plexiglass helmet with an apron of plastic sheeting. A vacuum pump connected to the helmet removed air from the helmet at a rate of 12 l/min, i.e. greater than the rate of delivery of expired air (7 l/min). Under these conditions ambient room air was continuously sucked into the helmet beneath the plastic apron, thereby preventing any loss of 14CO2. The area under the 14CO2 concentration curve was determined by
planimetry and was directly related to the amount of $^{14}$CO$_2$ eliminated by respiration during the 12-hour study. Acetate in whole blood and dialysate was measured by gas liquid chromatography.

Phospholipid, triglyceride and cholesterol analyses were done colorimetrically, free fatty acids by gas liquid chromatography and radioactivity in each of these fractions determined by scintillation counting after separation by thin layer chromatography (Morin, 1967).

RESULTS

The acetate concentration in whole blood obtained from the venous and arterial lines during dialysis are shown in Figure 1. The arterial and venous acetate reached a constant level by the end of dialysis. In the 6 uraemic dogs studied,

![Figure 1. Acetate concentration in the venous and arterial dialysis lines during a 4-hour haemodialysis in an uraemic dog](image)

the mean (± SE) acetate concentration at the end of dialysis was 15.8 ± 1.4 mM in the arterial line and 2.6 ± 0.4 mM in the venous line. The achievement of a constant arterial acetate level suggests that the delivery of non-radioactive acetate during dialysis did not exceed the maximum capacity to metabolise acetate. Acetate delivery in the last hour of dialysis averaged 86 millimoles per hour or 3.2 millimoles/hour/kg. At the end of dialysis arterial blood was drawn at frequent intervals (1, 2, 3, 5, 10, 15, 20 and 30 minutes) for the determination of acetate half-life. Acetate disappeared rapidly from arterial blood following
dialysis (Figure 1). Regression analysis of the acetate decay curve confirmed that degradation followed first order kinetics. The mean acetate half-life was 3.3 minutes (range: 2.7 to 4.2 minutes).

The continuous measurement of the oxidative metabolism of acetate-1-$^{14}$C to $^{14}$CO$_2$ during a 4-hour dialysis and for 8 hours following dialysis is shown in Figure 2. The appearance of $^{14}$CO$_2$ in expired air was observed within 3 minutes of the start of dialysis and acetate-1-$^{14}$C infusion. The $^{14}$CO$_2$ concentration rose rapidly during the first hour and then increased slowly until the end of dialysis. The level of radioactivity ($^{14}$CO$_2$) in expired air at the end of dialysis was approximately 1000 times background radiation. Following dialysis, $^{14}$CO$_2$ concentration fell at a rapid rate but was still well above background at 12 hours when the dogs were killed. The area under the $^{14}$CO$_2$ curve is proportional to the amount of acetate-1-$^{14}$C metabolised to $^{14}$CO$_2$ and expired during the 12-hour study.

The recovery of radioactivity in expired air and dialysate in the uraemic dogs is summarised in Figure 3. An average of 70.6 ± 1.7% of the infused acetate-1-$^{14}$C was recovered as $^{14}$CO$_2$ in expired air. An additional 13.1 ± 1.2% of the radioactivity was found in the volatile component of the dialysate; this com-
ponent consists of $^{14}$CO$_2$ and H$^{14}$CO$_3^-$ dialysed from the dog and represents acetate-1-$^{14}$C which had undergone oxidative metabolism. Thus, a total of 83.7% of the infused acetate-1-$^{14}$C has been metabolised via the citric acid cycle to $^{14}$CO$_2$. An additional $5.2 \pm 0.6$% of the infused radioactivity was found in the dialysate. This was the non-volatile component and consisted of unmetabolised acetate or other non-volatile products of acetate metabolism. Therefore, a total of 88.7% of the infused radioactivity was recovered in expired air and expended dialysate. The remaining 11.9% of unrecovered radioactivity remains in the animals as lipids, HCO$_3^-$, CO$_2$ and other metabolic products of acetate metabolism.

Serial analysis of plasma lipids before, during and after dialysis showed no statistically significant changes in the concentrations of free fatty acids, phospholipids, triglycerides, cholesterol and cholesterol esters.

Figure 4 shows the results of the measurements of acetate-1-$^{14}$C incorporation into plasma lipids during the course of the experiment. The height of each bar represents the incorporation of radioactivity into total lipids. Total plasma lipid radioactivity increased progressively during dialysis and remained elevated without further significant increase during the post-dialysis period. Incorporation of radioactivity into phospholipids, triglycerides and cholesterol esters increased significantly during dialysis and continued to increase during the post-dialysis period. Incorporation of radioactivity into free fatty acids and cholesterol increased during dialysis, but decreased following dialysis. Similar patterns of incorporation of radioactivity were observed in lipids of liver and aorta at 12 hours. Total incorporation of $^{14}$C into plasma and liver
lipids at 12 hours accounted for less than 1% of the infused radioactivity. Lipids in other organs may account for a portion of the remainder of the unrecovered radioactivity.

DISCUSSION

Mudge et al (1949) showed that sodium acetate could be used as an alkalinising agent in animals and man. Lundquist (1962) further studied the metabolism of acetate in man and calculated that the maximal rate of acetate metabolism is approximately 300 millimoles per hour. Mion et al (1964) described the use of sodium acetate as a substitute for sodium bicarbonate in dialysis solutions. Because of convenience, low cost and stability, acetate has become widely accepted and used as a substitute for bicarbonate in haemodialysis solutions. Until quite recently acetate has been assumed to be an ideal metabolisable anion for use in dialysis solutions. However, recent studies have suggested that acetate may not be rapidly metabolised to CO₂ by all uraemic patients (Novello et al, 1974). Additionally, recent studies by Gonzales et al (1974) have shown that the acetate load presented to a haemodialysis patient may approach 300 millimoles per hour (about 4–5 millimoles per kg per hour). Since the amount of acetate presented to patients may exceed their ability to metabolise it, it is quite possible that acetate accumulates in the circulation or enters other metabolic pathways. For
example, acetate is a precursor for many lipids and may enter into pathways leading to the synthesis of fatty acids, cholesterol and triglycerides.

Our studies in uraemic dogs have indicated that acetate delivered at the rate of 3.2 millimoles per kg per hour or 86 millimoles per hour was readily metabolised to CO₂. At the end of dialysis, arterial acetate concentration returned to baseline levels within 15 minutes. Most (83.7%) of the delivered acetate-1⁴C was metabolised to ¹⁴CO₂ within 8 hours of the end of dialysis. Of the 11.9% of the radioactivity not recovered in expired air or dialysate, a small amount was shown to be incorporated into lipids of plasma, liver and aorta. Serum lipid levels, however, did not change during dialysis. Therefore, it is certainly possible that the acetate-1⁴C being incorporated into tissue lipids is simply representative of a normal metabolic turnover and does not necessarily imply an increase in production or deposition in these tissues. It remains conceivable, however, that frequent small increases in lipid production and deposition induced by large acetate loads may, over time, contribute to the hyperlipidaemia and accelerated atherosclerosis seen in haemodialysis patients.

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