Biocompatibility pattern of a bicarbonate/lactate-buffered peritoneal dialysis fluid in APD: a prospective, randomized study

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Abstract

Background. In chronic ambulatory peritoneal dialysis, bicarbonate-buffered fluids, with their neutral pH and less advanced glycosylation end-products (AGE) and glucose degradation products (GDP), have better biocompatibility than conventional peritoneal dialysis (PD) solutions. That difference may be more beneficial in automated peritoneal dialysis (APD), due to its more frequent exchanges and longer contact times with fresh dialysate. We performed a prospective, randomized study in APD patients to compare the biocompatibility of conventional and bicarbonate/lactate-buffered PD fluids.

Methods. We randomized 14 APD patients to have APD with either conventional or bicarbonate/lactate-based fluids. After 6 months, both groups changed to the other solution. The overall observation period was 12 months. After 1 and 5 months and again after 7 and 11 months, phagocytotic and respiratory burst capacities of effluent peritoneal macrophages were determined. Plasma interleukin (IL)-6 and C-reactive protein (CRP) as well as effluent IL-6, CRP, transforming growth factor (TGF)-β1, AGE and CA125 concentrations were measured. Inflow pain was quantified using a patient questionnaire.

Results. Respiratory burst capacity remained unchanged and phagocytotic activity increased significantly during APD \((P < 0.001)\) with the bicarbonate/lactate fluid. Effluent IL-6 release was significantly lower than with the lactate fluid \((P < 0.05)\). While in the effluent TGF-β1 was unaffected, AGE concentration was lower after bicarbonate/lactate treatment \((P < 0.05)\). Effluent CA125 concentration, an indicator of mesothelial cell integrity, was higher \((P < 0.05)\) in neutral effluents. Finally, patients’ inflow pain diminished \((P = 0.05)\) when using the neutral fluid.

Conclusions. The use of a neutral PD fluid in APD improved patients’ inflow pain as well as biocompatibility parameters reflecting enhanced phagocytic activity of peritoneal macrophages, reduced constitutive inflammatory stimulation (IL-6), reduced AGE accumulation in the peritoneal cavity and better preservation of the mesothelial cell integrity. From the biocompatibility point of view, a neutral fluid with low GDP content can be recommended as the primary choice for APD.

Keywords: advanced glycosylation end-products; automated peritoneal dialysis; bicarbonate; biocompatibility; glucose degradation products; interleukin-6

Introduction

In peritoneal dialysis (PD), several in vitro and in vivo studies [1–3] have shown that an unphysiologically low pH and high glucose result in the formation of advanced glycosylation end-products (AGEs) [4] and glucose degradation products (GDPs) [5,6], which determine bio-in-compatibility. The cytotoxicity of low pH and high glucose is strongest immediately after the inflow of the solution and gradually declines within minutes of the dwell [3], for the residual intraperitoneal fluid and rapid equilibration within the peritoneal cavity diminish the adverse effects of the fresh dialysate. Thus, it may be hypothesized that automated peritoneal dialysis (APD), with its larger volumes of solution and its more frequent contact times with fresh and unphysiological dialysates, may increase the adverse effects of an acidic solution. This hypothesis is of clinical relevance, since the use of APD is becoming more common, due to potentially higher dialysis efficiency and ultrafiltration rates, lower rates of infections and higher quality of life [7,8].

The buffer bicarbonate, introduced recently, preserves a neutral pH in PD solutions [9]. The composition in a double-chamber bag resulted in a substantial
Clinically indicative of biocompatibility is the observation that neutral fluids buffered with either bicarbonate or a combination of bicarbonate and lactate are superior in terms of inflow pain in PD [9], an undesirable effect mostly attributed to the acidity. In vitro biocompatibility studies of neutral PD fluids have found diminished inhibition of cell viability and function [10]. Since peritoneal histology and function are often impaired by the long-term use of unphysiological solutions [11,12] and since PD necessarily means more frequent contact times with fresh dialysate, the use of neutral fluids low in GDP seems beneficial, especially in APD. However, to date, data on the long-term effects of neutrally buffered fluids with low GDP exist only for chronic ambulatory peritoneal dialysis (CAPD) [5,6] and not for APD patients. We, therefore, performed a prospective, randomized, cross-over study on chronic APD patients comparing a neutral, bicarbonate/lactate-buffered PD solution with an acidic, lactate-buffered fluid. The aim of the study was to assess the impact of a neutral fluid with low GDP content on intraperitoneal host defences, local inflammation, mesothelial cell integrity, AGE accumulation and inflow pain in APD.

Subjects and methods

Patients

The design of the study was prospective, randomized and cross-over. The study was not blinded, which would have been technically difficult and also did not seem to be necessary due to the A–B design, which allowed an intra- and inter-individual comparison of the data. Nevertheless, to minimize a bias in data analysis, all laboratory tests were performed blinded. After their written consents were obtained, 14 APD patients were randomized to have APD with either a conventional solution (Dianeal®; Baxter Healthcare SA, County Mayo, Ireland) (group A) or a bicarbonate/lactate-based neutral fluid (Physioneal®; Baxter Healthcare SA, County Mayo, Ireland) (group B). After 6 months, both groups changed to the alternate solutions, according to an A–B study design (Table 1). Of the original cohort, 12 patients completed the study. One patient was transplanted and another, initially having started with the neutral fluid, refused to accept the lactate fluid due to severe inflow pain. The baseline sociodemographic data and patients’ clinical characteristics are listed in Table 2. The patients were stable on PD, had no history of peritonitis and were adequately dialysed according to DOQI criteria (Kt/V ≥ 2.1 or wCrCl ≥ 63/l/1.73 m²). All patients had started dialysis with APD and were on PD for <1 year. Except for one patient, whose treatment volume increased from the standard 151 per day to 201, the APD regimen did not change during the observation period. That regimen was defined as ‘CCPD’ (continuous cycling PD) and consisted of a 151 1.36%-glucose, low-calcium (1.25 mmol/l) 8 h administration with various fill volumes. The CCPD protocol included, for the daytime, a last fill with 1.36% glucose that varied from 0.7 to 21 (1.52 ± 0.36 l) in fill volume. Additional daytime exchanges were not performed routinely. Due to high residual renal functions, glucose concentrations >1.36% were not needed. Glucose load did not differ between either group and no peritonitis was observed during the observation time. Except for pH (7.4 vs 5.5) and the buffer (bicarbonate 25 mmol/l + lactate 15 mmol/l vs lactate 40 mmol/l), the composition of the two fluids used did not differ.

Materials and methods

During the overall observation period of 12 months, biocompatibility tests were done after 1 and 5 months and after 7 and 11 months, respectively, according to the A–B design, changing the studied PD solution after 6 months (Table 1).

Peritoneal macrophages. Peritoneal macrophages were isolated from the 6-h dwell effluent of the last fill. To prevent cell adhesion to plastic materials, the effluent was drained into an ice-cold drainage bag, poured into tubes and immediately centrifuged (350 g, 5°C, 30 min). The cell pellets were resuspended in 1 ml phosphate-buffered saline (PBS; Serag Wiessner, Naila, Germany), recentrifuged and resuspended again. The number of monocytic cells was determined with light microscopy and cell viability was controlled with a conventional trypan blue test (Seromed, Pollenfeld, Germany). For phagocytosis assay, 2 \times 10^6 mononuclear

<table>
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<th>Group A</th>
<th>Lactate</th>
<th>Bicarbonate/Lactate</th>
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<tr>
<td>Group B</td>
<td>Bicarbonate/Lactate</td>
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Table 1. A–B study design with a total observation period of 12 months and a 6 month treatment period with each tested PD fluid. Biocompatibility tests were done after 1 (U1) and 5 months (U2) and after 7 (U3) and 11 months (U4)
cells were suspended in RPMI 1640 (Gibco BRL, Paisley, UK), which was supplemented with -glutamine (Gibco BRL) with a final concentration of 2 mM; penicillin/streptomycin (ICN Biomedicals, Meckenheim, Germany) with final concentrations of 50 U/ml and 50 lg/ml, respectively; amphotericin B (Gibco BRL) with a final concentration of 0.25 lg/ml; and 10% heat-inactivated fetal calf serum (FCS; Gibco BRL). The suspension was incubated (Inter Med Nunc, Nunclon Delta, Roskilde, Denmark) for 90 min with 5% CO2 at 37 °C. After washing with warm PBS, the cells were suspended in RPMI 1640 (Gibco BRL, Paisley, UK) for a period of 60 min, recording the relative light units every minute. After 60 min the suspension was incubated with 100 l of the zymosan solution. Unstimulated cells were washed with warm PBS and incubated for 30 min with 1 ml of a stock zymosan solution. The zymosan solution was made up of zymosan powder (Sigma-Aldrich Chemikalien GmbH, Steinheim, Germany) in 1 ml of KRPG buffer (Na2HPO4 + H2O 12.7 mM, d-glucose 11 mM, NaCl 120 mM, KCl 4.8 mM, CaCl2 0.7 mM, MgSO4 + H2O 1.2 mM) and 3 ml pooled human serum at 37 °C at a final concentration of 10 mg/ml. For phagocytosis, the zymosan solution was diluted 1:100 with the supplemented RPMI 1640. After incubation with zymosan, the cells were washed six times with warm PBS and air-dried overnight. After conventional cell-staining (Dade Diff-Quick; Dade AG, Düdingen, Switzerland), the number of cells that had incorporated one or more zymosan particles were counted under a light microscope.

To measure respiratory burst capacity by chemoluminescence, 0.25 × 106 cells in 50 l KRPG buffer and 50 l luminol solution in 50 l Aqua dest. (Sigma-Aldrich Chemikalien GmbH) were used in a white 96-well plate at 37 °C. Immediately prior to the test phase, macrophages were stimulated with 100 l of the zymosan solution. Unstimulated samples were used for control. Chemoluminescence was measured at 37 °C (MYL Microtiter Plate Luminometer; Dynex Technologies Inc., Chantilly, VA, USA) for a period of 60 min, recording the relative light units every minute. The results were integrated and expressed as ‘area under the curve’ (AUC).

Mediator concentrations in plasma and effluent. We measured plasma concentrations of interleukin (IL)-6 and C-reactive protein (CRP). IL-6 being a known marker of local peritoneal inflammation, was measured also in the effluent. Even though, to date, there is no evidence of its intraperitoneal production, in addition to measuring plasma CRP, we looked for CRP in the effluent. Mediator concentrations in the PD fluid were measured in the 6 h effluent of the last fill after nightly CCPD. The IL-6 and transforming growth factor (TGF)-β concentrations were determined by enzyme-linked immunosorbent assay (ELISA) with a double antibody DuoSet® system (R&D Systems Inc., Minneapolis, MN, USA), as described previously [13]. To measure and tag the specific instructions of the manufacturer, CRP was also determined by ELISA (CRP-ELISA KIT; Alpha Diagnostic Int., San Antonio, Texas, USA). The specificity of the assays, including potential matrix effects (dialysate), was tested by sample spiking and dilution. The assays were considered highly specific and no significant cross-reactivity was observed. All samples were tested simultaneously and in duplicate. The recovery rate of spiked samples was >92% for all assays used. The intra- and interassay variations were, respectively, 4.2% and 9.3% for IL-6, 7.4% and 10.6% for TGF-β and 6.2% and 10.8% for CRP. The sensitivity was 0.70 pg/ml for IL-6, 7 pg/ml for TGF-β and 0.35 ng/ml for CRP.

To measure effluent AGE concentrations, an ELISA plate was coated with AGE antigen (100 µl) and kept overnight at 4 °C (0.1%). The AGE antigen was composed by incubating 100 ml human albumin (20%) with 100 ml glucose (50%) for 6 weeks. After washing with 0.9% sodium chloride and 0.05% Tween-20 (Sigma-Aldrich Chemikalien GmbH), the plate was incubated with 300 µl of 4% bovine serum albumin (Sigma-Aldrich Chemikalien GmbH) in PBS for 2 h and air-dried. The effluent sample (50 µl) was mixed with 50 µl (0.25 µg/ml) mouse AGE antibody (Transgenic Inc., Ltd, Kumamoto, Japan), added to the plate and incubated for 1 h. After washing the plate, 100 µl biotinylated secondary antibody (Strept ABCComplex/HRP Duet Kit-mouse/rabbit; DAKO, Hamburg, Germany) diluted 1:10 in reagent diluent (PBS with 1.4% Opticlear; R&D Systems Inc.) and 0.05% Tween-20 was added and incubated for 1 h. After washing the plate again, 100 µl of streptavidin–horseradish peroxidase (ABCComplex/HRP; DAKO) 1:10 in reagent diluent was added and the plate was incubated for 15 min. After washing again and adding the detection reagent (1,2-phenylenedia-
mine-dihydrochloride), the generation of the immunocomplex was measured at 492 nm, the reference filter being of 620 nm. Since the ELI for the composed AGE ELISA is quantitative but without a definite concentration standard, the results are given as arbitrary units.

The effluent CA125 concentration was measured using an electro-chemoluminescence immunoassay (CECLIA) (Lecsys 2010; Roche Diagnostics, Heidelberg, Germany), the sensitivity of which was 0.60 U/ml.

**Dialysate inflow pain.** We used a patient questionnaire for quantifying pain during fluid inflow. The pain was quantified on a scale from 1 (no pain) to 5 (very intense). The subjects answered the questionnaire before beginning the study and at the end of the 5 month intervals of treatment with each of the neutral and the acidic solutions. Before randomization of the 14 patients, two patients were already with each of the neutral and the acidic solutions. Before

**Statistical analysis.** Results are expressed as means ± SD. The paired t-test and Wilcoxon analysis for non-parametric distribution of data were used for statistical analysis. An alpha error at $P < 0.05$ was judged to be significant.

**Results**

**Peritoneal macrophages**

The mean number of isolated peritoneal cells was $9.66 \pm 7.61 \times 10^6$ ($n = 48$) per draining bag. The number of cells did not change significantly during the observation period of 5 months and did not differ between the fluids. Cell viability was $96.7 \pm 2.3\%$ without significant differences. Respiratory burst capacity did not change during the observation period and did not differ between the bicarbonate/lactate- and the lactate-buffered fluid [bicarbonate/lactate 1 month: $189 \pm 126$ vs 5 months: $158 \pm 102$ (AUC); lactate 1 month: $171 \pm 102$ vs 5 months: $131 \pm 89$ (AUC)]. Phagocytic activity increased significantly during the 5 months on the neutral, double-chamber bag solution (from $14 \pm 9\%$ to $25 \pm 10\%$; $P = 0.001$) and was significantly higher after 5 months compared with the acidic fluid ($25 \pm 10\%$ vs $15 \pm 7\%$; $P = 0.013$; Figure 1).

**Mediator concentrations**

Plasma IL-6 concentrations did not change over time or differ between the two fluids (data not shown). IL-6 in effluent significantly increased during the observation period of 5 months with the acidic fluid ($33.2 \pm 21.6$ vs $41.2 \pm 21.3\, \text{pg/ml}$; $P < 0.05$; Table 3), whereas it remained stable during treatment with the bicarbonate/lactate fluid. After 5 months, the IL-6 concentration after APD with the acidic solution was significantly higher compared with the bicarbonate/lactate solution ($22.8 \pm 15.0$ vs $41.2 \pm 21.3\, \text{pg/ml}$; $P < 0.02$; Table 3). Plasma CRP concentrations did not change over time or differ between the two fluids (data not shown). Also, effluent CRP remained stable during treatment time with either fluid (Table 3). TGF-β1 effluent concentrations did not differ between the two fluids over time (Table 3). For both fluids, effluent AGE concentrations did not change significantly during the observation period (Table 3). However, after 5 months, the bicarbonate/lactate-buffered solution with reduced GDP content resulted in significantly lower effluent AGE concentrations compared with the acidic fluid ($1.68 \pm 0.12$ vs $1.80 \pm 0.10$ arbitrary units; $P = 0.01$; Table 3). CA125 did not change significantly during the observation period with either the lactate- or the bicarbonate/lactate-buffered solution. Comparing both solutions, CA125 was significantly higher after 1 and 5 months of treatment with the bicarbonate/lactate-buffered fluid with less GDPs (1 month: $14.5 \pm 4.9$ vs $10.8 \pm 3.8\, \text{U/ml}$; 5 months: $13.3 \pm 5.0$ vs $9.6 \pm 4.8\, \text{U/ml}$; $P < 0.05$; Table 3). The increase in CA125 after changing from the conventional to the neutral PD fluid, therefore, was already significant after 1 month of use.

**Dialysate inflow pain**

Dialysate inflow pain was significantly reduced using the neutral, bicarbonate/lactate-based fluid ($0.46 \pm 0.93$ vs $1.67 \pm 1.70$; $P = 0.05$; Figure 2). One patient refused the acidic fluid after the 6 months of bicarbonate/lactate treatment, due to severe inflow pain when using the conventional solution and, therefore, dropped out of the study.

**Discussion**

The results of our present study confirm the data in CAPD [5,6], which prove the beneficial effect of neutrally buffered PD fluids low in GDP with regard to peritoneal host defence parameters. Undisturbed phagocytic activity is important for an intact primary local host defence against bacteria and, hence, prevention of peritonitis. It is important to underline that even after the equilibration process of a 6 h dwell, the beneficial effect of the neutral fluid with respect to the peritoneal phagocytic capacity of macrophages was apparent. This observation is in contrast to that of a recent study, which did not find differences in cytokine release by peritoneal leucocytes isolated after an
Biocompatibility peritoneal dialysis fluid in APD

Table 3. Effluent concentrations of IL-6, CRP, TGF-β1, AGE and CA125 after 1 and 5 months of APD treatment with lactate- (pH 5.5) or bicarbonate/lactate-buffered (pH 7.4) fluids

<table>
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<tr>
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<th>Lactate (n = 12)</th>
<th>Bicarbonate/lactate (n = 12)</th>
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<tr>
<td></td>
<td>1 month</td>
<td>5 months</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>33.2 ± 21.6</td>
<td>41.2 ± 21.3α</td>
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<tr>
<td>CRP (ng/ml)</td>
<td>1.83 ± 1.92</td>
<td>3.32 ± 2.32</td>
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<tr>
<td>TGF-β1 (pg/ml)</td>
<td>169.4 ± 70.8</td>
<td>180.7 ± 57.2</td>
</tr>
<tr>
<td>AGE (arbitrary units)</td>
<td>1.82 ± 0.11</td>
<td>1.80 ± 0.10</td>
</tr>
<tr>
<td>CA125 (U/ml)</td>
<td>10.8 ± 3.8</td>
<td>9.6 ± 4.8</td>
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aLactate 1 month vs lactate 5 months; P < 0.05.
bLactate vs bicarbonate/lactate 1 month; P < 0.05.
cLactate vs bicarbonate/lactate 5 months; P < 0.02.
dLactate vs bicarbonate/lactate 5 months; P < 0.05.
eLactate vs bicarbonate/lactate 1 month; P < 0.05.
fLactate vs bicarbonate/lactate 5 months; P < 0.05.

overnight dwell of 8 h using either neutral or acidic fluids in CAPD [14].

As IL-6 is involved in the cytokine network within the peritoneal cavity, the constitutive intraperitoneal IL-6 release that we measured during 5 months of treatment with the neutral fluid was less than with the conventional solution. The decrease in peritoneal IL-6 synthesis could have been interpreted as either inhibition of cell function or less inflammatory stimulation of the cytokine-releasing cells, meaning the return to the ‘normal’ situation. Because no in vivo ‘physiological standard’ exists, since PD is an unphysiological situation per se, it is difficult to decide whether or not higher IL-6 levels indicate an inflammatory process or better cell function. In a recent in vitro study with mesothelial cells, we attempted to elucidate the underlying influence of uraemia as the ‘normal baseline situation’ before the start of PD. We found that mesothelial cells from patients on PD with conventional fluids showed an increase in IL-6 release compared with cells from uraemic patients before the initiation of PD [13], indicating that PD induced inflammation. These data as well as histological studies in rats [15] along with recent in vivo data by Cooker et al. [16] accord with the present results; they can most probably be interpreted as indicative of cellular stimulation induced by conventional PD fluids with relatively high GDP content. That effect is avoided by the use of a neutral fluid with lower amount of GDPs.

In accordance with our results, Cooker et al. [16] demonstrated lower IL-6 effluent concentrations in CAPD patients during treatment with a bicarbonate/lactate-based fluid. In that study of Cooker et al. during treatment with the conventional fluid effluent IL-6 concentrations tended to increase non-significantly, our present study shows a significant increase over time, indicating progressive inflammatory stimulation with the use of the conventional solution. It was demonstrated recently that the pro-inflammatory effect of conventional fluids, as measured by increased in vitro VICAM-1 expression and IL-6 release, is mediated by GDPs [17]. With regard to inflammation in PD patients, the importance of IL-6 has been underlined, pointing to an association between plasma IL-6 concentrations and poor clinical outcome [18]. One may hypothesize that, clinically, continuous inflammatory stimulation of the peritoneum followed by a potentially inadequate response to an additive stimulation might imply that peritoneal host defences have been compromised. Additionally, with the use of neutral fluids, lower background intraperitoneal inflammation might prolong the process of angiogenesis and peritoneal fibrosis in long-term PD patients, something which already has been proven in rats [15]. In this regard, in PD patients an association was reported between plasma IL-6 levels and high peritoneal solute transport, also suggesting the correlation of local inflammation, angiogenesis and peritoneal transport [19].

Whereas TGF-β1 effluent concentrations were unaffected, AGE concentrations after 5 months of treatment with the bicarbonate-buffered fluid, and use of the double-chamber system, were slightly but significantly reduced. Since, in good accordance with animal studies with neutral fluids [4], peritoneal AGE accumulation is thought to be substantially involved in ultrafiltration failure in long-term PD [20], it is not unreasonable to consider it an indicator of biocompatibility. With regard to the measurement of AGE concentrations, the authors are aware of the lack of standard controls.
in using the composed ELISA. On the other hand, the antibody used had broad specificity against different AGE compounds and the data are in accordance with what was expected, based on the reduction of AGE in the double-chamber bag. Furthermore, the data accord with experimental results in rats [4].

In line with data available for CAPD, CA125 was significantly higher after APD with the neutral fluid with low GDP content than with the conventional fluid. In a manner comparable to the known process of re-mesothelialization after peritonitis, the peritoneal mesothelial cell layer obviously seems to recover within a few weeks after the acidic fluid is replaced with a neutral one from a double-chamber bag containing lower GDP, which has been identified as the key factor in CA125 generation [5].

As the study was not performed blinded, the results of the patient questionnaires with regard to inflow pain need to be interpreted with caution. However, in APD most patients seemed to enjoy the lack of acidity, confirming CAPD data [9].

As we now believe to have proved the beneficial effect of a more biocompatible PD solution in APD, the question remains as to whether the benefit of using neutral fluids with low GDP is more pronounced in APD patients than in CAPD patients. As APD necessarily means larger volumes of solution with shorter dwell times and more frequent contact times with fresh and unphysiological dialysate, an additive negative impact on biocompatibility parameters might be possible. Since the present study did not look for an additive beneficial effect of a more biocompatible solution in APD compared with CAPD, no additional information on this issue is available and further comparative studies are needed.

We conclude that, in line with CAPD data, using a neutral PD fluid in APD over 5 months improved the patients’ inflow pain and biocompatibility parameters, as reflected in enhanced phagocytotic activity of peritoneal macrophages, reduced constitutive inflammatory stimulation (IL-6), a reduced AGE accumulation in the peritoneal cavity and better preservation of the mesothelial cell integrity.

Conflict of interest statement. None declared.

References
17. Welten AG, Schalkwijk CG, ter Wee PM, Meijer S, van den Born J, Beelen RJ. Single exposure of mesothelial cells to glucose degradation products (GDPs) yields early advanced glycation end-products (AGEs) and a proinflammatory response. Perit Dial Int 2003; 23: 213-221

Received for publication: 13.12.03
Accepted in revised form: 26.3.04