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Pyruvate-based peritoneal dialysate preserves neutrophilic oxygen consumption[1]

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KEY WORDS pyruvates; lactates; neutrophils; oxygen consumption; continuous ambulatory peritoneal dialysis; dialysis solution; acidosis; zymosan

AIM: To investigate effects of pyruvate- or lactate-based peritoneal dialysis solutions (P-PDS or L-PDS) on neutrophilic oxygen consumption and the role of the extracellular pH (pH_e) in cells' oxygen uptake. **METHODS**: Human neutrophils were incubated in P-PDS or L-PDS containing pyruvate or lactate 35 - 38 mmol·L^{-1} at various pH_e, respectively. Oxygen consumption rates by opsonized zymosan (OZ)-stimulated cells were measured polarographically, using a Clark-type oxygen electrode. **RESULTS**: L-PDS at an initial pH 5.2 dramatically inhibited the rate of oxygen consumption (2.2 nmol·$min^{-1}/10^6$ cells) by neutrophils, while the equally acidic P-PDS markedly improved the rate (6.4 nmol·$min^{-1}/10^6$ cells) ($P < 0.01$). However, P-PDS at pH_e 5.2 severely impaired the rate by cells, the same as pH_e 5.2 L-PDS. **CONCLUSION**: P-PDS preserved an oxygen consumption rate by OZ-stimulated human neutrophils, but in an acidi milieu it comparably deteriorated the ability of cells to consume oxygen, indicating that the pH_e of PDS plays an essential role in cellular oxidative metabolism. The superior biocompatibility of an acidic P-PDS was associated with its lower buffering capacity.

Continuous ambulatory peritoneal dialysis (CAPD) has been one of the conventional replacement therapies for chronic renal failure for more than two decades. But peritonitis that CAPD often complicates and to be considered one of the risk factors of patients' mortality remain a major problem. Commercially available peritoneal dialysis solutions (PDS) containing sodium lactate are considered to be one of major factors leading to susceptibility to CAPD-associated peritonitis[1-3]. Due to its bioincompatibility, mainly induced by its high concentration of lactate anions (35 - 40 mmol·L^{-1}) and low pH (5.0 - 5.5), PDS reduce phagocyte viability and induce apoptosis of mesothelial cells[1,2,4]. Of particular importance is that the low pH value of *dl*-lactate-based PDS (L-PDS) plays an essential role responsible for infectious peritonitis. L-PDS induced a prompt and substantial intracellular acidosis of phagocytes[5-7], resulting in marked inhibitions of superoxide production (O_2^-)[3,8], oxygen consumption[9], and various physiologic functions by peritoneal phagocytes and mesothelial cells[1,8]. In consequence, peritoneal phagocytes, as a first line of host defense in a peritoneal cavity, fail to kill invading microorganisms.

In pyruvate-based PDS (P-PDS), *dl*-lactate was replaced by equimolar pyruvate, the cytotoxicity was apparently neutralized[10,11]. P-PDS appreciably preserved O_2^- by neutrophils and evidently improved neutrophilic intracellular acidosis[12]. The purpose of this study was to elucidate the characteristics of neutrophilic oxidative metabolism by observing oxygen consumption rates in P-PDS and to demonstrate that the lower buffering capacity of P-PDS contributes to its superior biocompatibility.

MATERIALS AND METHODS

Preparation of test solutions P-PDS and L-PDS prepared in our laboratory had the same composition as the commercial Dianeal PD-2 PDS with 1.5 % dextrose and a pH of 5.2 (Baxter Healthcare, USA) except its lactate replaced by an equimolar pyruvate in P-PCS: sodium 132, calcium 1.75, magnesium 0.25, chloride 96, *dl*-lactate 40, and *d*-glucose 76 mmol·L^{-1}. The pH values were titrated with either HCl or

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NaOH 0.1 mol · L^{-1} to pH 5.2 and 7.4, respectively. The amount of HCl or NaOH required for each titration was < 2.0 mmol·L^{-1}.

Isolation of human neutrophils Neutrophils were isolated from the peripheral blood of healthy individuals, using Histopaque™-1077 gradient centrifugation at 500 × *g*, dextran sedimentation, and hypotonic lysis of erythrocytes[3]. Neutrophils were washed twice with Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution. A final suspension of neutrophils containing 1×10^{10} cells·L^{-1} was made in a pH 7.4, Ca^{2+}-free, phosphate-buffered saline solution (PBS). The viability of cells was evaluated with the trypan blue exclusion test.

Preparation of opsonized zymosan A Freshly opsonized zymosan A (OZ) was made on the day of each experiment[3]. Briefly, zymosan was boiled in physiologic saline for 30 min and resuspended in pH 7.4 PBS. The boiled zymosan was opsonized with an equal volume of fresh autologous serum at 37 ℃ for 30 min. After washing twice with PBS, OZ was resuspended in pH 7.4 PBS at a concentration of 20 g·L^{-1} and kept at 4 ℃ until just prior to use.

Measurement of oxygen consumption rate by neutrophils Oxygen consumption rates by neutrophils incubated in various PDS were measured polarographically[9]. Briefly, 6 mL of each PDS was placed in a Plexiglass chamber with a magnetic bar maintained at 37 ℃ with a warm water circulator on a stirring plate. A Clark-type oxygen electrode (YSI 4004 Clark Oxygen Probe, Yellow Springs Instrument, USA) was closely placed in the chamber just over the surface of the incubation solution, leaving no air space between them. Cell suspension and OZ at equal amount of 0.4 mL were injected separately into the chamber through a fine side port of the probe. The oxygen tension of the mixture was then monitored continuously for 10 min by polarography. The pH of each final neutrophil-riched mixture was determined before and after oxygen consumption tests. According to the calibration curve over temperatures, the oxygen concentration in an air-saturated buffered solution at 37 ℃ was in the realm of 220 mmol·L^{-1}[13], the rates of oxygen consumed by OZ-stimulated neutrophils incubated in various PDS were calculated by analyzing the slope of the oxygen disappearance curve obtained[9,13].

Oxygen consumption rate (nmol·min^{-1}/10^6 neutrophils) = 220 mmol·L^{-1} × Vol (L) × %/10^6 cells × min. Volume: total volume of mixture solution tested, *ie*, 6.8 mL, %: the rate of the slope of the oxygen disappearance curve obtained polarographically.

All the chemicals and reagents were purchased from Sigma, USA except a 60 % sodium *dl*-lactate from Pfanstiehl Laboratories, USA.

Experimental design

Effect of original pH value of PDS on oxygen consumption rate by neutrophils Neutrophils from each of 7 healthy subjects (age 31 ± 11 a) and OZ were incubated in pH 5.2 P-PDS and pH 5.2 L-PDS, respectively. Oxygen uptake was then recorded polarographically. Because of the presence of the pH 7.4 PBS, the resultant pH, *ie*, pH_e values of incubation mixture solutions rose to 7.0 ± 0.1 for the P-PDS mixture and 5.4 ± 0.2 for the L-PDS mixture, respectively. Both P-PDS and L-PDS at pH 7.4, therefore, the pH_e of the corresponding incubation mixture was 7.4 as well were also performed as controls. The final concentration of pyruvate or lactate in PDS was then diluted to 35 mmol·L^{-1} in this experiment.

The phenomenon that a pH value rose in mixture solutions including PDS occurs clinically. Ordinarily, when a 2-liter volume of a fresh conventional acidic L-PDS is instilled into the peritoneal cavity of patients, it meets a resident peritoneal fluid in 0.2-0.4 L with acid-base values approximating those of plasma. Consequently, the pH of the incoming acidic PDS is invariably higher.

Effect of PDS at pH_e 5.2 on oxygen consumption rate by neutrophils The pH values of both P-PDS and L-PDS were initially adjusted to < 5.2, using HCl 0.1 mol·L^{-1} (the quantities of HCl used for titration were < 1.0 mmol·L^{-1}). Neutrophils from another 7 healthy subjects (age 40 ± 13 a) were separately pre-incubated in the PDS at a constant pH_e of 5.2 for 5 min. Immediately after the preinubation, OZ and a trace amount of NaOH were injected individually into the pH_e 5.2 PDS/cell mixtures, resulting in an immediate increase in pH values of the mixture to 7.4, and oxygen uptake was recorded continuously. As controls, both PDS at pH 7.4, *ie*, the pH_e of PDS/cell mixture was

7.4 as well, were also tested for a 5-min preincubation. The concentration of pyruvate or lactate in PDS during the preincubation was 38 mmol·L^{-1} in this test. Statistical analyses were carried out, using ANOVA and the Bouferroni multiple comparison test.

RESULTS

The viable isolated neutrophils were >95 %. There were no significant changes in the pH of the mixtures at the end of incubations. The rate of oxygen consumption was dramatically suppressed in pH 5.2 L-PDS (2.2 nmol·min^{-1}/10^6 cells), compared with its pH 7.4 counterpart (7.6 nmol·min^{-1}/10^6 cells) ($P<0.01$), while the rate of pH 5.2 P-PDS was slightly lower ($P>0.05$) (Tab 1). P-PDS and L-PDS at pH 7.4 indeed showed a comparable rate of oxygen uptake. However, the rate of pH 5.2 P-PDS was appreciably higher (6.4 nmol·min^{-1}/10^6 cells) than that of pH 5.2 L-PDS ($P<0.01$). In this instance, cells were actually exposed to the pH_e of P-PDS mixture, which was increased to 7.0, but the pH_e of the corresponding mixture of L-PDS with an equally original pH of 5.2 was only converted to 5.4.

When cells were pre-exposed to a constant pH_e of 5.2 for 5 min, the rates of oxygen consumption were completely inhibited in spite of whether P-PDS or L-PDS was used as a medium and final pH value had neutralized after adding OZ (Tab 1).

Tab 1. Oxygen consumption rates (nmol·min^{-1}/10^6 cells) by neutrophils exposed to PDS. n = 7 human subjects, $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$ *vs* corrsponding pH 7.4 (a: pH_e=7.0, c: pH_e=5.4). $^fP<0.01$ *vs* L-PDS. $^iP<0.01$ *vs* corrsponding pH_e 7.4.

pH	P-PDS	L-PDS
PDS original pH		
5.2	6.4±2.6^{af}	2.2±2.1^c
7.4	7.1±2.7	7.6±3.7
Cells/PDS mixture pH_e for 5-min preincubation		
5.2	1.4±1.1^i	1.3±0.8^i
7.4	10.2±2.0	8.6±2.3

Regardless of the pH value, no difference in rates of oxygen consumption by neutrophils was seen between the two PDS ($P>0.05$) when pH_e values of cells in cells/PDS mixtures were comparable.

DISCUSSION

Results in this study showed that an acidic P-PDS had a rate of oxygen consumption close to that of a neutralized P-PDS and the rate of the acidic P-PDS was significantly higher than that of the equally acidic L-PDS (Tab 1). In this regard, the pH_e of the former rose actually to 7.0, and that of the latter amounted only to 5.4. Our previous data also indicated that O_2^- of an acidic P-PDS was similar to that of a neutralized P-PDS and markedly greater than that of the equally acidic L-PDS (data not shown). These results strongly confirm that the biocompatibility of P-PDS is quite superior to that of L-PDS as indicated by Mahiout *et al*[10,11]. However, the present data also demonstrated that oxygen consumption rates would be comparable as long as the pH_e to which neutrophils exposed, in fact, was equal despite whether P-PDS or L-PDS was a medium. This study indicated again that the pH_e of PDS played a crucial and fundmantal role in the biocompatibility at a given high concentration of the base (35 – 38 mmol·L^{-1} in present experiments).

The oxidative metabolism of cells is closely related to their intracellular pH (pH_i), which directly modulates O_2^- of neutrophils. Although the mechanism remains incompletely understood, the optimal pH value of the major oxidoreduction enzyme, NADPH, which is located in the plasma membrane and involved in cell's respiratory burst and oxidative metabolism, is between 7.0 and 7.5. Evidence showed that an acidic P-PDS brought about higher pH_e and pH_i values of neutrophils as compared to an equally acidic L-PDS, however, the acidic P-PDS and L-PDS at an identical pH_e could induce comparable degrees of intracellular acidosis in neutrophils[12]. It is reasonable to presume that the higher oxygen consumption rate of P-PDS is related to its higher pH_e through the correspondingly higher pH_i modulation.

Present results are consistent with the hypothesis that an H^+/lactate$^-$ cotransport system exists in the plasma membrane of human neutrophils[14]. The specialized cotransporter in human neutrophils, which accounts for all of

lactate anion uptake, is strongly pH-dependent: extracellular acidification enhances the rate of lactate influx across the membrane of cells, while alkalinization inhibits the process. Along with lactate uptake, equivalent amounts of H^+ transport in symport into the cytoplasm by the carrier, resulting in an accumulation of intracellular protons and a resulting suppression of respiratory burst response and oxidative metabolism of cells. The affinity of the carrier for pyruvate is identical to that for lactate, and no significant undissolved molecules of pyruvic or lactic acid influx could be detected in human neutrophils[14,15]. In peritoneal macrophages, most lactate uptakes were also mediated by the proton cotransport system in a pH-dependent manner. Although the improvement of cellular energy metabolism in P-PDS might be involved[10,11], our results demonstrated that in the presence of a high concentration of the bases, the rate of oxygen consumption by neutrophils depended largely on the pH_e of PDS, to which cells actually exposed, via pH_i. It should be noted that because the pK_a of pyruvic acid is 2.49, while the pK_a of lactic acid is 3.9, the buffering capacity of the buffer pair in P-PDS is weaker than that of buffer pair in L-PDS[12]. The acidic P-PDS was unable to resist the alkalining influences of a pH 7.4 PBS in this experiment as efficiently as an equally acidic L-PDS, so that the resultant pH of the mixture, *ie*, the pH_e of incubated neutrophils was higher in P-PDS (7.0) than in L-PDS (5.4). Clinically, during CAPD cycles the residual fluid with an acid-base value similar to that of corresponding plasma will raise the pH of the residual fluid/PDS mixture to a higher level if the PDS is pyruvate-based than when it is lactate-based. A higher pH of PDS in a peritoneal cavity, *in vivo*, should preserve the function of peritoneal phagocytes and mesothelial cells, thus enabling the acidic P-PDS to be superiorly biocompatible.

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丙酮酸盐腹膜透析液改善中性白细胞的氧耗量[1]

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关键词 丙酮酸盐; 乳酸盐; 中性白细胞; 氧消耗; 非卧床腹膜透析, 透析液; 酸中毒; 酵母聚糖

目的: 观察丙酮酸盐和乳酸盐腹膜透析液 (P-PDS, L-PDS) 在不同 pH 条件下对中性白细胞氧耗量的影响. **方法**: 中性白细胞分别与 P-PDS 及 L-PDS 在细胞外液 pH 5.2 和 7.4 及 PDS 原始 pH 5.2 的条件下温育, 观察经调理的酵母聚糖为刺激剂的细胞氧耗量. **结果**: 与 L-PDS 相比较, 酸性的 P-PDS 仍能显著保持细胞的耗氧功能, 但在相同的细胞外液 pH 条件下, 两者的耗氧能力无差别. **结论**: PDS 的 pH 值对中性白细胞氧化代谢有重要影响, P-PDS 优越的生物相容性与其低缓冲容量有关.