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Contribution of the monocyte to thrombotic potential

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Abstract

The circulating monocyte exhibits the capacity to initiate and accelerate the coagulation cascade. We have devised a simple whole blood clotting assay which quantitates the monocyte's contribution of procoagulant (a marker of monocyte activation) to the clotting process and in addition, measures the *in vivo* activation of the cell. Citrated whole blood is added to saline and the recalcification time (RT) determined without incubation (RT control), with two hours incubation (RT saline), or added to endotoxin with incubation (RT endotoxin). The reduction in value between RT control and RT saline is a measure of monocyte activation *in vivo*. The reduction in time between RT saline and RT endotoxin is a marker of monocyte activation *in vitro*. This simple test enables the measurement of monocyte activation and this cells contribution to the hypercoagulability described in many disease states.

Introduction

Recent evidence has stressed the important contributory role of the monocyte to the extrinsic pathway of the coagulation process. Isolated monocytes have been shown to generate procoagulant activity in both healthy individuals as well as in individuals with a variety of diseases and conditions, even if not stimulated with endotoxin. Techniques for demonstrating this property of the monocyte have been laborious, expensive and not adaptable to routine clinical application [1]. Incubation of citrated blood with endotoxin at increasing concentrations for increasing periods of time results in proportionately reduced recalcification time (RT) due to procoagulant generation [2]. It is well established that many disease states are associated with accelerated coagulation, which may be related to generation of monocyte procoagulant activity when compared to normal controls. Since the monocyte is the only circulating cell capable of generating significant procoagulant activity, addition of endotoxin to whole blood provides procoagulant release which is also a marker of monocyte activation. It should be emphasized that procoagulant activity is directly related to prior activation of this cell *in vivo*. This information has led to the development of a simple, reliable and clinically applicable test to measure endotoxin activated RT in a variety of disease states in which hypercoagulability plays a basic pathophysiologic role [3–8]. In earlier studies, the RT were determined after incubation of blood with saline and with endotoxin. Alteration in the RT when compared with healthy controls indicated the degree of tissue factor release by the monocyte. However, by this technique, assessment of prior activation of the monocyte *in vivo* could not be assessed. In order to determine if prior monocyte activation had taken place an additional parameter in which the RT was

determined after the addition of saline but without incubation was studied (RT control). Experience with this modification of the test is presented in this communication.

Materials and methods

Peripheral venous blood from 21 apparently healthy volunteers was drawn into 5 ml siliconized glass tubes containing 0.5 ml of 3.8% buffered citrate. Citrated whole blood 1.0 ml was added to 12×75 mm plastic tubes containing (1) 20 µl saline, (2) 20 µl saline, (3) 20 µl saline containing 10 µg of *E. coli* endotoxin 055:B5W. Sample 1 was brought to 37 degrees and the RT determined (RT control). Samples (2) (RT saline) and (3) (RT endotoxin) were incubated at 37 degrees for two (2) hours after which the RT was determined on each sample.

Results

The RT $\pm$ SD (min) for 21 blood samples are as follows: RT control 6.33 ± 1.3, RT saline 5.33 ± 1.0, and RT endotoxin 4.21 ± 0.9 min. There was a significant difference $p < 0.001$ for RT control vs RT saline and RT saline vs RT endotoxin (paired *t*-test).

Discussion

The RT control value is a measure of the *in vivo* coagulation state. The RT saline values are reduced versus RT control values because the *in vivo* stimulated monocytes continue to generate procoagulant activity during incubation even without stimulation. In other words, the difference between RT control and RT saline values is a measure of prior activation of the monocyte *in vivo*. RT endotoxin values are reduced further since the *in vitro* stimulation by endotoxin results in increased monocyte activation with resultant procoagulant generation.

That these RT values are significantly different demonstrates the prominent role of the monocyte in the overall clotting process, which is not evaluated by the conventional plasma clotting tests and assays. This simple test enables the measurement of monocyte activation and the contribution of this cell to hypercoagulability.

References

[1] R. L. Edwards, F. R. Rickles and M. Cronlund, *Abnormalities of blood coagulation in patients with cancer*. J. Lab. Clin. Med. *98*, 917–928 (1981).

[2] B. Osterud and E. Bjorklid, *The production and availability of tissue thromboplastin in cellular populations of whole blood exposed to various concentrations of endotoxin*. Scand. Haematol. *29*, 175–184 (1982).

[3] C. R. Spillert, C. T. Curtis and E. J. Lazaro, *Assessment of monocyte function in the diagnosis of breast disease*. South Med J *81*, 164–166 (1988).

[4] J. N. McCullough, C. R. Spillert and E. J. Lazaro, *A direct correlation between injury severity and two markers of the functional status of the immune system*. Circ. Shock. *31*, 309–316 (1990).

[5] C. R. Spillert, J. T. Milazzo, W. D. Suval and E. J. Lazaro, *Hypercoagulability in arterial disease*. Angiology. *40*, 886–889 (1989).

[6] C. R. Spillert, V. J. Milazzo, J. A. Pastena and E. J. Lazaro, *Prediction of clinical course of diabetes utilizing a simple coagulation test*. J. Natl. Med. Assoc. *81*, 81–83 (1989).

[7] K. S. Dasmahapatra, N. R. Cheung, C. R. Spillert and E. J. Lazaro, *An assessment of monocyte procoagulant activity in patients with solid tumors*. J. Surg. Res. *43*, 158–169 (1987).

[8] C. R. Spillert, M. P. Hafstein, V. Lanke and E. J. Lazaro, *Increased thromboplastin production in multiple sclerosis: An immunologic defect?* Ann. NY Acad. Sci. *475*, 345–346 (1986).