Citrasate® dialysis concentrate

In vitro tests and results of the Citrasate® concentrate use in *in vivo* bicarbonate haemodialysis and on-line haemodiafiltration

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Content

1. Use of Citrasate® concentrate in conventional dialysis machines – feasibility *in vitro* study

1.1 Introduction

Most of the contemporary haemodialysis machines displays the value of sodium and bicarbonate ions in the produced dialysis solution although those variables are not really measured. What is really is only the total conductivity of the solution. However, detailed composition of the concentrate used is entered in machines´ memory in service mode and from that data and from known values of concentrate/water proportioning ratio, dissociation and conductance constants of each component and total conductivity, concentration of both ions can be calculated. Usually, service technicians can enter only amounts of predefined ingredients in concentrates, not the type of ingredients. The list of concentrate ingredients in contemporary machines includes only currently used substances (sodium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium acetate, acetic acid (AA), sodium bicarbonate, and glucose). The citric acid (CA) contained in the Citrasate® concentrate has not been included in that list in any machine yet.

1.2 Aims of the in vitro tests

The aim of the *in vitro* testing was thus to verify whether it is technically feasible to use the CA-containing A-concentrate in machines preset for conventional AA-containing A-concentrate types. Taking into account the double-stage proportioning of concentrates in a dialysis machine (first, the A-concentrate is mixed with water and final dialysate appears in the second stage where the B-concentrate is added), it was necessary to verify that there will not appear any conductivity alarms in either of the two proportioning stages and that also the possibility to regulate sodium and bicarbonate concentrations in final dialysate will be preserved over the entire currently used range (typically 130 to 150 mmol/l for sodium and 24 to 38 mmol/l for bicarbonate).

1.3 Material and methods

The *in vitro* testing was performed in in the following haemodialysis machines: Dialog Plus manufactured by B. Braun, 4008S and 5008 manufactured by Fresenius, AK200 and AK200S manufactured by Gambro, and DBB 05 manufactured by Nikkiso.

Two types of the “acidic” A-concentrate were used, giving either 3 mmol/l of acetic acid (BIC-F08) or 2,4 mmol of citric acid and 0,75 mmol/l of sodium acetate (BIC-F322) in final dialysate for standard proportioning ratio (1:1,225:32,775) – see Tab. 1.

concentrate	BIC-F08	BIC-F322
NaCl (g)	214,15	214,15
KCl (g)	7,83	7,83
$CaCl_2.2H_2O$ (g)	9,01	9,01
$MgCl_2.6H_2O$ (g)	3,56	3,56
$C_6H_{12}O_6.H_2O$ (g)	--	--
CH_3COOH (g)	6,31	--
$CH_3COONa.3H_2O$ (g)	--	1,43
$C_6H_8O_7.H_2O$ (g)	--	5,88
Aqua purificata (ml)	ad 1000	ad 1000

Tab. 1 Composition of both „acidic“A-concentrates used in the in vitro tests

Concentrations od Na^+, K^+ a Ca^{2+} in final dialysate were measured by an ion-selective analyzer (Ionometer EF, Fresenius), HCO_3^- concentrations by a blood-gas analyzer (ABL-5, Radiometer). Measurement was performed for 4 different combinations of sodium and bicarbonate set values covering the whole range of practically used setting (134/ 24, 134/38, 148/24 and 148/38 mmol/l).

The measured concentration values of all ions were compared with those obtained by theoretical calculations – both assuming complete dissociation of all salts in the solution and considering dissociation and conductance constants of all the salts (the latter calculation was performed at the Gambro R&D department by means of a program used for development of conductivity measurement and control circuitry).

1.4 Results:

The Na^+ a HCO_3^- concentration control worked with both A-concentrates normally and practically the same values of most ingredients were obtained. Complete results from all machines are shown in Tab. 2a to 2f.

Performance of all tested dialysis machine types was problem-free without any alarms, including the first proportioning stage in which the A-concentrate is mixed with water.

Lower ionized calcium concentration was found in all machines with the CA-containing A-concentrate (by 0,35-0,55 mmol/l, depending on the set values of the Na^+ a HCO_3^- concentrations).

Bicarbonate concentration exhibited tendency towards slightly higher values with the the CA-containing A-concentrate (the difference ranged from 0 to 2,5 mmol/l, depending on the machine type and the Na^+ a HCO_3^- concentrations set values). This is apparently caused by the fact that acetic acid in conventional A-concentrate reacts in dialysis solutions preferential-

4008S Fresenius

measurements with conc. **BIK-F 300/BIC-F8,4%**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
134/24	136	2,98	1,11	7,18	23
134/38	131,4	2,51	0,89	7,39	35
148/24	148,6	3,28	1,22	7,11	22
148/38	145,6	2,84	1	7,36	35

measurements with conc. **BIK-F 08/BIC-F8,4%**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
134/24	133,6	2,93	1,59	7,08	22
134/38	130,6	2,45	1,24	7,35	35
148/24	145,2	3,26	1,75	7,01	22
148/38	140,5	2,83	1,39	7,26	35

Tab. 2a

5008 Fresenius

measurements with conc. **BIK-F 322/BIC-F8,4%**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
132/24	129,8	2,83	1,06	7,22	22
132/38	127,7	2,41	0,85	7,46	35
148/24	147,3	3,2	1,17	7,15	22
148/38	143,8	2,78	0,98	7,39	35

measurements with conc. **BIK-F 08/BIC –F8,4%**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
132/24	130,4	2,78	1,5	7,12	22
132/38	127,2	2,4	1,21	7,37	35
148/24	144,7	3,16	1,68	7,03	22
148/38	143,2	2,78	1,39	7,29	34

Tab. 2b

ly with bicarbonate and bicarbonate concentration in the final solution is thus decreased by the bicarbonate amount consumed in that reaction. Contrary to this, citric acid contained in the Citrasate® concentrate preferentially creates complexes with calcium ions and bicarbonate concentration in the final dialysate thus correspond to full amount of bicarbonate added in B-concentrate in the second proportioning stage.

AK200 Gambro

measurements with conc. **BIK-F 300/BIC-F8,4%**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
132/24	132	2,79	1,05	7,25	24
132/38	131,2	2,42	0,87	7,47	36
148/24	148,1	3,21	1,18	7,18	23
148/38	147	2,81	0,99	7,42	35

measurements with conc. **BIK-F 08/Sol-Cart B**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
134/24	132,6	2,81	1,5	7,12	23
134/38	130,1	2,41	1,2	7,34	36
148/24	148,6	3,2	1,69	6,99	22
148/38	146,2	2,78	1,38	7,23	35

Tab. 2c

AK200S Gambro

measurements with conc. **BIK-F 300/BIC-F8,4%**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
132/24	134,4	2,83	1,04	7,22	25
132/38	132,5	2,44	0,84	7,45	37
148/24	150,4	3,27	1,21	7,15	24
148/38	149,3	2,93	1,03	7,38	37

measurements with conc. **BIK-F 08/BIC-F8,4%**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
132/24	136,5	2,94	1,59	7,09	23
132/38	130,7	2,47	1,22	7,34	37
148/24	149,5	3,23	1,71	7	22
148/38	147,2	2,81	1,4	7,26	35

Tab. 2d

Dialog Plus, B. Braun

measurements with conc. **BIK-F 300/Sol-Cart B**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
132/24	129,8	2,81	1,05	7,11	23
132/38	133,3	2,46	0,86	7,37	40
148/24	148,5	3,07	1,1	7,07	22
148/38	150,1	2,84	0,99	7,33	38

measurements with conc. **BIK-F 08/Sol-Cart B**

setting Na/HCO_3	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
134/24	129,9	2,83	1,49	7,06	23
134/38	133,8	2,53	1,27	7,32	40
148/24	146	3,15	1,68	7,01	23
148/38	149,1	2,81	1,39	7,25	38

Tab. 2e

DBB 05 Nikkiso

measurements with conc. **BIK-F 300/BiCart**

setting celk. a bik. vodivosti	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
13,4/2,5	132,9	2,88	1,09	7,2	24
13,4/3,5	131,5	2,5	0,88	7,38	37
15,4/2,5	155,9	3,38	1,25	7,13	23
15,4/3,5	155,3	3,05	1,07	7,28	35

measurements with conc. **BIK-F 08/Bicart**

setting celk. a bik. vodivosti	Na^+ mmol/l	K^+ mmol/l	Ca^{2+} mmol/l	pH --	HCO_3^- mmol/l
13,4/2,5	130,4	2,75	1,48	7,11	24
13,4/3,5	130,1	2,45	1,23	7,3	37
15,4/2,5	153,4	3,44	1,84	6,98	23
15,4/3,5	155,1	3,05	1,53	7,2	35

Tab. 2f

The finding of significantly lower Ca^{2+} concentration and tendency towards higher HCO_3^- concentration in dialysate produced with the Citrasate® concentrate was confirmed by comparison of measured values with theoretical calculations - see Tab. 3.
Measured concentration (3rd column i Tab. 3) is with AA-containing concentrate (left part of the Tab. 3) by about 16% lower than the value calculated assuming total dissociation and zero interaction between ingredients (1st column). With the Citrasate® concentrate, this difference is significantly higher, cca 40% (compare columns 3 and 6 of the Tab. 3). In both cases, there is a good correspondence with theoretical prediction accounting for both limited dissociation and interaction between dialysate ingredients (compare the 2nd and the 4th columns of both halves of the Tab. 3).

	dialysate with F08 (AA)			dialysate with F322 (CA)		
	CD	CD_{theor}	CD_{meas}	CD	CD_{theor}	CD_{meas}
Na^+ (mmol/l)	140	140	139,2	140	140	138,6
K^+ (mmol/l)	3	3	2,8	3	3	2,81
Ca^{2+} (mmol/l)	1,75	1,46	1,43	1,75	1,05	0,96
Mg^{2+} (mmol/l)	0,5	0,42	nm	0,5	0,31	nm
Cl^- (mmol/l)	112,5	112,5	nm	110	110	nm
acetate (mmol/l)	3	3	nm	0,3	0,3	nm
citrate (mmol/l)	--	--	nm	0,8	0,19	nm
$HCO3^-$ (mmol/l)	32	30,77	30,5	35	33,75	32,1
pH	nc	nc	7,25	nc	nc	7,39

np – non calculated, nm – not measured

Tab.3

Measured and calculated values shown in Tab. 3 were obtained for standard proportioning ratio A-concentrate : B-concentrate : water = 1 : 1,25 : 32,275 on the AK200S Gambro machine.

1.5 Conclusions:

■ HD machines preset for use with conventional A-concentrates acidified with AA can be used without any adjustments for BHD with A-concntrate acidified with CA (Citrasate®) *(practically tested for the following types: Dialog of B. Braun, 4008/5008 of Fresenius, AK 100/200/200S of Gambro and DBB05 of Nikkiso)*

■ Concentration of ionized Calcium in dialysate prepare with the Citrasate® concentrate is by 0,35-0,55 mmol/l lower as compared to dialysate prepared with the conventional AA-acidified A-concentrate *(documented by both practical measurement and theoretical calculation)*

■ Practically no change is seen in concentration of Sodium ion in dialysate prepared with the Citrasate® concentrate and conventional AA-acidified type *(documented by both practical measurement and theoretical calculation)*

■ Concentration of bicarbonate ion in dialysate tends to be slightly higher when using the Citrasate® concentrate as compared to conventional AA-acidified type – by about 0,5 to 2,5 mmol/l (0,2-0,8%), depending on the type of mixing system (volumetric or conductometric) of the HD machine in question *(documented by both practical measurement and theoretical calculation)*

2. Bicarbonate haemodialysis with Citrasate® concentrate - *in vivo* study

2.1 Introduction, balance considerations

After successful verification of technical feasibility of the Citrasate® concentrate problem-free use in contemporary dialysis machines, *in vivo* haemodialyses were started (At that time, the new concentrate already had the CE mark).

The questions, which this part of the study was planned to answer, were related to lower ionised calcium level in dialysate found in the *in vitro* tests. In presence of citric acid (CA), part of the Ca^{2+} ions from the dissociated $CaCl_2$ form complexes with citrate anions (see Chapter 1 of this Report – levels lower by 0,35-0,55 mmol/l found during the measurement). This diminishes Ca^{2+} concentration gradient between plasma and dialysate, and consequently also transfer of Ca^{2+} into blood. Depending on plasma levels this transfer may even take the opposite direction. This change is on the other side compensated by diffusion of the Ca-citrate complexes from dialysate into blood. Diffusion of those complexes will, however, be as fast as diffusion of calcium ions from blood, due to higher molecular weight of the complexes (several hundred Daltons). During subsequent metabolisation of the Ca-citrate complexes in the Krebs´s cycle, calcium ions will be liberated and will join the body pool of free calcium. The principal question thus is what the final balance of all those phenomena would be.

2.2 Aim of the study

The aim of the *in vivo* study was to evaluate impact of longer use of the Citrasate® concentrate in a smaller group of patients on bicarbonate haemodialysis (BHD), namely its impact on plasma levels of calcium and bicarbonate and on coagulation of blood in the extracorporeal circuit, and to look into phenomena related to changes in coagulation.

2.3 Material and methods

Five patients dialysed for chronic renal failure were monitored during an interval of 4 weeks, of which BHD with conventional acetic acid (AA)-acidified A- concentrate was used followed by three weeks of BHD with the citric acid (CA)-containing Citrasate® A-concentrate. Prescribed parameters (dialyser type, blood flow, dialysis time, heparinisation mode and heparin dose) remained in each patient unchanged during the whole monitored period. Patient characteristics and prescription data are shown in Tab. 2-1.

	Patient				
	1	2	3	4	5
Gender	M	M	F	F	F
age (years)	75	87	67	72	75
„dry weight“ (kg)	62	78	58,5	81	74
HD time (hours)	4	4	4	4	4
blood flow (ml/min)	320	320	320	320	320
dialyser	FDX120GWS	PFL 14L	PES150 DL	PES150DL	Sureflux170L
A-concentrate with AA	F61	F61	F61	F64	F51
Citrasate® concentrate	B320	B320	B320	B320	B320

Tab. 2-1 Characteristics of monitored patients and BHD prescription data

The following parameters were measured during the whole 4 weeks-interval: pre- and post-HD plasma levels of Na^+, K^+, Ca^{2+}, HCO_3^- and pH value. Intradialytic changes in those levels were assessed as markers of changes in overall balance, although the balances themselves were not evaluated. Special attention was paid to trends in pre-HD values of the parameters.

The spKt/V was calculated from the pre- and post-HD plasma urea levels using the standard Daugirdas formula (2^{nd} generation).

Haemocoagulation status of all patients during each dialysis was assessed by means of the time constant of the exponential decrease (K_e) of Activated Clotting Time (ACT). The K_e was calculated from ACT values prior to administration of the initial heparin bolus (ACT1), 5 minutes after the bolus (ACT2) and after cca 2 hours of dialysis (ACT3), all measured by the coagulometer Hemochron 401 (International Technidyne Corp., USA). The K_e constant characterises steepness of the clotting time decay after the initial bolus administration towards the base-line value (before the bolus administration) and is expressed in $hour^{-1}$. The shorter its value is the slower is the ACT decay. With intermittent heparinisation, K_e is calculated as

$$K_e = (\ln((ACT2 - ACT1)/(ACT3 - ACT1)))/\Delta t \qquad [2\text{-}1]$$

In continuous heparinisation, the following equation was used

$$K_e = I*(ACT2 - ACT1)/(M0*(ACT3 - ACT1)) \qquad [2\text{-}2]$$

In Eq [2-1] the Δt denotes time interval between ACT2 and ACT3 measurements (usually 2 hours), M0 in Eq [2-2] gives the initial heparin bolus (I.U.) and I the heparin infusion rate (I.U./hour). Both equations can be derived from heparin kinetics assuming linear relationship between the administered dose and increase in ACT – see reference (5, 6).

2.4 Results

Tab. 2-1 gives mean values of the displayed parameters in individual patients, in which a significant change was seen with the change of the A-concentrate used - intradialytic change

patient	**ΔCa_{tot} (mmol/l)**		**spKt/V**		**K_e (ACT) ($hour^{-1}$)**	
	A-concentrate used		A-concentrate used		A-concentrate used	
	BIC F-08	BIC F-300	BIC F-08	BIC F-300	BIC F-08	BIC F-300
1	0,233	0,163	1,41	1,43	0,636	0,513
2	0,430	0,320	1,30	1,34	0,660	0,567
3	0,273	0,038	1,43	1,63	0,562	0,459
4	0,203	0,182	1,11	1,25	0,486	0,439
5	0,380	0,217	1,49	1,56	0,706	0,663
mean	**0,304**	**0,184**	**1,348**	**1,442**	**0,610**	**0,528**
change (%)		**-39,4**		**+6,97**		**-13,41**

Tab. 2-1 Parameters, in which a significant change was seen between dialyses with the AA-containing concentrate and those with Citrasate®: intradialytic change in plasma level of total calcium (ΔCa_{tot} calculated as pre-HD minus post-HD value), spKt/V and the ACT time decay constant (K_e). Number given in lines 1to 5 are mean values seen in individual patients over one week monitoring interval, i.e. 3 dialyses.

in total plasma calcium (ΔCa_{tot}), spKt/V and K_e values. For the conventional A-concentrate, mean values calculated over the initial one-week interval are given, for Citrasate® the figures are the means over the following three weeks interval.

Fears of a significantly negative calcium balance proved unsubstantiated in all monitored patients, pre-HD calcium plasma levels remained practically unchanged. Although there was a slight intradialytic decrease in total plasma calcium (but not in ionised fraction) after transition to the Citrasate® concentrate, rather surprisingly this change did not bring about any changes of pre-HD ionised calcium levels. Just slight decreasing tendency (0,1-0,2 mmol/l) was noticed in total calcium level - see Fig. 2-1. The plot displays typical time-course of both total and ionised calcium in one of the monitored patients, situation in other patients was practically identical.

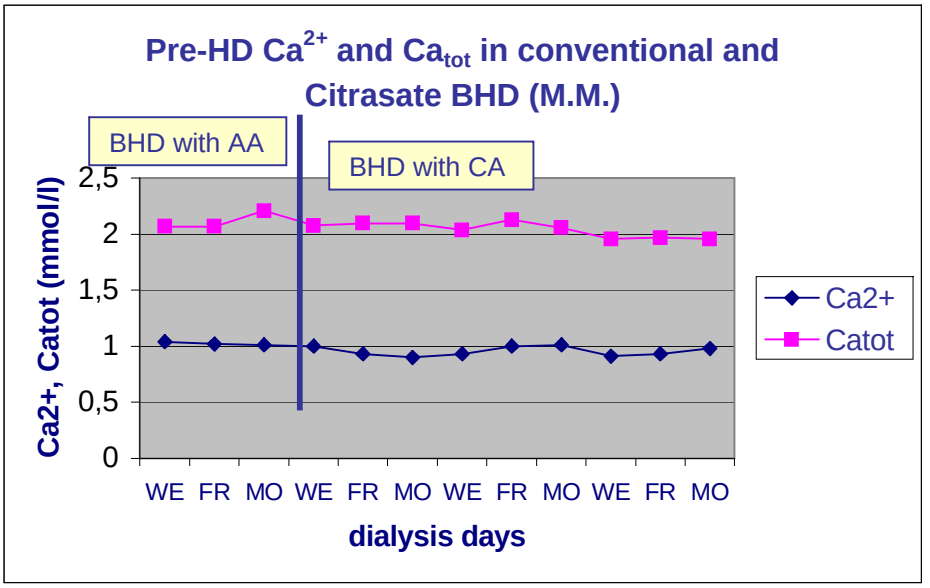

Fig. 2-1 Time course of total and ionised Ca pre-HD plasma level in one of the monitored pateints over the entire monitored interval of 4 weeks

Also in other ions (Na^+, K^+, Mg^{2+}) no changes in pre-HD plasma levels were seen over the entire 3 weeks interval after the change over to the Citrasate® concentrate. Slight increase

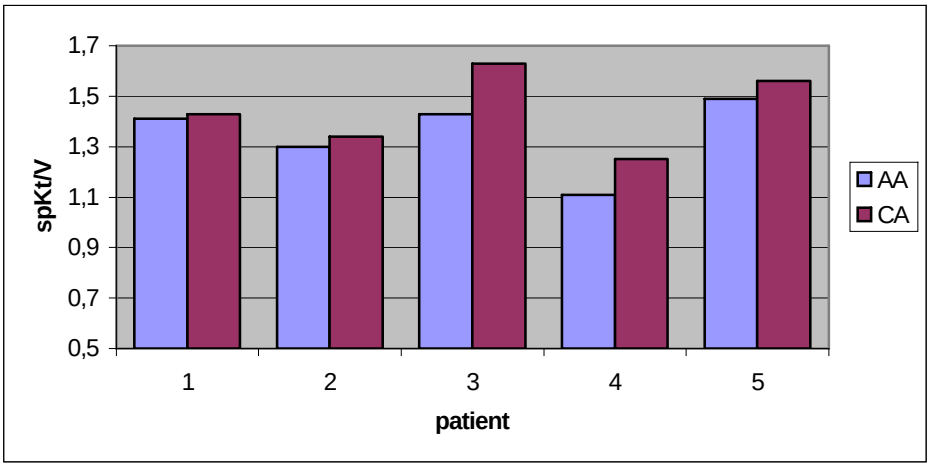

Fig. 2-2 spKt/V in monitored patients (mean values for the entire monitored interval)

by about 1 mmol/l occurred only in pre-HD level of bicarbonate.

Contrary to this, there was a marked increase in dialysis dose Kt/V in all 5 monitored patients, the dose increased on average by 7% - see Fig. 2-2..

Similarly positive change associated with transition over to the Citrasate® concentrate was seen in the coagulation time constant K_e, shown already in Tab. 2-1. With the Citrasate® concentrate, the mean K_e value was in all patients smaller than with the conventional A-concentrate – see Fig. 2-3. This positive effect of Citraste® is in correspondence with previously reported possibility of heparin dose reduction (2, 4). However, calculation of the K_e, provides more objective data than by trial and error carried out reduction in heparin dose. Analysis of the exponential ACT decay enables to calcualte that possible percentage reduction of heparin dose in continuous heparinisation corresponds to the percentage difference in the K_e value. One can even speculate whether use of the Citrasate® concentrate could not replace technically cumbersome regional citrate anticoagulation used in cases where heparin cannot be used.

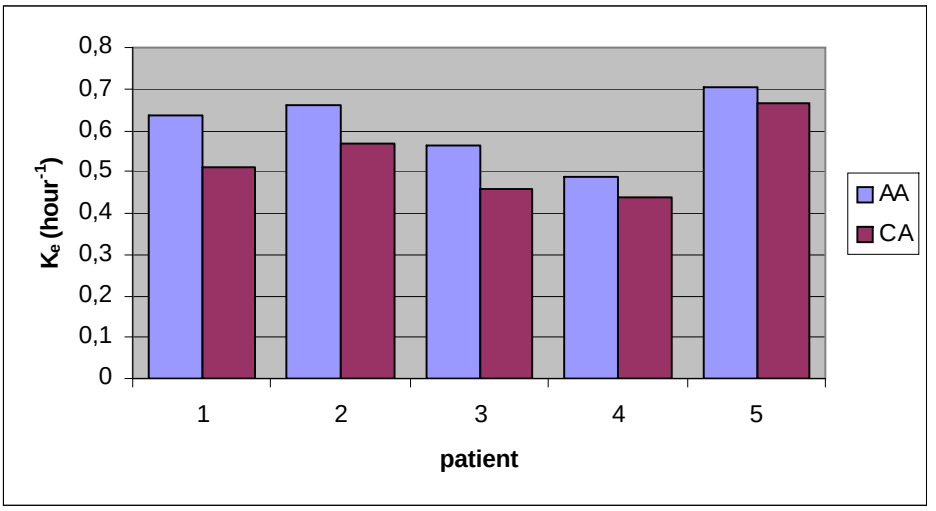

Fig. 2-3 Average value of the ACT decay time constant in all 5 monitored patients during the interval with conventional A-concentrate with AA and with the Citrasate® concentrate

2.5 Conclusions

■ No significant changes were seen in pre-HD plasma level of sodium, bicarbonate and ionised calcium over the 3 weeks interval of *in vivo* BHD with the Citrasate® concentrate in any of the 5 patients monitored.

■ Slight decrease in total plasma calcium level, which may occur when dialysate with lower Ca content (1,25 a 1,5 mmol/l) is used, can be avoided during transition over to the Citrasate® concentrate by using Citrasate® concentrate with higher Ca content (e.g. 1,25 instead of 1,5 mmol/l).

■ All five patients exhibited an increase in spKt/V after they started using the Citrasate® concentrate (all other dialysis prescription parameters being kept unchanged).

■ In BHD with the Citrasate® concentrate, less steep decay of the ACT values was seen (with unchanged heparin dose), suggesting the possibility of heparin dose reduction. This was, however, not practically attempted during this study.

3. On-line haemodiafiltration with Citrasate® concentrate - *in vivo* study

3.1 Introduction

Despite rather small molecular weight of both the citrate anion and the calcium-citrate complexes (272-816), which speaks for primarily diffusive transport across the membrane in the extracorporeal depurative techniques of renal replacement therapy and presumably minor role of convection in haemodiafiltration (HDF) and thus also no or negligible difference in overall balance of those substances in HD and HDF, we still considered separate verification of the impact of Citrasate® concentrate use in on-line HDF mandatory because practically all reports on Citrasate® use until now have been of American origin and on-line HDF is not routinely used in the United States.

3.2 Aims of the study:

The aim of the *in vivo* study with the Citrasate® concentrate use in on-line HDF thus was, similarly to the previous study with HD, to evaluate impact of the Citrasate® concentrate use on HDF efficacy and changes in coagulation status. Additionally, overall calcium balance was evaluated and compared to HDF with conventional acetic acid containing A-concentrate. Also the risk of citrate accumulation in plasma possibly induced by its insufficiently rapid metabolisation in Krebs´s cycle was assessed. This study was performed in a form of BSc. project (J. Jumr, 2009).

3.3 Material and methods:

All measurements were performed in 3 patient treated by on-line hemodiafiltration. Patient characteristics and standard HDF parameters are summarised in Tab. 3-1.

	patient		
	1	2	3
gender	M	M	M
age (years)	64	62	52
„dry weight“ (kg)	75	82	81,5
HDF mode	post-dilution	pre-dilution	pre-dilution
HDF session time (hours)	5	5	5
blood flow (ml/min)	370	320	300
tot. subst. volume (l)	19	25	25
dialyser	Rexeed 18UX	PES 210DH	PES 210DH
A-concentrate with AA	BIK-F59	BIK-F51	BIK-F51
A-concentrate with CA	BIC-318	BIC-313	BIC-313

Tab. 3-1 Characteristics of monitored patients and HDF prescription data

Each patient was monitored for a period of two weeks (altogether 6 HDF sessions), one week on HDF with conventional A-concentrate with acetic acid and the following one on HDF with the Citrasate® A-concentrate (with identical amount of calcium chloride). All HDFs were performed using the AK200S Ultra machines of Gambro with fixed dialysate production rate

of 500 ml/min. Dialysate flow through the dialyser is thus by the substitution fluid flow lower than the 500 ml/min value.

Efficacy of each monitored HDF was assessed by the „dialysis dose“ spKt/V, calculated from the pre- and post-HDF plasma urea level by means of the Daugirdas equation (II. generation). For comparison, mean values over one week were used in all patients.

Further, pre-HDF concentrations of both ionized and total calcium and of bicarbonate were evaluated to detect possible changes after the change in the A-concentrate.

Total calcium and citrate balance over one HDF session was evaluated by means of partial dialysate collection over the entire HDF procedure. Hundred ml/hour of dialysate were continuously taken from the dialysate drain tube with an infusion pump and collected in a special bag. Total balance of the substance in question was calculated from concentrations in fresh dialysis/substitution solution (CS) and in a sample of fluid taken from the collection bag at the end of the monitored HDF (CDo) by means of the following equation:

$$m = QD*CS*Td - (QD + UFR)*CDo*Td \qquad [3\text{-}1]$$

where m (mmol) denotes the total amount of a substance delivered to the patient during the entire monitored HDF (if the value is positive) or total removed amount (if the value is negative), QD means total produced dialysate flow rate (500 ml/min), UFR ultrafiltration rate (ml/min) and Td (min) is the session time of the monitored HDF.

Impact of the Citrasate® concentrate upon coagulation status of the patient was assessed in the same way as in the BHD study (see chapter 2 of this report, Equations [2-1] a [2-2]), i.e. by means of the time constant K_e of the exponential decay of Activated Clotting Time (ACT), again averaged for each patient over the entire week 1 and week 2.

We assumed that positive impact of changes in coagulation status on efficacy of the extracorporeal procedure (spKt/V) found in the BHD study was due to less coating and deposits forming on the membrane or lower number of dialyser fibres clotted during the procedure. During the HDF part of the Citrasate® study we therefore tried to verify this by means of measuring the dialyser blood path volume (FBV – „fibre bundle volume“) at the beginning and towards the end of each HDF. This technique was developed by N. Krivitski (N. Krivitski, 1998) at the Transonic Systems and practically verified in our department within frame of one of the previous BSc projects (E. Slavíčková, 2005).

To assess possible accumulation of citrate in plasma, the so-called „calcium-gap“ concept (Gabutti, 2009) was used. The Ca-gap is defined as a difference between the intradialytic change of total Ca concentration (C_PCa^{tot}) and that of its ionised fraction (C_PCa^{2+}) (value at the HDF end minus HDF start)

$$\text{Ca-gap} = (C_PCa^{tot}(post) - C_PCa^{tot}(pre)) - (C_PCa^{2+}(post) - C_PCa^{2+}(pre)) \qquad [3\text{-}2]$$

Gabutti assumes that the value of in this way defined Ca-gap is proportional to accumulation of yet unmetabolised citrate in plasma.

As we used the citrate measurement technique developed in the Clinical Biochemistry Institute of the General University Hospital in Prague for measurement of citrate in urine, i.e. in basically protein-free environment, we did prior feasibility testing of the technique for plasma. Citrate-free plasma samples were loaded with pre-defined amounts of citrate, its concentration measured and conversion coefficient between the pre-defined and measured concentation was established. This coefficient was then used to convert citrate values measured during the Citrasate® study into the real ones.

3.4 Results:

Changes in the mean spKt/V value over three HDFs in the week with the conventional A-concentrate and the week with the Citrasate® concentrate are displayed in Fig. 3-1. The same value is seen in patient 2, the other two patients showed an increase of the order of several %.

Behaviour of pre-HDF plasma level of total and ionised calcium over the whole two-weeks period is shown in Fig. 3-2a (patient 1) 3-2b (patient 2) a 3-2c (patient 3). Slight decrease in

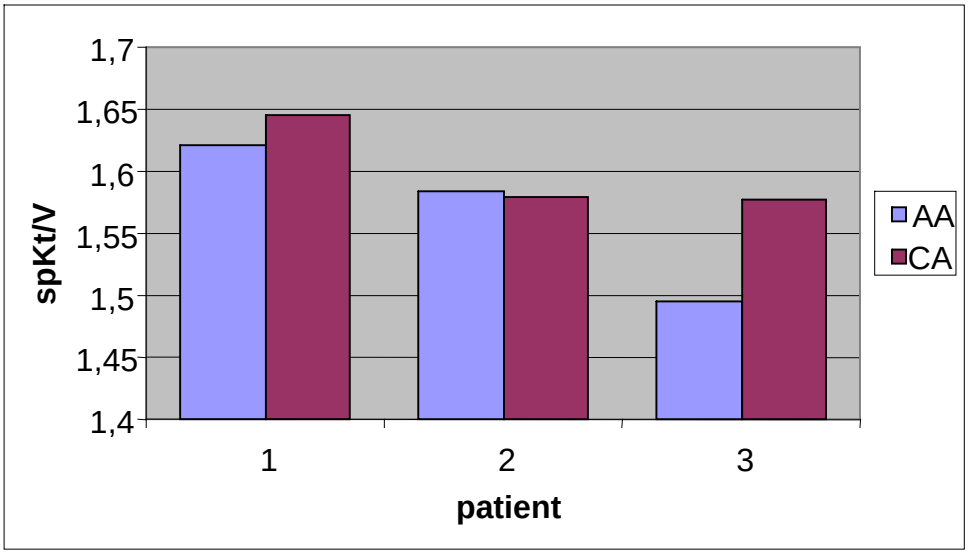

Fig. 3-1 Comparison of the mean spKt/V values in HDF in all three patients over one week with conventional (AA) A-concentrate and one week with the Citrasate® (CA) concentrate

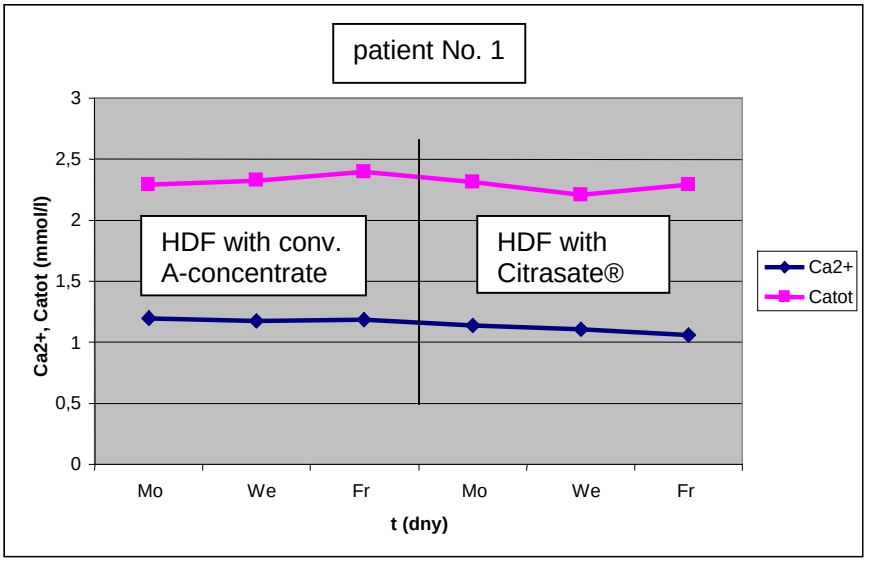

Fig. 3-2a Pre-HDF plasm Ca^{tot} and Ca^{2+} concentration in patient 1 during the 2 weeks´ monitoring (1^{st} week with conventional A-concentrate, 2^{nd} week with Citrasate®) in patient 1

both total and ionised calcium is seen in patient 1 only. The other two patients exhibited no change with the change-over to Citrasate®. It should be noted that patient 1 used A-concentrate with Ca-level of 1,25 mmol/l (value assuming total dissociation of $CaCl_2$). Real value of ionised calcium in dialysate thus was always lower than his plasma level at each HDF start. The other two patients were haemodiafiltered with dialysate Ca-content of 1,5 mmol/l and it was always higher than their pre-HDF plasma levels of ionised calcium.

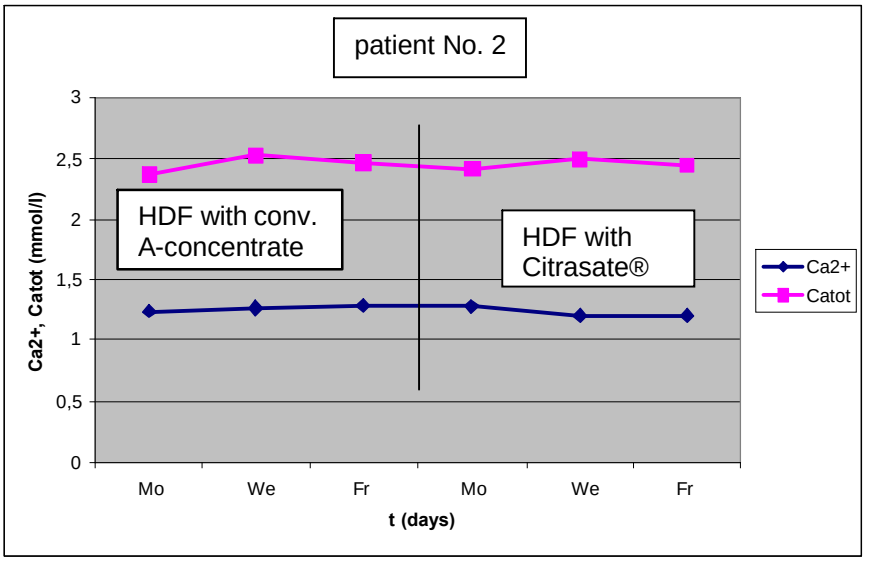

Fig. 3-2b Pre-HDF plasm Ca^{tot} and Ca^{2+} concentration in patient 2 during the 2 weeks´ monitoring (1st week with conventional A-concentrate, 2nd week with Citrasate®

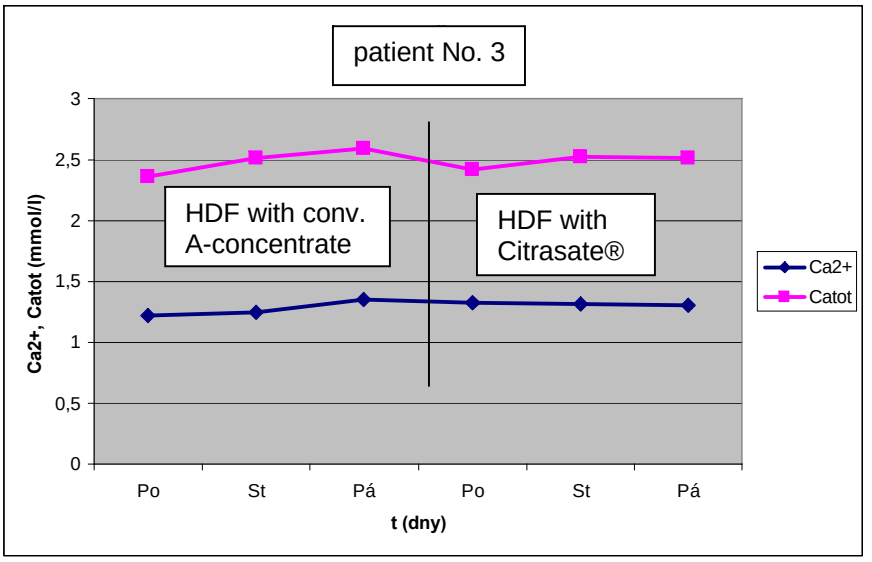

Fig. 3-2c Pre-HDF plasma Ca^{tot} and Ca^{2+} concentration in patient 3 during the 2 weeks´monitoring (1st week with conventional A-concentrate, 2nd week with Citrasate®)

Also overall intra-HDF calcium balance corresponded to changes in pre-HDF calcium levels – see Tab. 3-2 (calculated by means of Eq. [3-1]). The balances were in all patients slightly negative and got more negative after the change-over to the Citrasate® concentrate.

The highest calcium loss during HDF already with the conventional A-concentrate was seen in patient 1. The overall Ca-loss during one HDF increased by about 10 mmol. It is sufficiently low figure to consider transition to Citrasate® in the on-line HDF safe. Only in patients with conventional A-concentrate with Ca-level of 1,25 mmol/l, an increase to 1,5 mmol/l may be reasonable when that transition is attempted.

	Ca balance (mmol) in HDF*		
	patient 1	patient 2	patient 3
convent. A-concentrate	-10,05	-0,82	-6,89
Citrasate®	-19,57	-10,27	-11,25

(each value in the Table is a mean of all 3 HDFs during the given week)*

Tab. 3-2 Mean one HDF calcium balance (averaged over the whole week interval)

With regard to the interrelation of calcium and phosphorus kinetics, also phosphorus removal was evaluated (removed amounts were calculated using again Eq. [3-1], leaving out the first member in the equation since there was no phosphate contained in fresh dialysate. The whole week removal of phosphorus was in all 3 patients higher when the Citrasate® concentrate was used (by 10% in patient 1, by 6% in patient 2 and by 5% in patient 3).

	removed amounts of phosphorus (mmol) in individual HDFs and over the week							
	HDF with conventional s A-concentrate				HDF with Citrasate® concentrate			
	1. HDF	2. HDF	3. HDF	whole week	1. HDF	2. HDF	3. HDF	whole week
pat. 1	62,9	66,5	76,5	**205,9**	78,2	77,1	71,7	**227,0**
pat. 2	42,1	41,9	40,3	**127,5**	42,1	44,8	45,1	**132,0**
pat. 3	54,4	53,9	44,7	**153,0**	56,0	52,5	52,5	**161,0**

Tab. 3-3 Removed amount of phosphorus during each HDF and during the whole week

No systematic changes were seen in plasma levels of sodium, bicarbonate, and phosphorus either after change-over to Citrasate® - see Tab. 3-4. Although there was slight intradialytic increase in plasma bicarbonate level in individual patients after that change-over, this change

	HDF	pre-HDF plasma level of Na, HCO3 and P (mmo/l)					
		conventional A-concentrate (AA)			Citrasate® A-concentrate (CA)		
		Na	HCO_3	P	Na	HCO_3	P
patient 1	1	141,1	20	3,25	137,2	20	4,08
	2	139,2	21	3,87	140,2	24	3,74
	3	140,2	21	3,78	138,9	24	3,62
patient 2	1	137,8	19	1,44	136,5	17	1,73
	2	138,1	20	1,68	139,9	21	1,97
	3	137,5	21	142	136,4	21	1,71
patcient 3	1	135,7	21	1,92	135,5	23	1,80
	2	134,1	23	1,80	134,4	22	1,89
	3	136,9	23	1,81	134,0	24	1,97

Tab. 3-4 Pre-HDF plasma concentrations of Na, HCO_3 and P

was, however, apparently too small to influence the pre-HDF level. Patient 1 exhibited persisting hyperphosphatemia. Noteworthy is the interindividually different pre-HDF plasma sodium, which howerer shows surprising stability during the subsequent HDFs. This suggests existence of an individual sodium "set-point" exploitable for individualisation of dialysate sodium concentration in patient with high intradialytic body weight increments (although this finding is not related to the issues investigated in this study).

Blood sampling with the ACT measurements had rather high failure rate so that the time constant K_e of the ACT exponential decay could not be established in each of the monitored HDF. The procedures with failed K_e measurements are marked by two dash lines in Tab. 3-5. Sufficient data for comparison was available in patients 1 and 3 only. Both exhibited significant shortening of the K_e constant in HDFs done with Citrasate® concentrate as compared to procedures with conventional A-concentrate type. This suggests the possibility of heparin doses reduction. Similarly to the BHD study, also in this short study the reduction was not practically attempted.

	Time constant K_e of the exponential ACT decay ($hour^{-1}$)							
	HDF with conventional A-concentrate				HDF with Citrasate® concentrate			
	HDF 1	HDF 2	HDF 3	mean	HDF 1	HDF 2	HDF 3	mean
Pat. 1	--	0,547	0,580	**0,563**	0,400	0,357	--	**0,377**
Pat. 2	--	0,931	1,200	**1,065**	--	--	--	**--**
Pat. 3	0,814	1,313	1,238	**1,121**	0,825	--	0,604	**0,715**

Tab. 3-5 Time constant K_e of the exponential ACT decay

Because of the delay in delivery of the special commercially unavailable software for the measurement of the dialyser blood path volume (FBV- fibre bundle volume), measurements were performed in two patients only. The first measurement in each HDF was done in the 40th minute of the procedure and the second one 20 minutes before its end. Tab. 3-6 shows the mean FBV values seen in all 3 HDFs in a given patient with each A-concentrate type.

	patient 1		patient 2	
	HDFwith conventional A-concentrate	HDF with Citrasate®	HDFwith conventional A-concentrate	HDF with Citrasate®
FBV at 40´ (ml)	118,2	124,3	148,3	151,3
FBV at 280´ (ml)	128,8	127,0	165,0	153,2
ΔFBV (ml)	**10,6**	**2,7**	**15,7**	**1,9**

Tab. 3-6 Mean FBV in each of the 3 evaluated HDF sessions

The results were rather surprising. The expected higher drop in FBV towards the end of the session in HDF with conventional A-concentrate as compared to that with Citrasate® was not found. Instead, lowered FBV values were seen in HDF with conventional A-concentrate in the 40th minute measurement, which increased towards the end. Practically no change in the 40th minute and HDF end FBV measurement was seen with Citrasate® and that value was about the same as the FBV value at the end in HDF with conventional A-concentrate. It means that

there is negligibly or no transient secondary membrane created with Citrasate® and it is thus the first hour during which the procedure with Citrasate® is more effective, not the end part of the procedure – see Fig. 3-3.

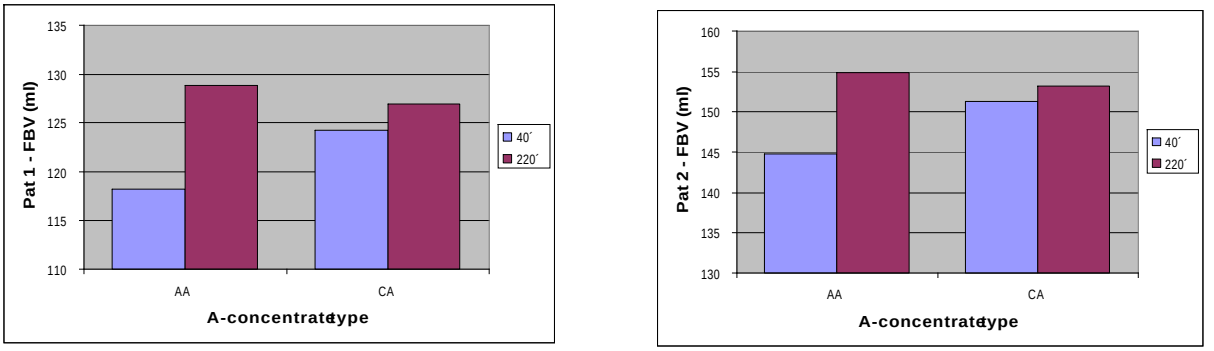

Fig. 3-3 Fibre bundle volume (FBV) measured in patient 1 (left plot) and patient 2 (right plot) at 40th minute of the HDF and towards its end with conventional (AA) and Citrasate® (CA) A-concentrate

The last assessed parameter was accumulation of citrate in plasma during HDF. It was evaluated from values of Ca-gap in each HDF session by means of Equation [3-2]. The Ca-gap values in individual HDF with conventional A-concentrate and with Citrasate® are given in Tab. 3-7. Differences in values with both concentrates were very small and in most sessions

	Ca-gap							
	conventional A-concentrate				Citrasate® concnetrate			
	HDF 1	HDF 2	HDF 3	mean	HDF 1	HDF 2	HDF 3	mean
Patient 1	0,16	0,13	0,09	**0,13**	0,09	0,23	0,02	**0,11**
Patient 2	0,30	0,17	0,13	**0,20**	0,36	0,09	0,23	**0,23**
Patient 3	-0,04	-0,06	-0,18	**-0,09**	0,20	0,02	-0,01	**0,07**

Tab. 3-7 Ca-gap values (at each HDF and wholw week means) in individual patients with use of conventional A-concentrate and Citrasate® concentrate

did not exceed the value of 0,2 mmol/l, chosen by Gabutti (2009) as the threshold between fast and slow citrate metabolisers. It can thus be concluded that there has been no significant accumulation of citrate in plasma during HDF, i.e. patients were able to metabolise the citrate, which diffused into plasma, quickly enough.

3.5 Conclusions:

■ Similarly to HD, use of the Citrasate® concentrate in the on-line HDF leads to an increase in Kt/V value of the order of several percent (under otherwise unchanged HDF procedure parameters).

■ Results of the fibre bundle volume measurement during the first hour and towards the end of the HDF procedure indicate that the increase in efficacy with the Citrasate® concentrate as compared to the use of conventional A-concentrate with acetic acid is caused by less transient plaguing of the dialyser blood path during the first part of the procedure.

■ Assumption that the above behaviour of fibre bundle volume is caused by beneficial effect of Citrasate®-based dialysate appears further supported by the finding of less pronounced transient leukopenia when the Citrasate® concentrate is used.

■ Equally to HD also in on-line HDF performed with the Citrasate® concentrate, lower time constant of the ACT decay was found, suggesting the possibility of heparin dose reduction.

■ With a calcium level of 1,5 mmol/l or higher in dialysate, change in dialytic calcium balance after a switch-over to the Citrasate® concentrate is so small that pre-dialysis level of both total and ionised calcium will likely not be affected.

■ Patients using acetic acid-acidified dialysate with calcium level of 1,25 mmol/l may need an increase to 1,5 mmol/l when switched to the use of the Citrasate® concentrate.

■ Amount of phosphorus removed by HDF over the whole one-week interval was in all three patients slightly higher with the Citrasate® concentrate HDF as compared to HDF with conventional acetic acid containing concentrate. On average, the increase was about 7,5%.

■ Accumulation of citrate in plasma during on-line HDF with the Citrasate® concentrate, which would suggest an unbalanced ratio between citrate delivery into blood and its subsequent metabolic rate, was not detected in any of the monitored patients (all patients enrolled for the study had normal liver function).

3.6 Addenum

The unexpected result of the FBV measurement in HDF indicated, that the acute phase reactions responsible for adhesion of certain cell types and of some plasma proteins onto the dialyser membrane can be modified when citric acid containing dialysate is used instead of the conventional one with acetic acid. During the subsequent literature search, one older work was found, which documented impact of citrate infusion during regional citrate anticoagulation on the blood-membrane interactions (Böhler, 1996). That is why we performed a simple test with the aim to verify whether such phenomena occur also with the use of citrate-containing dialysis solution (and conventional heparin anticoagulation).

Material and method:

Although this test was performed within the frame of the HDF study with Citrasate®, for the sake of higher number of patients tested and thus more reliable results, this test was performed in 10 patients on bicarbonate haemodialysis with longitudinal use of the Citrasate® concentrate (see further Chaper 4 of this report). In those patients, the drop in leukocytes count in the 20th minute into dialysis was measured, compared to the pre-HD count. This drop is known to be mediated by the complement activation induced by the blood-membrane interaction in the initial part of HD. The measurement was done as paired in each patient – during one dialysis the conventional A-concentrate (F61) was used, while during the other the Citrasate concentrate® was taken. All other dialysis prescription parameters (dialyser type, blood flow, heparinisation mode and heparin dose were kept unchanged in each patient.

Results:

Because of exclusive use of dialysers with synthetic membrane in all patients investigated, mean values of the transient drop in leukocyte counts were relatively low, nevertheless statistically significantly different when conventional and Citrasate® concentrates were used – 26,6±12,9% with conventional A-concentrate against 21,6±10,5% with the Citrasate® concentrate – see Fig. 3-4. The difference was even more pronounced in median values

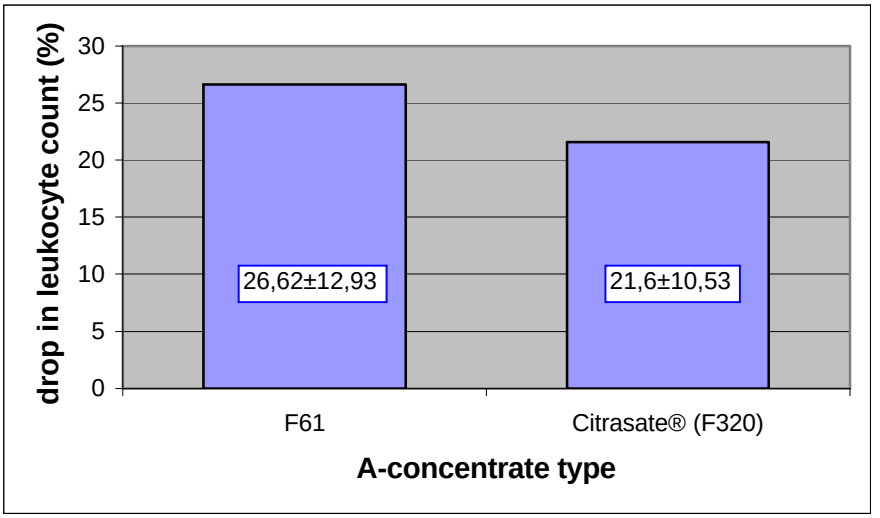

Fig. 3-4 Transient drop in leukocyte count in the 20th minute of the HD session (expressed as percentage of the value at HD start), mean values in the whole cohort

(29,1% against 20,6%). However, interindividual variability in the investigated cohort was quite high as can be seen from the standard deviation values. Despite this, statistical significance in the mean value reached a high level of p= 0,02. It clearly shows that use of the Citrasate® concentrate apparently improves biocompatibility of dialytic procedure.

Discussion:

The relatively high scatter of the transient leukocyte count drop can be – besides the above mentioned interindividual differences in complement system excitability – attributed also to non-uniform dialyser types (different membrane materials) used in patients of the investigated cohort. The difference in the drop seen in individual patients can be a consequence of local decrease in ionised calcium level in plasma in the dialyser, caused partly by its transfer from blood to dialysate but also by calcium ion complexing with citrate anions entering blood, which in turn modifies the acute phase reactions time course. Separate assessment of the impact of both phenomena could be done in future by careful measurement of ionised calcium concurrently at the dialyser inflow and outfow, both on the dialysate and blood side.

Conclusions:

■ Transient drop in leukocyte count during the initial HD part, which is a rough marker of complement activation, is statistically significantly lower with use of the Citrasate® than with the use of conventional A-concentrate type with acetic acid.

■ This finding is in correspondence with the previously described lower or missing decrease of the fibre bundle volume during the first hour of HDF with the Citrasate® concentrate. It suggests that higher overall efficacy of HD /HDF when performed with Citrasate® is not merely consequence of less clotting tendency in the extracorporeal circuit documented by prolonged ACT values.

4. Long-term results of BHD with the Citrasate® concentrate

4.1 Introduction

Following the above described successfull short-term BHD and HDF studies, 10 stable patient in chronic dialysis program were transferred from conventional A-concentrate to the Citrasate® type. The aim of this study was to extent monitoring time and to look also to parameters where changes can be expected in long-term perspective only.

4.1 Patients and evaluated parameters

Tab. 4-1 gives an overview of the patient characteristics and BHD prescription parameters of patients in whom long-term use of the Citrasate® concentrate was instituted. The amount of calcium in dialysate was preserved with the change of the A-concentrate type. Except for patient No2 who was treated by postdilutional on-line haemodiafiltration, all patients were on haemodialysis. All dialyser types used (both low- and high-flux) had synthetic membrane.

pat.	gender (M/F)	age (roky)	RRT dur. (month)	HD time (hod)	dialyzer type	A-conc. with AA	A-conc. with CA
1	M	79	18	4	low-flux	BIK F-61	BIC 320
2	F	83	12	4	high-flux	BIK F-61	BIC 320
3	F	73	18	4	low-flux	BIK F-61	BIC 320
4	F	82	37	4	high-flux	BIK F-61	BIC 320
5	F	70	10	4	low-flux	BIK F-61	BIC 320
6	F	61	10	4	high-flux	BIK F-61	BIC 320
7	F	3	3	4	low-flux	BIK F-61	BIC 320
8	M	76	21	4	high-flux	BIK F-61	BIC 320
9	M	88	52	4	low-flux	BIK F-61	BIC 320
10	F	73	10	4	low-flux	BIK F-61	BIC 320
mean		74,8	19,1	4			

Tab. 2-1 Characteristics of enrolled patients and BHD prescription data

During the year, the following standard biochemical and haematological parameters were measured in the investigated patients in two-month intervals: predialysis plasma levels of urea, creatinine, total calcium, phosphates, alcaline phosphatase, albumin and total plasma protein, CRP and haemoglobin. Also doses of heparin and erythropoietin were recorded an trends analysed. The spKt/V value was evaluated from the pre- and postdialysis plasma urea (Daugirdas equation, 2nd generation). The investigated parameters were averaged over the year period before the change-over to Citrasate® and compared to those reached during the year of the Citrasate® use – see the paragraph 4.3 Results below.

Flat decrease of heparin dose by 10% was undertaken in all patients after 3 months of the Citrasate® use and another decrease of 5-10% was done after another 2 months in part of the patients. Clinical acceptability of those decreases was based on routinely performed visual inspection of the dialyser appearance after rinse-back (three-stage assessment “clear-partially clotted-grossly red/clotted” was used).

4.3 Results

The results of successful reduction in heparin doses confirmed conclusions from the measurements of Activated Clotting Time (ACT) performed within the short-term study of bicarbonate haemodialysis and subsequently also of the on-line haemodiafitration, which suggested that prolonged ACT values and less steep decrease of ACT indicate feasibility of heparin dose reduction without any negative effects on coagulation.

Tab. 4-2 gives the mean values of selected biochemical and haematological parameters averaged over the period preceding the change-over to the Citrasate® concentrate (upper part of the Table) and over the period after it (lower part of the Table). Statistical significance of both means is displayed on the last line of the Table.

reference interval with conventional A-concentrate (with acetic acid)										
patient	S-Alb g/l	S-Prt. g/l	S-Ca mmol/l	S-P mmol/l	S-Alp μkat/l	PTH pmol/l	spKt/ V	S-Hb g/l	preHD TV(kg)	EPO U/týd
1	39,6	70,2	2,26	1,86	3,448	12	1,55	124,2	72,0	3600
2	37,2	65,6	2,33	1,94	1,592	14,8	1,52	122,2	77,8	
3	41,2	66,6	2,36	1,85	1,168	19,6	1,56	117,4	73,3	3600
4	34,4	65,6	2,36	1,85	1,412	31,6	1,54	119,0	70,4	
5	36,6	69,6	2,40	2,25	0,774	4,6	1,37	113,6	85,2	8000
6	32,4	57,4	2,28	2,38	1,344	3,6	1,74	109,4	53,2	7200
7	34,3	65,3	2,16	2,12	1,175	12,8	1,24	104,3	100,2	4000
8	32,4	56,0	2,13	1,42	1,71	20,4	1,66	103,0	59,4	
9	37,2	62,2	2,00	1,90	1,47	40,2	1,39	108,0	84,3	
10	36,8	74,2	2,17	2,30	1,012	8,6	1,25	108,2	84,7	10800
mean	36,21	65,27	2,24	1,99	1,51	16,82	1,48	112,9	76,0	5314
SD	2,887	5,598	0,126	0,284	0,735	11,64	0,167	7,48	13,60	3576
evaluated yearly interval with the Citrasate® concentrate										
1	38,4	67,7	2,17	1,89	2,78	19,3	1,58	115,2	73,3	2167
2	33,5	59,5	2,23	1,93	1,70	30,3	1,66	99,2	79,7	
3	37,7	61,3	2,29	1,73	1,47	23,8	1,70	111,3	72,7	4333
4	33,2	61,3	2,45	2,43	1,43	32,2	1,62	106,3	72,0	
5	37,3	66,8	2,33	1,91	1,04	9,0	1,43	111,3	86,9	6667
6	36,2	61,5	2,27	2,34	1,31	8,8	1,77	107,7	54,3	9000
7	36,0	66,2	2,19	2,15	1,55	22,5	1,33	117,3	105,3	4000
8	32,7	55,5	2,03	1,88	1,87	28,0	1,61	106,8	57,7	
9	38,0	63,3	2,03	1,95	1,73	46,3	1,41	117,0	86,7	
10	37,0	70,3	2,12	1,85	0,85	16,3	1,24	119,8	85,1	9000
mean	36,0	63,4	2,21	2,00	1,57	23,7	1,53	111,2	77,4	5024
SD	2,13	4,42	0,13	0,23	0,53	11,34	0,173	6,34	14,9	3397
stat. sgn..	ns	0,05	0,01	ns	ns	0,001	0,01	ns	ns	ns

Tab. 4-2 Mean values of selected biochemical and haematological parameters during the use of conventional A-concentrate (upper part of the Table) and during the yearly period of the Citrasate® A-concentrate use (lower part of the Table)

Despite the reduction in heparin doses, there was no decrease in the Kt/V. On the contrary, corresponding to the results of the previous short-term studies, the mean Kt/V slightly increased (from 1,48 to 1,53 with p= 0,01)

Very low, yet statistically significant decrease was detected in the total calcium level (the whole group mean decreased from 2,24 to 2,21 mmol/l). Possibly related to this was the increase in PTH level (from 16,2; range 12,0-40,2 to 23,7; range 19,3-46,3 pmol/l). This increase, however, still remains within recommended limits of the latest international guidelines and despite its statistical significance does not appear clinically relevant.

Although the total plasma protein level change reached the lowest level of statistical significance, both positive and negative changes were seen in individual patients. No change was seen in serum albumin and the mean body weight showed even slight increase.

Neither the mean haemoglobin level in the group nor their EPO doses exhibited any change with their transfer onto Citrasate® (slight decrease did not reach statistical significance).

Neither the mean level of Hb nor erythropoietin doses exhibited any change with the change in the A-concentrate (slight decrease seen did not reach level of statistical significance). However, erythropoietin therapy was not needed in all patients and evaluation of possible influence on that parameter was thus hampered by small size of the investigated cohort.

At the time of writing of this report, all the enrolled patients continue their treatment with the Citrasate® A-concentrate.

4.4 Conclusions:

■ Long-term follow-up confirmed the previously found slight increase in dialysis dose spKt/V after patients transfer to the Citrasate® concentrate.

■ Flat decrease in heparin dose (10%) was performed in all patients of the investigated cohort and another 10% decrease was found feasible in a subgroup of the whole cohort without any impact on visual appearance of the dialyser after rinse-back.

■ Assessment of nutritional parameters (serum albumin, total plasma proteins, body weight) did not reveal any unambiguos impact of the Citrasate® concentrate use.

■ Long-term use of the Citrasate® concentrate with unchanged content of calcium chloride in dialysate lead to only clinically insignificant decrease in pre-dialysis plasma levels of total calcium (assessed from the mean of the whole cohort).

■ In contrast to the changes seen in total plasma protein content (positive in some and negative in other patients), the PTH slightly increased in all patients after their transfer to Citrasate®.

5. Summary and conclusions

This paragraph of the report merely summarizes conclusions from separate studies with Citrasate® described in previous chapters, i.e. feasibility of the Citrastae® concentrate use in HD/HDF machines set for use of the conventional A-concentrate types with acetic acid (Chapter 1), short-term (pre-HD/pre-HDF and intradialytic) changes in biochemical parameters, procedure efficacy and coagulation status (Chapers 2, 3) and also impact of long-term use of citric acid-containing dialysate (Chapter 4 – focused in particular on possible reduction of heparin dose, consumption of erythropoiesis stimulating agents as well as parameters evaluated in the previous two short-term studies).

■ The A-concentrate acidified with citric acid instead of until now exclusively used acetic acid can be used with any dialysis machines marketed in the Czech Republic without any changes in machines settings. Also the control of sodium and bicarbonate concentration in dialysate remains fully functional over the entire built-in range.

■ Creation of the calcium-citrate complexes in dialysate when the Citrasate® concentrate is used results in lowered level of ionized calcium (by about 0,4-0,5 mmol/l) as compared to use of conventional A-concentrate with acetic acid. Due to absence of the reaction between the acid and sodium-bicarbonate, concentration of bicarbonate anion in the final dialysate is slightly higher (by about 1 – 1,5 mmol/l) than in dialysate prepared with any conventional A-concentrate type.

■ Both the short- and long-term studies with the Citrasate® concentrate revealed an increase in the dialysis dose Kt/V, both in haemodialysed and in haemodiafiltered patients.

■ Use of the Citrasate® concentrate decreases tendency to coagulation in the extracorporeal circuit, which has been quantitatively evaluated by means of changes in the time constant of the Activated Clotting Time.

■ In correspondence to this finding, possibility of reduction in heparin doses without any negative impact on coagulation in the extracorporeal circuit was shown in the long-term study.

■ Despite the above findings, measurement of the fibre bundle volume changes during the procedure (although their number was quite low) suggests that lower haemocoagulation may not be the reason or may not be the sole reason of higher efficiency of dialytic procedure when the Citrasate® concentrate is used, as the blood-membrane interactions during the first hour of dialysis appear modified, too.

■ Impact of the creation of calcium-citrate complexes with resulting lower ionised calcium concentration upon overall dialytic calcium balance appear negligible, at least with concentrate types in which amount of calcium chloride in final dialysate corresponds to 1,5 or 1,7 mmol/l. Only with the Cirasate® concentrate types with lower content of calcium chloride (1,25 mmol/l), slight decrease in pre-dialysis calcium levels can be expected. Partial increase in PTH levels seen in the long-term study may have been caused by this balance changes.

■ With regards to the results of the fibre bundle volume measurements (Chapter 3) and investigation of the transient leukopenia (Addenum 3.6, Chap. 3), study of the Citrasate® concentrate impact on general markers of biocompatibility appears highly desirable.

6. References

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7. **List of presentations and publications from the Strahov dialysis department related to the Citrasate® concentrate use**

Presentations:

Lopot F, Švára F, Polakovič V: Citrátový dialyzační roztok – technické aspekty, 32. kongres České nefrologické společnosti, Olomouc, 19.-21. 6. 2008, poster *(in Czech)*

Lopot F, Švára F, Polakovič V: Bicarbonate haemodialysis using A-concentrate with citric acid, 19th Danube symposium on Nephrology, Krems, August 2008, presentation

Lopot F: Use of citric acid containing A-concentrate in conventional haemodialysis machines, 37th EDTNA/ERCA Conference, Praha, September 2008, invited lecture

Bieliková L, Krejčová M, Veverková J: Citrátový dialyzační roztok CITRASATE, Brodův nefrologický den, Poděbrady, June 2009, presenation *(in Czech)*

Lopot F, Polakovič V: Bicarbonate haemodialysis and haemodiafiltration using citric acid-containing A-concentrate, 9th International Nephrological Symposium: Metabolic changes in chronic renal failure, Tatranská Lomnica, 21. 10. 2009, invited lecture

Published abstracts and articles

Lopot F: Use of citric acid containing A-concentrate in conventional haemodialysis machines, 37th EDTNA/ERCA Conference, Prague, September 2008, Abstract Book, p. 18 (abstract)

Lopot F, Švára F, Polakovič V: Bicarbonate haemodialysis using A-concentrate with citric acid (Citrasate), Aktuality v nefrologii, 15, 2009, č. 2, 105-110 *(in Czech, only abstract in English)*

Polakovič V, Lopot F: Bicarbonate haemodialysis and haemodiafiltration using citric acid-containing A-concentrate, 9th International Nephrological Symposium: Metabolic changes in chronic renal failure, in Aktuality v nefrologii, 16, 2010, 13 (abstract)

Švára F, Polakovič V, Lopot F: Long-term use of the Citrasate® A-concentrate in bicarbonate haemodialysis and haemodiafitrationi, Czech Nephrological Congress, Praha, June 2010 *(in Czech)*

BSc thesis

Jumr J: Balance studies on total and ionised calcium and citrate during the on-line haemo-diafitration with citric acid containing A-concentrate, BSc. Thesis, study programme „Health Care Technology“, 1st Medical faculty, Charles University, Prague, September 2009 *(in Czech, only abstract in English)*

8. Enclosures

presentations and publications on Citrasate® use in BHD and on-line HDF from the Strahov Dialysis Department as listed in Chapter 7 of this report