



Clinical and analytical changes in conventional hemodialysis without acetate

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SUMMARY

Background: the purpose of this study was to evaluate blood acetate levels and its correlation with clinical and analytical changes in hemodialysis patients treated with standard bicarbonate dialysate compared to treatment with acetate-free bicarbonate dialysate. **Methods:** Fourteen patients on hemodialysis (11 male) with mean age of 61 ± 15 years were treated with conventional bicarbonate dialysate for 1 month and then switched to acetate-free bicarbonate dialysate for another month. Blood samples were drawn at the third session of first and fourth week of each type of dialysis. **Results:** Pre-dialysis blood acetate levels were similar in both groups, whereas post-dialysis blood acetate levels were higher in patients treated with conventional bicarbonate dialysate (0.48 ± 0.64 vs 0.18 ± 0.23 mmol/L, $p = 0.024$). Moreover, both periods had similar percentage of patients with pre-dialysis blood acetate levels in the pathologic range, whereas this percentage was higher in post-dialysis samples from patients treated with conventional bicarbonate dialysate respect to acetate-free dialysate (67% vs 21%, $p = 0.001$). Serum levels of interleukin-6 were statistically higher in the period with conventional bicarbonate dialysate (31.7 ± 24.7 vs 18.7 ± 10.3 pg/ml, $p = 0.014$), even though other inflammatory markers such as LBP, TNF- α and CRP failed to increase in the same period. We didn't found significant differences in the other parameters studied except for the changes in serum concentrations of sodium, chloride and bicarbonate. **Conclusions:** Acetate-free bicarbonate dialysate does not expose patients to a big amount of acetate and allows that the majority of patients finished hemodialysis with blood acetate levels in the physiologic ranges. Acetate-free dialysate was safe and well tolerated by our hemodialysis patients, although clinical advantages derived from its use should be evaluated in long-term prospective studies.

Key words: **Hemodialysis. Dialysate. Acetate. Inflammation. Hypotension.**

CAMBIOS CLÍNICOS Y ANALÍTICOS AL SUSTITUIR EL LÍQUIDO DE DIÁLISIS CONVENCIONAL POR UNO SIN ACETATO

RESUMEN

Introducción: El objetivo de dicho estudio fue evaluar los cambios en la acetatemia durante la hemodiálisis (HD) en pacientes con líquido de diálisis (LD) convencional con acetato y en pacientes con LD con clorhídrico (HCl) y analizar sus efectos sobre la clínica y sobre distintos parámetros analíticos. **Material y métodos:** 14 pacientes en programa de HD estable (11 hombres) de 61 ± 15 años de edad fue-

ron dializados durante 1 mes con el LD convencional con acetato y durante el segundo mes con el LD con HCl (sin acetato). Se obtuvieron análisis pre y post-diálisis la tercera sesión de las semanas 1 y 4 en cada uno de los períodos (con y sin acetato). **Resultados:** Las medias de los acetatos pre-diálisis fueron similares en ambos grupos, mientras que las medias de los acetatos post-diálisis fueron significativamente superiores en el grupo tratado con el LD convencional ($0,48 \pm 0,64$ vs $0,18 \pm 0,23$ mmol/L, $p = 0,024$). Tampoco hallamos diferencias significativas entre los 2 grupos en cuanto a la presencia de valores de acetato pre-diálisis patológicos, mientras sí hallamos un mayor porcentaje valores patológicos de acetato post-diálisis en el grupo tratado con LD convencional respecto al grupo del HCl (67% vs 21%, $p = 0,001$). Los niveles plasmáticos de IL-6 fueron significativamente superiores en el período de diálisis con acetato ($31,7 \pm 24,7$ vs $18,7 \pm 10,3$ pg/ml, $p = 0,014$), aunque no se acompañaron de un aumento de otros marcadores inflamatorios como la LBP, el TNF- α o la PCR dializante el mismo período. No hallamos diferencias estadísticamente significativas en los otros parámetros evaluados excepto en la variación de las concentraciones de sodio, cloro y bicarbonato. **Conclusiones:** El LD sin acetato no expone a los pacientes a concentraciones elevadas de acetato consiguiendo que la mayoría de pacientes (79%) termine la HD con una acetatemia dentro del rango fisiológico. El uso de LD sin acetato es seguro y bien tolerado por los pacientes en hemodiálisis, aunque su traducción clínica debe ser evaluada en estudios prospectivos a más largo plazo.

Palabras clave: **Hemodiálisis. Dializado. Acetato. Inflamación. Hipotensión.**

INTRODUCTION

The new advances in HD aim at improving the biocompatibility and safety, and achieving better tolerability of dialysis sessions.

In this sense, it has been shown that the presence of acetate in the dialysis fluid (DF) at concentrations of 35-45 mmol/L induces several intra-dialysis events such as nausea, vomiting, cramps, and hypotensive episodes, as well as dizziness after the dialysis session.^{1,2} This led to the development of bicarbonate-based DF in the 1970s, containing just 3-4 mmol/L of acetate. The presence of acetic acid is required to maintain the pH of the HD fluid within 7.1-7.6 to prevent precipitates of calcium and magnesium.³ However, during conventional HD with bicarbonate, when passing through the dialyzer, the patient's blood is exposed to an acetate concentration 30-40 fold higher than the normal concentration of 0.1 mmol/L.

The changes induced by hyperacetatemia are well known. For instance, in young healthy volunteers it has been observed that the increase of acetatemia in 1 mmol/L induces tachycardia and decreases diastolic blood pressure.⁴ Since the enzyme responsible of acetate, acetyl CoA synthetase has a Km of 0.7 mmol/L, acetatemia increase in 0.1-0.7 mmol/L will increase 7 fold the rate of acetate metabolism.⁵

Conversion of sodium acetate to sodium bicarbonate, after administering sodium acetate to experimental animals, produces similar adverse effects than the metabolic changes seen in hemodialysis patients, such as intolerance to carbohydrates, hypertriglyceridemia, and hyperphosphatemia.⁶

In spite of the better tolerance obtained with bicarbonate-based HD, recent studies show that the presence of 3 a 4 mmol/L of residual acetate may lead to the generation of inflammatory mediators such as interleukins, potentially responsible of long-term complications.^{7,8}

The aim of this study was to assess the effect of changing conventional DF with acetate to another one with HCl (acetate-free) on acetatemia and its correlation with the clinical and analytical changes.

MATERIAL AND METHODS

Patients

We studied 14 adult patients with end-stage chronic renal failure (CRF) on stable dialysis program. All patients signed an informed consent and the study was approved by the Hospital's Ethical Committee.

The patients were 11 males and 3 females, aged 61 \pm 15 years (range: 32-81) and average time on HD the-

rapy of 59 ± 92 months (range: 3-341). The causes of CRF were: vascular ($n = 4$), diabetes ($n = 2$), interstitial ($n = 3$), polycystic renal disease ($n = 1$), and unknown ($n = 4$). About associated morbidity, 3 were diabetic, and 4 had a positive serology for the hepatitis C virus.

Excluded from the study were all those patients having received blood transfusion within the previous 3 months and/or had received corticosteroids, anti-inflammatory drugs, or immunosuppressants. We also excluded those cases with neoplasms. Besides, we did not detect any new infection or inflammation during the 2 months that the study lasted.

Dialysis technique and study design

Usually all patients got dialysis 3 times a week, in 3-4 h sessions, with conventional concentrate of bicarbonate with acetate (BHD3 A4, Fresenius Medical Care, Bad Homburg, Germany or Dialisan –Bicart Ca 30, Hospal, Lyon, France). All HD devices were equipped with filters against endotoxins (Diasafe, Fresenius, Bad Homburg, Germany) (Hospal, Lyon, France) (Bellco, Mirandola, Italy). All patients had diuresis lower than 150 mL/24 h, and their mean dry weight was 62.2 ± 13 kg (41-91). The mean Kt/V (Daugidas 93) was 1.37 ± 0.38 , mean Qb 345 ± 40 mL/min, the Qd was the same for all at 500 mL/min, and the mean weight gain inter-dialysis was 1.9 ± 0.85 kg. Mean sodium conductivity was 13.9 ± 0.1 ms/cm and mean bicarbonate conductivity was 3.1 ± 0.1 ms/cm, both of them being kept constant throughout the study. Seven patients used synthetic membranes (polysulphone) for dialysis, and the remaining 7 used cellulose-based membranes (cellulose diacetate and triacetate).

Among prescribed medications there were phosphate chelators, vitamin D, folic acid, vitamins B and C, iron supplements, and erythropoiesis-stimulating factors.

The patients were followed-up for one month, being treated with conventional DF with acetate, and then all were switched to acetate-free DF (611 acetate-free, Bellco, Mirandola, Italy).

Acetate-free DF was used in three types of HD machines: Formula (Bellco, Mirandola, Italy), Fresenius 4008 (Fresenius Medical Care, Bad Homburg, Germany), and Integra (Hospal, Lyon, France).

HD tolerability was assessed as the number of sessions with some episode of hypotension, headache, or cramps (assessing all sessions performed each month of the follow-up period).

The hypotension episodes were defined as systolic blood pressure < 95 mmHg, associated to clinical symptoms requiring the intervention of the health care personnel.

Blood analyses were extracted pre- and post-dialysis at the third weekly session of the first and fourth weeks of each period.

Blood analysis performed pre- and post-HD included the determination of BUN, creatinine, sodium, potassium, chloride, bicarbonate, acetate, calcium, phosphate, glucose, uric acid, cholesterol, triglycerides, albumin, and full blood count. We also analyzed pre-albumin, ferritin, transferrin saturation index, parathyroid hormone, b2-microglobulin, C reactive protein (CRP), interleukin-6 (IL-6), tumoral necrosis factor alpha (TNF-alpha), and pre-dialysis liposaccharides binding protein (LBP), and post-dialysis complement activity.

The serum for IL-6, TNF- α , and LBP determination was frozen at -20° C.

Serum LBP was determined using the technique of specific immunoassay (Immulite $\text{\textcircled{O}}$) with a detection limit of 1 mg/mL and a variation coefficient of 5.2%. The normality values obtained in 20 healthy volunteers were 3-12 mg/mL.

Serum IL-6 was determined using an ELISA technique (Human IL6 Elisa, IBL, Hamburg, Germany) with a detection limit of 1 pg/mL, range de 1-400 pg/mL. The variation coefficient was lower than 6.2%. The normality value sin 20 healthy controls were lower than 5 pg/mL.

Serum TNF- α was determined using the technique of specific immunoassay (Immulite $\text{\textcircled{O}}$ EURO/DPC) with a detection limit of 1.7 pg/mL, range 1.7-1000 pg/mL. The normality value sin healthy controls were lower than 8.1 pg/mL.

The CRP was analyzed by nephelometry (ultrasensitive CRP, Behring Diagnostics, GMBH, Rarburg, Germany) with a detection limit of 0.1 mg/mL and reference range of 0.1-0.4 mg/L. The variation coefficient was lower than 4%.

Serum acetate was determined using an ultraviolet radiation method: the synthetic enzymatic reaction of acetate yields NADH+ that is measured as an increase of absorbance. The normality values in a healthy population were lower than 0,1 mmol/L with a detection limit of 0.01 mmol/L.

Preparation of acetate-free DF

All the electrolytes but bicarbonate are included in a concentrate when the acetic acid is replaced by HCl (Bellco-Soludia, Fourquevaux, France). The pH of the concentrate with HCL is < 1 (table I).

Statistical analysis

The patients were their own controls.

The data are expressed as mean \pm standard deviation.

Table I. Calculated composition of electrolytes of acetate-containing DF and acetate-free DF

	Conventional DF with acetate	Acetate-free DF (with HC1)
Sodio (mmol/L)	140	140
Potassium (mmol/L)	1.5	1.5
Calcium (mmol/L)	1.5	1.5
Magnesium (mmol/L)	0.5	0.5
Chloride (mmol/L)	106.5	111.5
Bicarbonate (mmol/L)	35	35
Acetate (mmol/L)	4	0

All the variables followed a normal distribution but CRP and IL-6.

The Kolmogorov-Smirnov analysis was carried out to assess the normal distribution of the variables. CRP and IL-6 followed an exponential curve and required logarithmic transformation.

For parametric variables, the paired t test was used for two measurements, and the ANOVA test for more than two measurements.

For non-parametric variables, the Wilcoxon's test was used for two measurements, and the Dunett's test for more than two measurements.

All the data were analyzed by the SPSS software, version 10. Statistical significance was defined as $P < 0.05$.

RESULTS

The evaluation period took 2 months during which the 14 patients remained clinically stable, and all

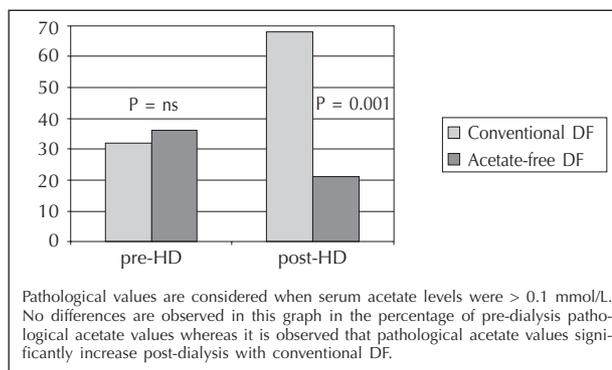


Fig. 1. — Percentage of pathological values of serum acetate pre- and post-HD during the period with acetate and without acetate.

completed the follow-up period. About HD tolerability, a lower incidence of hypotensive episodes was observed during the second period, with the acetate-free dialysis fluid, although it did not reach statistical significance (10.2% of hypotensions with the acetate-free dialyzate versus 13.3% of hypotensions with the conventional dialyzate; $P = 0.22$).

There were no significant differences in most of the analytical parameters studied, with the exception of pre- and post-dialysis sodium and chloride values, and pre-dialysis bicarbonate values. Dry weights and inter-dialysis weight gains remained stable during the two months of the study (table II).

Serum pre-dialysis acetate levels were similar in both groups, whereas post-dialysis acetate serum levels were statistically higher in patients treated with conventional DF (0.48 ± 0.64 vs. 0.18 ± 0.23 mmol/L, $p = 0.024$) (table III). Besides, both groups

Table II. Follow-up of the different biochemical parameters pre- and post-dialysis

Week	HD with acetate		HD without acetate	
	First	Fourth	Fifth	Eighth
Dry weight (Kg)	62.2 ± 13.1	62.2 ± 13	62 ± 13.2	61.9 ± 13.1
Sodium pre-HD (mEq/L)	136.9 ± 3.8	137.2 ± 2.5	137.6 ± 4.2	139.8 ± 4.1 ^{a,b}
Sodium post-HD (mEq/L)	138 ± 2.3	138.6 ± 1.6	136.2 ± 3.6	137.5 ± 1.7
Chloride pre-HD (mEq/L)	99.4 ± 4.6	99.4 ± 3.6	99.9 ± 5	102.3 ± 5.5 ^{a,b}
Chloride post-HD (mEq/L)	99.7 ± 1.9	99.9 ± 1.7	97.5 ± 4.6	98.1 ± 2.6 ^{a,b}
Bicarbonate pre-HD (mEq/L)	20.2 ± 2.9	19.6 ± 3.6	18.8 ± 3.9 ^a	17.5 ± 3.8
Bicarbonate post-HD (mEq/L)	24.7 ± 3.5	24.8 ± 2.2	24.2 ± 2	24.8 ± 1.9
Calcium pre-HD (mg/dL)	8.78 ± 1.04	9.29 ± 0.70	8.89 ± 0.96	9.07 ± 0.62
Phosphate pre-HD (mg/dL)	4.65 ± 1.38	5 ± 1.60	4.52 ± 1.20	5.17 ± 1.74
Hemoglobin pre-HD (mg/dL)	13.55 ± 1.97	12.17 ± 1.23	12.29 ± 2.15	12.27 ± 1.62

^a $p < 0.05$ compared with the baseline value (first week).

^b $p < 0.05$ compared with the value at the fourth week.

presented a percentage of patients with pre-dialysis acetate levels within the physiological range, whereas the percentage of patients with a pathological post-dialysis acetate level was clearly higher in those patients treated with acetate-containing DF (67% vs 21%, $p = 0.014$) (fig. 1).

Besides, it was observed that patients dialyzed with acetate presented IL-6 serum levels of higher than patients dialyzed without acetate [21.6 ($15.23-42.75$) vs 15.75 ($12.83-20.88$) pg/mL, $p = 0.014$], whereas plasma levels of TNF- α and LBP were lower during the period with conventional Acetate-containing DF (TNF- α 8.8 ± 2.9 vs 10.7 ± 3.3 pg/mL, $p = 0.024$, LBP 11.5 ± 5.6 vs 14.9 ± 5.9 mg/mL, $p = 0.030$) (table IV).

Patients dialyzed with cellulose membranes had post-dialysis serum levels of complement (C3, C4, and properdin) lower than those of patients dialyzed with synthetic membranes, suggesting their activation with the former, without observing any change by type of dialysis fluid used (table V).

DISCUSSION

In our study, replacement of acetic acid by HCl had more effects at the analytical level than at the clinical one.

In the first place, we observed an increase of serum pre-dialysis levels of sodium and chloride and a decrease in serum pre-dialysis levels of bicarbonate after one month on treatment with HCl in spite of the fact that in each period the conductivity obtained with each of dialyzate (with and without acetate) for sodium and bicarbonate was similar. This finding using acetate-free dialyzate has not been previously described. It is important to keep in mind that throughout the study the conductivities for sodium and bicarbonate were kept stable. Besides, the change in these electrolytes was not accompanied by changes in dry weight or higher inter-dialysis weight gain. Unfortunately, we did not measure the electrolyte levels in the dialyzate during the study. The decrease in pre-dialysis bicarbonate levels is in agreement with the absence of acetate in the DF and could be easily corrected by increasing the bicarbonate conductivity of the machine. The different composition of both dialyzates (table I), with components with different ionic force, may explain the differences in sodium, chloride, and bicarbonate levels in spite of using the same conductivity. However, we do not rule out the possibility that this may be due to an error inherent to the variation coefficient of analytical determinations since the sample size is relatively small.

On the other hand, this study shows that the presence of 4 mmol of acetate in the conventional dialy-

Table III. Changes in acetate serum levels during the period of acetate-containing DF and during the period of acetate-free DF

	Acetate-containing DF	Acetate-free DF	p
Acetateemia pr-HD (mmol/L)	0.19 ± 0.21	0.17 ± 0.21	NS
Acetateemia post-HD (mmol/L)	0.48 ± 0.64	0.18 ± 0.23	0.024

The values shown in this table are the result of adding the values obtained pre- and post-dialysis at the first and fourth weeks of each period.

zate can increase acetateemia by 2-3 fold over normal values after receiving HD for 3-4 hours. This effect was limited by exposure time since the patients recovered their initial acetate level by the beginning of the next HD session. Some patients did not have increased acetateemia with conventional dialyzate, probably because they were fast acetyl metabolizers. Our results are in agreement with those obtained by Fournier *et al.* that observed an important increase in acetateemia when using a conventional dialyzate.⁹ Besides, they showed that they could control chronic hyperacetateemia, which is common in HD patients, by using an acetate-free dialyzate.⁹ In this sense, there are studies comparing conventional HD with AFB (acetate free biofiltration) that have found similar results.^{10,11} Recently, Pizzarelli *et al.* have described that the acetate body gain is particularly high in convective therapies and that plasma acetate levels return to baseline values after two hours of having ended the treatment.¹² In our work, we did not find differences by dialysis shift (the patients coming at 7 a.m. were fasting, and those doing so at 7 p.m. were on a post-prandial state).

We have not either found a significant decrease in acute complications from HD, although the number of hypotension episodes, headache, or cramps was lower during the acetate-free period. Recently, Ver-

Table IV. Evolution of inflammatory parameters

	Acetate-containing DF	Acetate-free DF	p
Albumin (g/dl)	3.19 ± 0.47	3.07 ± 0.30	0.248
Ferritin (ng/ml)	287.79 ± 218.75	277.62 ± 170.39	0.890
CRP (mg/L)	0.69 (0.40-1.81)	0.70 (0.20-1.20)	0.565
IL-6 (pg/mL)	21.6 (15.23-42.75)	15.75 (12.83-20.88)	0.007
TNF- α (pg/mL)	8.81 ± 2.67	10.76 ± 3.21	0.005
LBP (μ g/mL)	11.51 ± 3.67	14.98 ± 5.44	0.031

The values shown in this table are the result of adding the values obtained pre- and post-dialysis at the first and fourth weeks of each period. IL-6 and CRP are expressed as the median value (percentiles 25-75). Albumin, ferritin, TNF- α and LBP are expressed as mean \pm standard deviation.

Table V. Post-dialysis complement values using synthetic or cellulosic membranes

	C3 (mg/dL)	C4 (mg/dL)	Properdin (mg/dL)
Acetate-containing DF + cellulosic MB	92.015 ± 17.66	26.03 ± 8.89	28.57 ± 7.25
Acetate-containing DF + synthetic MB	108.61 ± 37.36	34.81 ± 12.82	34.30 ± 9.96
Acetate-free DF + cellulosic MB	84.61 ± 24.42	23.79 ± 8.67	25.86 ± 6.26
Acetate-free DF + synthetic MB	106.62 ± 20.99	35.33 ± 10.65	33.13 ± 4.40
P	0.081	0.012	0.020

The values shown in this table are the result of adding the values obtained pre- and post- dialysis at the first and fourth weeks of each period.

zetti *et al.* observed that AFB was able to decrease intra- and extra-dialysis symptoms in patients with diabetic nephropathy.¹³

In hemodialysis patients, inflammatory markers predict hospitalization and mortality.¹⁴⁻¹⁶ In our study, we observed a decrease in IL-6 levels after one month of acetate-free therapy. This suggests that in spite of the fact that hemodialysis-induced hyperacetatemia is limited to the session duration in most of the patients, repeated exposure to high acetate levels may induce cytokines release. This finding is in agreement with an *in vitro* study showing an increase in interleukin-1 production when incubating human monocytes with sodium acetate.⁷ Moreover, another study showed that IL-6 levels were lower at the end of acetate-free on-line hemodiafiltration (HDF) as compared to on-line HDF with conventional dialyzate.¹⁷ Similarly, AFB does not induce the production of IL-1Ra, by contrast to what occurs with bicarbonate HD or with HDF with conventional dialyzate.¹⁸ Another study done on HD patients could not detect interleukin activation (analyzed by standard ELISA), although it observed a slight increase in TGF- α levels after 4 hours of conventional HD as compared to acetate-free HD, with persistently elevated levels in the acetate group.¹⁹

In our study, both LBP and TNF- α slightly increased after one month of therapy with acetate-free DF, although these two parameters were not corrected with volume changes (concentration/dilution) generated with body weight gain/loss. This finding disagrees with that obtained by Amore *et al.*, who showed that endothelial cells increased their TNF- α synthesis in the presence of acetate.²⁰ Other inflammatory parameters assessed in our study, such as the CRP, ferritin or albumin were not modified with the dialyzate change.

As expected, patients dialyzed with cellulose membranes showed a decrease in complement as compared to patients dialyzed with synthetic membranes. We could not show, however, that this effect would be higher in the presence of acetate. Besides, the inflammatory parameters were not affected by the type of membrane.

One of the limitations of our study was the small sample size and the short follow-up period. In order to confirm these data, larger studies with longer follow-up period would be required.

To conclude, our study shows that a higher percentage of patients (67%) end up dialysis with serum acetate levels within the physiologic range when using acetate-free DF. These analytical findings were not accompanied by significant changes in acute complications from HD. Long-term prospective studies with larger sample sizes are needed in order to assess the clinical and analytical significance of using an acetate-free dialyzate.

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