Physiol. Res. 54: 697-699, 2005

RAPID COMMUNICATION

Apoptosis as an Early Event in the Development of Multiple Organ Failure?

J. PACHL, F. DUŠKA, P. WALDAUF, M. FRIC, J. FANTA[1], E. ŽĎÁRSKÝ[2]

Department of Anesthesiology and [1]Surgery, [2]Institute of Pharmacology, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Received May 10, 2005
Accepted May 27, 2005
On-line available April 26, 2005

Summary

We have recently developed a simple method of plasma free DNA detection, which enables us to distinguish between apoptotic and genomic (necrotic) DNA. After applying this method to the critically ill, we revealed apoptotic DNA on the day of admission to be higher than later when multiple-organ failure developed. Moreover, apoptotic DNA contributed to total plasma DNA much more than DNA from necrotic cells and its increase predicted future development of multiple-organ failure and death.

Key words

Multiple-organ failure • Early apoptosis • Apoptic DNA • Plasma free DNA

Introduction

Multiple-organ dysfunction syndrome (MODS) is the most common cause of death in intensive care units. According to the classical etiologic concept, the initial insult damages target organs and leads to tissue necrosis, which initiates a systemic inflammatory response and an alteration of whole body hemodynamics, microcirculation and oxygen metabolism. This leads in turn to distant organ damage (by both necrosis and apoptosis). The role of cell doom in the process is reflected by plasma levels of DNA which has been proven as a prognostic tool in the critically ill patient (Wijeratne *et al*. 2004). We recently developed a new plasma DNA assay based on electrophoretic separation on agarose gel after a single phenol extraction step. This method enables us to distinguish between DNA released from apoptotic and necrotic cells (aDNA, gDNA). Although other types of tissue-specific mRNAs which circulate in the plasma (Taback *et al.* 2004) may be obtained, this is beyond the scope of our investigation. Hotchkiss *et al.* (1999) showed that aDNA is present in the plasma of intensive care patients. To our knowledge, this is for the first time that both aDNA and gDNA are separated and quantified independently in intensive care patients. When we optimized our new assay in comparison with the nowadays most frequently used methodology – glass milk based DNA isolation and real-time PCR quantification of β-globin gene fragment (gmRT-PCR) – we found that there is a higher proportion of gDNA over aDNA in gmRT-PCR assay. This is obviously due to a higher absorption of longer

(genomic) DNA on a glass surface (Krawetz *et al.* 1986, Pachl and Žďárský 2004). However, the trend of the plasma DNA content to decrease, as measured by gmRT-PCR, is due to the fact that the gDNA usually forms less than 10 % of DNA content of the plasma.

In an attempt to reveal how apoptosis and necrosis contribute to secondary organ damage, we measured the respective DNA plasma levels in the broad spectrum of intensive care unit patients during the course of their critical illness. Our pilot study was performed at a Department of Critical Care of the University Hospital and included 94 critically ill (APACHE II 22.7±8.8 (SD). Forty-seven patients with major trauma and 47 patients admitted from medical or surgical departments (79 survived and 15 died) and 86 age-matched healthy controls. After the protocol had been approved by the local Ethics Committee and the studied subjects (or their nearest of kin) gave their informed consent, we measured plasma DNA levels released from apoptotic and necrotic cells on the day of admission as well as on the 3rd and 5th consecutive days (DNA I, II, III).

Withdrawal from the central venous catheter provided 2.7 ml of blood in collection tubes (Sarstedt Monovette EDTA K) containing 1.6 mg EDTA as anticoagulant. The blood was centrifuged at 1000 g/5 min at room temperature. After centrifugation the plasma was collected with a pipette, placed into Eppendorf tubes and frozen to –40 °C until analysis.

For the analysis the plasma was defrosted and 150 µl were centrifuged at 24 000 x g (18,000 rpm/min in a Hereaus M3 centrifuge) for 5 min. Sixty microliters of plasma were mixed with 40 µl of a phenol/$CHCl_3$/2-butanol (4:1:1) mixture and stirred thoroughly.

Ten microliters of the centrifuged product were then mixed with 1 µl of diluted SYBR Green solution (1:5000) and 1 µl of 40 % dextran blue and stirred well. After that, the mixture was transferred to a 12-cm plate 8 % agarose gel of 0.5 mm thickness. Electrophoresis (sequencer ABI Prism 377) in TBE buffer was run 2 hours at 1000 V voltage and temperature maintained by a water cooling circuit at 25 °C. Readout of results was performed in a fluorescence window of 550 nm.

In accordance with previous publication (Wijeratne *et al.* 2004) we found substantially increased total aDNA and gDNA levels in the critically ill when compared with healthy controls ($p<0.001$). The contribution of aDNA to the surplus of total plasma DNA in the critically ill patients was ~16fold greater than the contribution of gDNA from necrotic cells. Even more surprisingly, aDNA levels were highest on the day of admission and declined thereafter ($p<0.001$), while the opposite was true for gDNA derived from necrotic cells ($p<0.001$) (Fig. 1). Apoptotic DNA concentration in samples collected from medical patients on the day of admission significantly differed in survivors and non-survivors (Fig. 2) with mortality prediction ($p<0.05$, logistic regression, ROC: AUC 0.71, for the level of apoptotic DNA 250 % - sensitivity 70 %, specificity 85 %) and development of MODS expressed as SOFA score in the whole evaluated group (Vincent *et al.* 1996) on the 3rd day ($p<0.05$, $r^2 = 0.05$, Spearman r = 0.22). The APACHE II score on the day of admission predicted a necrotic DNA value on day 5 in a group of major trauma patients ($p=0.01$, $r^2 = 0.08$, Spearman r = –0.28).

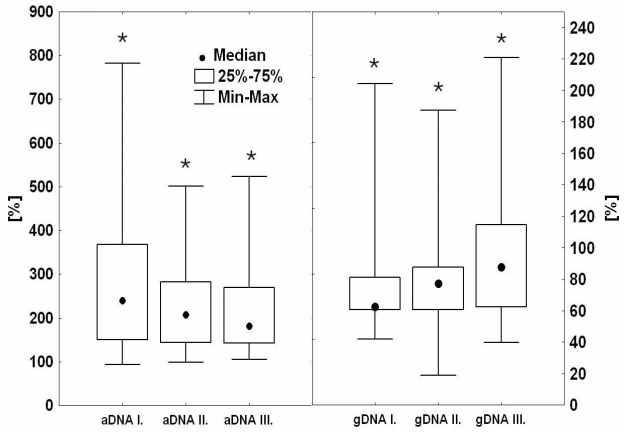

Fig. 1. Plasma concentrations of DNA derived from apoptotic (aDNA) and necrotic (gDNA) cells. Data are expressed in % of values obtained in healthy controls (aDNA, 129±10 ng/ml, gDNA 8.4±1.0 ng/ml, means ± S.D). P-value from the Mann-Whitney test.

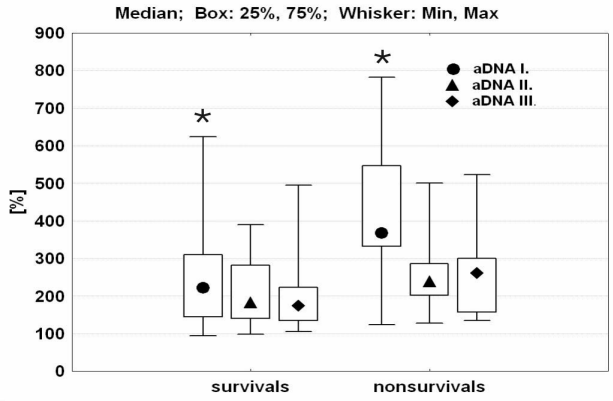

Fig. 2. Levels of plasma apoptotic DNA (aDNA) in patients (survivals and nonsurvivals) during the time course of critical illness expressed as % of the values obtained in healthy controls. P-value by ANOVA and the Fischer LSD test.

The most surprising fact in our study was the trend in concentrations of both aDNA and gDNA. Apoptotic DNA is highest at the time of admission to the intensive care unit, i.e. usually within 120 min after the time of injury in the multiple trauma group and sometimes as early as 20 min. This time seems to be insufficient for the induction of apoptosis by extracellular signals. Possibly, the primary insult causes partial cellular damage, e.g. mitochondrial effects (Crouser *et al*. 2002) by inducing apoptosis in the cell *via* an intrinsic pathway. We expected gDNA to reflect the severity of primary organ damage and apoptosis to play a role later when secondary organs are impaired by systemic inflammation followed by Systemic Inflammatory Response Syndrome (SIRS). In fact, the opposite was true. According to our results, it seems that the primary insult induces apoptosis to an extent related to its severity which subsequently declines in both survivors and non-survivors while the increment of DNA from necrotic cells during the course of the illness seems to reflect secondary organ damage.

Our study is the first human study showing the role of apoptosis in the earliest events of critical illness and confirms previous animal data (Guan *et al.* 2002). We also wish to bring an attention to this field as further studies are urged to confirm our results and detect which tissues are the sources of plasma apoptotic DNA. Possibly, new therapeutic strategies for the prevention of multiple-organ damage syndrome could result.

Acknowledgements

The project was supported by the IGA MZd 7737-3.

References

CROUSER ED, JULIAN MW, BLAHO DV: Endotoxin-induced mitochondrial damage correlates with impaired respiratory activity. *Crit Care Med* **30**: 276-284, 2002.

GUAN J, JIN DD, JIN LJ: Apoptosis in organs of rats in early stage after polytrauma combined with shock. *J Trauma* **52**: 104-111, 2002.

HOTCHKISS R, SWANSON PE, FREEMAN BD, TINSLEY KW, COBB JP, MATUSCHAK GM, BUCHMAN TG, KARL IE: Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. *Crit Care Med* **27**: 1230-1251, 1999.

KRAWETZ SA, STATES JC, DIXON GH: Isolation and fractionation of total nucleic acids from tissues and cells. *J Biochem Biophys Methods* **12**: 29-36, 1986.

PACHL J, ŽĎÁRSKÝ E: Both circulating DNA and RNA data in plasma are simply available and useful in critical care. *Intensive Care Med* **30** (Suppl 1): 69, 2004.

TABACK B, POON DS: Circulating nucleic acids and proteomics of plasma/serum: clinical utility. *Ann N Y Acad Sci* **1022**: 1-8, 2004.

VINCENT JL, MORENO R, TAKALA J, WILLATTS S, DE MENDONCA A, BRUINING H, REINHART CK, SUTER PM, THIJS LG.: The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive Care Med*. **22**: 707-710, 1996.

WIJERATNE S, BUTT A, BURNS S, SHERWOOD K, BOYD O, SWAMINATHAN R: Cell-free plasma DNA as a prognostic marker in intensive treatment unit patients. *Ann N Y Acad Sci* **1022**: 232-238, 2004.

Reprint requests

J. Pachl, University Department of Anesthesiology and CCM, Third Faculty of Medicine, Charles University, Šrobárova 50, 100 34 Prague 10, Czech Republic. Fax: +420.267163125. E-mail: pachl@fnkv.cz.