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19 年入学的医检本科，需要修改学校名称为“湖北民族大学科技学院”

湖北恩施学院

文献翻译

旋转血栓造影仪在人体纤维蛋白溶解和凝血系统激活模型中的验证

学　　院　宋体小 3 号

专　　业　宋体小 3 号

班　　级　宋体小 3 号

学　　号　宋体小 3 号

姓　　名　宋体小 3 号

指导教师　宋体小 3 号

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摘　要

背景：血栓弹力图(TEG)是一种全血检测方法，用于评估血栓形成和血栓溶解过程中的粘弹性特性。旋转血栓造影仪(ROTEM)已经克服了传统 TEG 的一些限制，并在一些凝血疾病的临床环境中作为护理点设备使用。内毒素血症导致全身凝血系统和纤溶酶的激活。目的：我们验证了旋转式血栓弹力图对内毒素的诱导，组织因子触发的凝血和纤溶反应敏感，其测量是否与凝血和纤溶的生物体液标记物相关。患者和方法：20 名健康男性志愿者参加了这项随机安慰剂对照试验。志愿者接受 2ng kg^{-1} 个国家参考内毒素或生理盐水。结果：内毒素血症可显著缩短病程。凝血时间(CT)下降 36%(CI：0.26-0.46;P< 0.05)与血浆凝血酶原片段峰值水平(F_{1+2})呈明显的负相关(r=-0.83,P<0.05)。此外，内毒素输注 2h 后最大溶出量(ML)比安慰剂或基线提高 3.9 倍(CI:2.5-5.2)(P<0.05)。峰值 ML 与峰值组织型纤溶酶原激活物(t-PA)值明显相关(r=0.82,P<0.05)。ROTEM 参数凝块形成时间和最大凝块硬度不受 LPS 输注影响，而血小板功能分析仪(PFA-100)关闭时间减少。结论：旋转血栓造影仪(ROTEM)可以检测全身体内凝血激活的变化，重要的是，它是一种护理点设备，对人的纤溶变化很敏感。体外测量 CT 和 ML 分别与体内建立的凝血激活(F_{1+2})和纤溶(t-PA)标记物相关。

关键词：凝血；内毒素；纤溶；血栓弹力图

1 介绍

血栓弹力图(TEG)是一种评价血栓形成和溶栓过程中，粘弹性特性的方法。通过使用全血，可以获得凝血过程和纤溶过程各阶段的各种参数(血浆因子，血小板和白细胞)累积效应相关的信息[1-2]。因此，与标准的凝血分析方法相比，TEG的应用具有几个优点，前者反映出了止血过程的分离，还包括部分非生理性的激活剂。

最近，改进的旋转血栓造影仪(ROTEM）它克服了传统 TEG 的一些局限性。ROTEM 非常坚固，不受振动或机械冲击的影响。通过使用电子移液管，再现性和性能得到了提高。此外，根据测量的参数，ROTEM 结果可早于 15 分钟至 1 小时。因此 ROTEM 的应用会使临床更快速的做出适当决策。

血栓弹力描记术已经运用在多种临床当中。例如用于监测肝移植过程中的止血和心脏外科手术，已被证明在高凝和高纤溶情况是一种快速诊断和鉴别的护理点设备[3-4]。

内毒素血症导致人体凝血系统的全身激活和纤维蛋白溶解[5-6]。因此，它是一个很好的检测抗凝药物的模型[7-9]，以及诊断性凝血试验和纤溶标志物[10-12]。

目前尚不清楚 ROTEM 对体内凝血的全身性变化敏感，或者其凝血和纤维蛋白溶解的措施是否分别与凝血酶形成和组织纤溶酶原激活物（t-PA）或纤溶酶原激活物抑制剂（PAI-1）水平的内源性变化相关。因此，当前的研究只是在定义明确的人类内毒素诱导的，模型中表现 ROTEM 的功能特性，该模型由内毒素诱导，组织因子（TF）引发凝血[8-13]和肿瘤坏死因子（TNF-a）引发纤维蛋白溶解[14]。我们假设 ROTEM 可能是一种全血检测方法，它不仅可以像其他两种检测方法一样检测凝血的变化[11-12]，而且还可以检测人内毒素血症中纤维蛋白溶解的变化。

2 患者和方法

2.1 研究设计

该研究得到维也纳医科大学伦理委员会的批准，所有参与者都给予了书面知情同意。20 名健康男性志愿者[年龄 26 岁(95%可信区间 22-29)]参加了一项安慰剂对照试验。对照组为 4 名志愿者(4:1 随机)。医学筛选包括病史、体格检查、实验室参数、病毒学和标准药物筛选。此外，对研究对象进行遗传性血栓性血友

病检测，即因子 VLeiden、蛋白 C 和 S 缺乏，以减少内毒素诱导的凝血激活[8]的潜在风险。排除标准是定期或近期服用药物，包括非处方药(OTC)，以及病史或实验室参数的临床相关异常。我们的内毒素模型的实验过程已经在其他试验中详细描述了[7-8]。简单地说，志愿者们在经过一夜的禁食后，于早上 8 点进入研究病房。在整个研究期间，参与者被限制卧床休息，并在注射 LPS 后禁食 8.5 小时。在目前的研究中，志愿者接受了 2ng/kg^{-1} 的血红蛋白或 1 个国家参考内毒素（LPS，大肠杆菌；USP，Rockville，MD，美国）或 0.9％NaCl 作为安慰剂。给予生理盐水溶液（200mL h^{-1}），以维持所有受试者在最初 6 小时内禁食的充足水分。

2.2 采样和分析

根据先前试验[8, 15-17]（F_{1+2}: 0, 2, 4, 6, 8, 24；t-PA：0、2、4、24；PAI-1：0、2、3、4、24；纤溶酶抗纤溶酶复合物（PAP）：0、1、2、4、8、24；CEPI-CT：0、2、4、8、24；VWF-Ag：0、4、24，血小板计数：0、1、2、3、4、6、8、24；ROTEM 参数：0、1、2、3、4、6，LPS 输注后 8 和 24 小时。通过反复静脉穿刺将血样收集到柠檬酸或乙二胺四乙酸（EDTA）抗凝真空管中（Becton Dickinson，维也纳，奥地利）。通过在 2000g（在 4℃下 15 分钟）离心获得血浆，并在 80°C 下以 0.5mL 等分试样保存直至分析。大多数凝血和炎性参数通过酶免疫测定法（EIA）进行测定。凝血酶原片段的血浆水平（Enzygnost F_{1+2} micro；Dade Behring，德国马尔堡）用作体内凝血酶生成的标志物和血浆 t-PA 水平（t-PA Kit；Technoclone，维也纳，奥地利），纤溶酶激活剂抑制剂（PAI-1，Actibind Kit；Technoclone，维也纳，奥地利）和 PAP（PAP micro；DRG International，East Mountainside，NJ，美国）使用酶联免疫吸附测定法（REAADS VWF Ag Test Kit；Corgenix，CO，美国）测量血浆中血浆血管性血友病因子的水平。

2.2.1 ROTEM 修正的旋转血流弹性成像

TEG 和 ROTEM 凝血分析仪(Pentapharm,Munich,Germany)之前已经详细描述过[1-18]。简单地说，TEG 凝块形成和随后的纤维蛋白溶解过程中测量剪切弹性模量。ROTEM 使用滚珠轴承系统进行功率转换，使其更不容易受到机械应力、运动和振动的影响。

全血采集于 3.8%柠檬酸钠管中。在文献中，对于室温保存后的样品的稳定性和重现性存在不一致的文献。根据文献[9-21]，在 30 分钟至 4 小时内，TEG 测量产生了准确和可重复性的结果。我们在室温下储存血液后 1 至 2 小时进行了 TEG 测量。在运行试验之前，柠檬酸盐血样用 20 微升的 $CaCl_20.2_M$(Start-TEG; 诺比斯，恩丁根，德国)，测试开始了。为了尽可能地调整生理条件，并量化 TF 触发凝血的内在变化，我们没有向测试系统中添加激活剂[所谓的非激活 TEM(NATEM)]。以下 ROTEM 参数分析：凝血时间(CT)、凝血形成时间(CFT)、最大凝血硬度(MCF)、最大溶血量(ML)。

2.2.2 PFA-100 吸附法

外周静脉血收集于含 3.8%柠檬酸钠的试管中，PFA-100(Dade Behring, Deerfield, IL, USA)的功能特性已在其他地方详细描述[22-23]。简而言之，该系统由一个一次性测试盒组成，其中一个血小板的塞子阻塞了显微镜孔径，该孔被切成覆盖着胶原蛋白和肾上腺素(CEPI)或胶原蛋白和 ADP(CADP)的膜。塞子的形成在恒定真空产生并由毛细管控制的高剪切流条件下发生。阻塞所需的时间（闭合时间，CT）指示血小板功能和原发止血能力。

3 数据分析

数据表示为平均值和 95％置信区间，以在文本中进行描述。应用了非参数统计。所有统计比较均使用 Friedman ANOVA 和 Wilcoxon 符号秩检验进行事后比较。<0.05 的两尾 P 值被认为是显着的。Spearman 秩相关检验用于计算联想。全部统计计算使用商用统计软件(Statistica Vers.5.0;Stat Soft，塔尔萨，OK，美国)。

4 结果

注射 LPS 后未观察到严重、意外的不良事件。在 ROTEM 组间基线参数(表 1)。

内毒素对 ROTEM 的影响，CT,CFT 与凝血活化的关系(F_{1+2})。与安慰剂组和基线组相比，注射 LPS 后 6 小时的 CT 缩短了 36%(95%CI0.26-0.46)[CT:441s(95%CI 373-509)](组间和时间间 P<0.05)(图 1)。24 小时后，LPS 引起的变化减少，凝血时间恢复到基线值。与安慰剂相比，CFT 在时间过程中没有明显变化。

CT 的最大缩短与凝血酶原片段(F_{1+2})测量的最大凝血激活值一致，在 4 小时后，与基线值相比大约增加了 7 倍[(F_{1+2}:3.4nmol L^{-1}[95%CI 2.4-4.4)](P<0.05)。内毒素血症组 CT 最低值与 F_{1+2} 峰值呈强负相关(r=-0.83,P<0.05)。

内毒素对 ROTEM MCF 的影响与通过 PFA-100，VWF 抗原水平和血小板计数测量的 CEPI 闭合时间（CEPI-CT）相关。输注脂多糖对血凝块强度无明显影响，血凝块强度以 MCF 衡量，MCF 被认为是衡量血小板功能的指标[24-25](图 2)。在时间过程中没有变化，与安慰剂相比也没有变化。

这些结果与内毒素血症对用 PFA-100 装置[26]测量的封闭时间的影响形成对比。输注脂多糖可显著减少 CEPI 闭合时间。

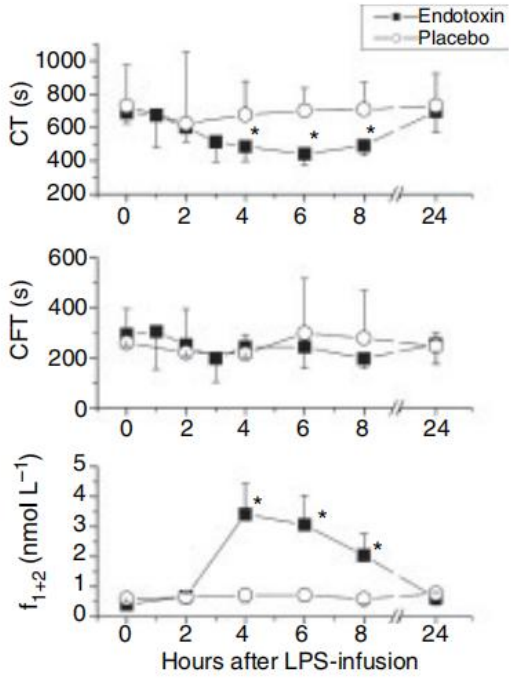

图 1 内毒素对 ROTEM 凝血时间（CT）和凝块形成时间（CFT）与凝血活化（F_{1+2}）的关系。健康的男性志愿者接受内毒素（LPS）推注（2ng kg^{-1}）（■，n=16）或安慰剂（◯，n=4）。内毒素血症导致 ROTEM，CT 显着瞬时缩短 36%（CI 0.26-0.46），与血浆 F_{1+2} 的峰值呈负相关。Data 表示平均值±95%CI。*P<0.05 vs.安慰剂。

约 50%在 4 小时后达到最低水平[80 秒(95%CI 73-88)];与基线和安慰剂相比 P<0.05)。血管性血友病 Ag 因子水平较基线水平增加近 3 倍(229IUdL^{-1}；95%CI187-271)。如之前发表的[26-28]，VWF 峰值水平与 CEPI-CT 的最低点负相关(r=0.56, P<0.05)。输注脂多糖可导致血小板计数在 3 小时后短暂下降 16%，达到最低水平[血小板计数：168·109L)1(95%CI144-192);P<0.05 与基线相比]。在安慰剂期，我们无法观察到时间进程的变化。

内毒素对 ROTEM 的影响。ML 与纤溶标志物(t-PA、PAI-1、PAP)的关系：内毒素输注对 ML 有明显的短期激活作用，可使其增加 3.9 倍(CI:2.5-5.2)，达到峰值。

表 1 ROTEM 凝血和纤溶参数和标记物

	Placebo, mean (±95% CI); $n = 4$	Endotoxin, mean (±95% CI); $n = 16$	*P*-value
CT (s)	729 (484–972)	689 (625–753)	ns
CFT (s)	260 (125–396)	293 (245–341)	ns
MCF (mm)	54 (46–61)	53 (50–56)	ns
CT plus CFT (s)	990 (609–1370)	998 (891–1104)	ns
ML (% of MCF)	14 (8–21)	14 (12–16)	ns
Prothrombin fragment 1 + 2 (nmol L^{-1})	0.41 (0.34–0.48)	0.61 (0.42–0.80)	ns
PFA-100, CEPI-CT (s)	165 (130–200)	161 (120–202)	ns
VWF-Ag levels (IU dL^{-1})	81 (68–94)	83 (64–102)	ns
Platelet count (G L^{-1})	200 (177–224)	194 (135–253)	ns
t-PA (ng mL^{-1})	1.7 (−1.0–4.6)	1.1 (−0.5–2.7)	ns
PAI-1 (ng mL^{-1})	8.1 (4.2–12.0)	7.3 (−2.6–16.1)	ns
PAP (μg L^{-1})	476 (338–616)	468 (307–627)	ns

CT, clotting time; CFT, clot formation time; MCF, maximum clot formation; VWF-Ag, von Willebrand factor-antigen; t-PA, tissue plasminogen activator; PAI-1; plasminogen activator inhibitor; PAP, plasmin antiplasmin complexes.

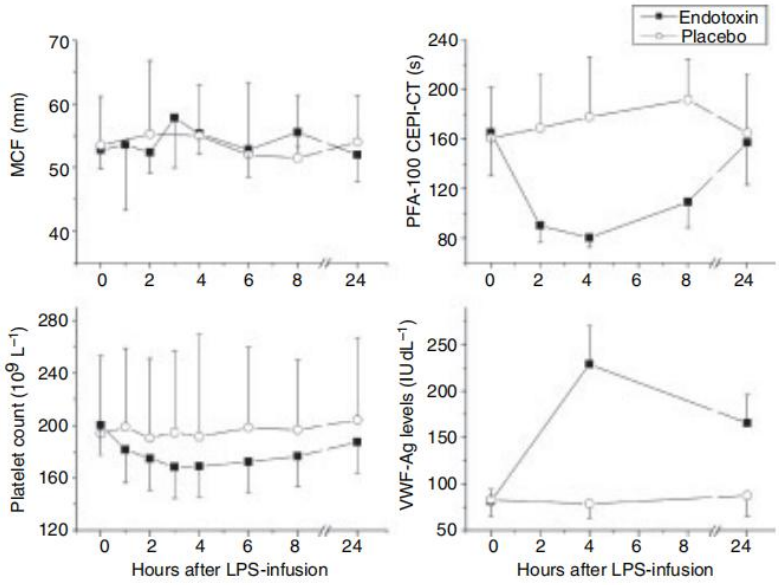

图 2 内毒素对 ROTEM 的影响用 PFA100、血管性血友病因子(VWF)抗原水平和血小板计数测量最大凝块硬度(MCF)与 CEPI 闭合时间(CEPI-ct)的关系。健康男性志愿者接受内毒素(LPS) bolus 输注(2ng kg^{-1})(■,n=16)或安慰剂(◯,n=4)。MCF 没有受到内毒素血症的影响。数据代表平均值±95%CI;*P<0.05vs 安慰剂。

53%的 MCF 与安慰剂相比(P>0.05 比基线和安慰剂)(图 3，95%可信区间：35-72)。2 小时后松解作用减弱。

这些结果与内毒素血症对血浆 t-PA 水平的影响一致，2 小时后血浆 t-PA 水平短暂升高 13 倍[t-PA:23.3ng mL^{-1})1(95%CI14.0-32.6)]。t-PA 峰值与最大裂解峰值(ML)有很好的相关性(r=0.82,P<0.05)。纤溶酶-抗纤溶酶复合物(PAP)显示了类

似的时间过程，在 2 小时后达到峰值，比基线增加 4.4 倍[PAP:2095ng mL^{-1}(95%CI1480-2711)]。与 t-PA 相比，纤溶酶原激活剂抑制剂的延迟时间增加了约 2 小时，并在 4 h 后达到峰值[PAI：60ng mL^{-1}（95％CI 43.5–76.5），增加了七倍（95％CI5.3–9.4）与基准。

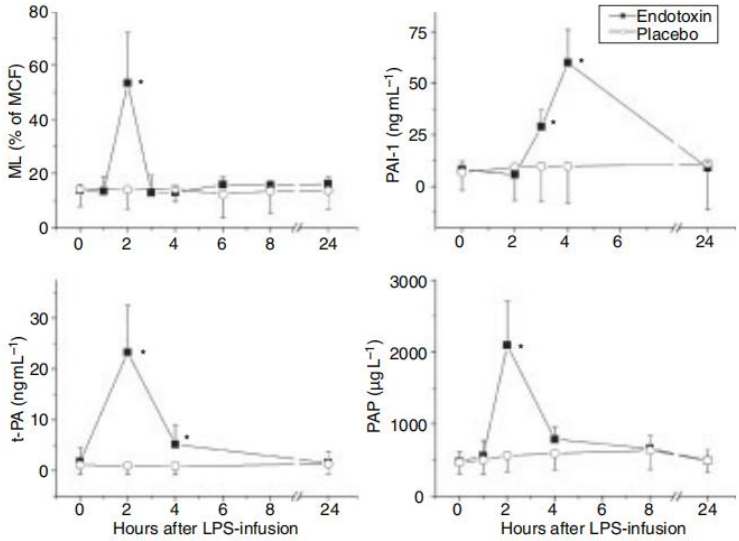

图 3 LPS 输注后数小时内毒素对纤维蛋白溶解标志物[组织纤溶酶原激活物（t-PA），纤溶酶原激活物抑制剂（PAI-1）]的影响，对 ROTEM 最大溶解度（ML）[最大血凝块形成率（MCF）的百分比]，纤溶酶抗纤溶酶复合物（PAP）]。健康的男性志愿者接受内毒素（LPS）推注（2ng kg^{-1}）（■，n=16）或安慰剂（◯，n=4）。与安慰剂相比，在 2 小时后，内毒素输注引起短期 ML 的明显增加，与安慰剂相比增加了 3.9 倍（CI：2.5–5.2）。峰值 ML 和峰值 t-PA 值具有极好的相关性（r=0.82，P<0.05）。数据表示平均值±95％CI；*P<0.05 秒安慰剂。

5 讨论

本研究表明，ROTEM 不仅能检测 LPS 诱导的体内凝血和 TF 触发的体内凝血的变化，还能检测纤维蛋白溶解的变化。此外,ROTEM，CT 和 ML 参数分别与凝血激活标记物(凝血酶原片段，F_{1+2})和纤溶系统标记物(t-PA,PAP,PAI-1)相关。

内毒素血症导致 CT 显著短暂缩短(图 1)。我们观察到 CT 值的最低点与血浆凝血酶原片段峰值水平(F_{1+2})(r=0.83)之间存在极好的相关性，这是 LPS 诱导凝血激活的标志。类似地，Rivard 等人[29]最近描述了凝血酶/抗凝血酶(TAT)复合物生成与计算的 TEG 参数总血栓生成(TTG)之间的良好相关性。此外，我们的结果证实并扩展了先前两项研究的单独观察结果，这两项研究验证了两种不同的医疗点

设备在同一人类内毒素血症模型中测量 TF 凝血时间和凝血起始时间[11-12]。然而，TEG 具有测量包括纤溶在内的凝血过程的所有部分的优势。

与 CT 相比，CFT 不受内毒素血症的影响。因此，起病时间(CT)，即凝血的开始，而不是凝血的增殖(CFT)似乎对低剂量内毒素血症比较敏感。

特别令人感兴趣的是，我们在内毒素模型中观察到 2 小时后裂解（ML）的短暂持续激活，这与纤维蛋白溶解的激活，以及随后对纤维蛋白溶解的抑制（通过 t-PA，PAI-1 和 PAP 测量）相平行（图 3）。峰值 ML 和峰值 t-PA 值具有极好的相关性（r=0.82）表明 ML 是循环 t-PA 活性的量度。据我们所知，这是首次在人体实验性内毒素血症中使用整体床旁检测监测纤维蛋白溶解系统，并与已建立的纤维蛋白溶解标记物（t-PA）交叉验证的首次报告。到目前为止，通过 TEG 监测高纤蛋白溶解状态已经在其他临床环境中进行，例如在临床上。原位肝移植[30-32]和 TEG 已被证明可用于早期检测高纤蛋白溶解[33-34]。

此外，MCF 主要依赖于血小板功能和血小板计数[24-25]，虽然血小板计数略有下降，但 MCF 未受影响，VWF 水平显著升高。这些结果与内毒素血症对 PFA-100 闭合时间(CEPI-CT)[26]的影响形成对比(图2)。这些结果可能有一些解释。首先，与基线相比，血小板计数仅略有下降(16%)，这可能不足以对 MCF 产生影响。第二，血小板的激活，发生于人类内毒素血症[35]，可能抵消血小板计数的轻微下降。最后，由于 TEG 不是一个高剪切系统，VWF-GPIb 相互作用不太可能是可测量的。然而，一种改良的 TEG 方法仍可能是评估抗血小板药物(包括非甾体抗炎药物、氯吡格雷或 GPIIb/IIIa 抑制剂)效果的有价值的工具[24, 36-37]。

本研究的一个局限性是，人类低级别内毒素血症模型不是脓毒症模型，如前面讨论的[9]。因此，很难推断 TEG 是否在临床上监测脓毒症和弥散性血管内凝血是一种有用的工具。另外，我们只验证了 ROTEM 由于上述原因，在没有任何激活剂的情况下，使用 TF 激活的 TEG 可能比未激活的试验数据散射更低[20, 24]。总之，旋转 TEG(ROTEM)不仅能检测体内凝血激活的系统性变化，还能检测人内毒素血症中纤维蛋白溶解的变化。体外凝血时间与凝血酶原片段水平有很好的相关性，凝血酶原片段是体内凝血酶生成的一个标记，与体内纤维蛋白溶解标记 t-PA 最大溶出量。

6 作者和单位信息

原文作者：A.O. SPIEL，* F. B. MAYR，* C. FIRBAS，* P. QUEHENBER and B.JIMLA*

原文依托单位：奥地利维也纳医科大学临床药理学系，奥地利维也纳医科大学医学和化学实验室诊断临床研究所

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ORIGINAL ARTICLE

Validation of rotation thrombelastography in a model of systemic activation of fibrinolysis and coagulation in humans

A. O. SPIEL,* F. B. MAYR,* C. FIRBAS,* P. QUEHENBERGER† and B. JILMA*

**Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria, and †Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, Vienna, Austria*

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Summary. *Background:* Thrombelastography (TEG) is a whole blood assay to evaluate the viscoelastic properties during blood clot formation and clot lysis. Rotation thrombelastography (e.g. ROTEM®) has overcome some of the limitations of classical TEG and is used as a point-of-care device in several clinical settings of coagulation disorders. Endotoxemia leads to systemic activation of the coagulation system and fibrinolysis in humans. *Objectives:* We validated whether ROTEM® is sensitive to endotoxin induced, tissue factor-triggered coagulation and fibrinolysis and if its measures correlate with biohumoral markers of coagulation and fibrinolysis. *Patients and methods:* Twenty healthy male volunteers participated in this randomized placebo-controlled trial. Volunteers received either 2 ng kg^{-1} National Reference Endotoxin or saline. *Results:* Endotoxemia significantly shortened ROTEM® clotting time (CT) by 36% (CI 0.26–0.46; $P < 0.05$) with a strong inverse correlation with the peak plasma levels of prothrombin fragments (F_{1+2}) ($r = -0.83$, $P < 0.05$). Additionally, endotoxin infusion enhanced maximal lysis (ML) 3.9-fold (CI: 2.5–5.2) compared with placebo or baseline after 2 h ($P < 0.05$). Peak ML and peak tissue plasminogen activator (t-PA) values correlated excellently ($r = 0.82$, $P < 0.05$). ROTEM® parameters clot formation time and maximal clot firmness were not affected by LPS infusion, whereas platelet function analyzer (PFA-100) closure times decreased. *Conclusions:* Rotation thrombelastography (ROTEM®) detects systemic changes of *in vivo* coagulation activation, and importantly it is a point of care device, which is sensitive to changes in fibrinolysis in humans. The *ex vivo* measures CT and ML correlate very well with established *in vivo* markers of coagulation activation (F_{1+2}) and fibrinolysis (t-PA), respectively.

Correspondence: Bernd Jilma, Department of Clinical Pharmacology, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria.
Tel.: ++43 1 40400 2981; fax: ++43 1 40400 2998; e-mail: bernd.jilma@meduniwien.ac.at

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Introduction

Thrombelastography (TEG) is a method to evaluate the viscoelastic properties during blood clot formation and clot lysis. By using whole blood, it is able to yield information relating to the cumulative effects of various parameters (plasma factors, platelets and leukocytes) of all phases of the coagulation process and fibrinolysis [1,2]. Therefore, application of TEG could have several advantages compared with standard coagulation analysis, which reflect only a part of the hemostatic process and additionally include partially unphysiologic activators.

Recently, the modified rotation thrombelastogram analyzer (ROTEM®; Pentapharm, Munich, Germany), has overcome some of the limitations of classical TEG. The ROTEM® is very robust and not susceptible to vibrations or mechanical shocks. By using an electronic pipette, reproducibility and performance has increased. Also, depending on the parameters measured, ROTEM® results are available as early as 15 min up to 1 h. Therefore, ROTEM® application may lead to accelerated and more appropriate clinical decision making.

Thrombelastography is already utilized in several clinical settings, e.g. for monitoring of hemostasis during liver transplantation and cardiac surgery and has been shown to be a point of care device for rapid diagnosis and differentiation of hypercoagulable and hyperfibrinolytic situations [3,4].

Endotoxemia leads to systemic activation of the coagulation system and fibrinolysis in humans [5,6]. Hence, it is a good model to test anticoagulants [7–9], as well as diagnostic coagulation tests and fibrinolytic markers [10–12].

It is currently unknown whether ROTEM® is sensitive to systemic changes of *in vivo* coagulation or if its measures of coagulation and fibrinolysis correlate with endogenous changes in thrombin formation and tissue plasminogen activator (t-PA) or plasminogen activator inhibitor (PAI-1) levels, respectively. Thus, the current study aimed to characterize the functional properties of ROTEM® in a well-defined human model of endotoxin induced, tissue factor (TF) triggered coagulation

[8,13] and tumor necrosis factor (TNF-α) triggered fibrinolysis [14]. We hypothesized that ROTEM® may be a whole blood assay, which not only detects changes in coagulation like two other assays [11,12], but may also detect changes in fibrinolysis in human endotoxemia.

Patients and methods

Study design

The study was approved by the Ethics Committee of the Medical University of Vienna and all participants gave written informed consent. Twenty healthy male volunteers [aged 26 years (95% CI 22–29)] participated in a placebo-controlled trial. The control group consisted of four volunteers (4:1 randomization). Medical screening included medical history, physical examination, laboratory parameters, and virologic and standard drug screening. In addition, study subjects were tested for hereditary thrombophilia i.e. factor V Leiden, protein C and S deficiency, to minimize potential risks of endotoxin-induced coagulation activation [8]. Exclusion criteria were regular or recent intake of medication including over the counter (OTC) drugs, and clinically relevant abnormal findings in medical history or laboratory parameters.

The experimental procedures of our endotoxin model have been described in detail in other trials [7,8]. Briefly, volunteers were admitted to the study ward at 8:00 AM after an overnight fast. Throughout the entire study period, participants were confined to bed rest and kept fasting for 8.5 h after LPS infusion. In the current study, volunteers received either a bolus of 2 ng kg^{-1} National Reference Endotoxin (LPS, *Escherichia coli*; USP, Rockville, MD, USA) or 0.9% NaCl as placebo.

Physiologic saline solution (200 mL h^{-1}) was administered to maintain adequate hydration for all subjects, who were kept fasting during the first 6 h.

Sampling and analysis

Sampling times were selected based on the kinetics of coagulation and fibrinolysis seen in subjects challenged with LPS in previous trials [8,15–17] (F_{1+2}: 0, 2, 4, 6, 8, 24; t-PA: 0, 2, 4, 24; PAI-1: 0, 2, 3, 4, 24; plasmin antiplasmin complexes (PAP): 0, 1, 2, 4, 8, 24; CEPI-CT: 0, 2, 4, 8, 24; VWF-Ag: 0, 4, 24, platelet count: 0, 1, 2, 3, 4, 6, 8, 24; ROTEM® parameters: 0, 1, 2, 3, 4, 6, 8 and 24 hours after LPS-infusion). Blood samples were collected by repeated venipunctures into citrated or ethylenediaminetetraacetic acid (EDTA) – anticoagulated vacutainer tubes (Becton Dickinson, Vienna, Austria). Plasma was obtained by centrifugation at 2000 *g* (15 min at 4°C) and stored in 0.5 mL aliquots at −80°C until analysis.

Most coagulation and inflammatory parameters were measured by enzyme immunoassays (EIA). Plasma levels of prothrombin fragment (Enzygnost® F_{1+2} micro; Dade Behring, Marburg, Germany) were used as markers of *in vivo* thrombin generation and plasma levels of t-PA (t-PA Kit; Technoclone, Vienna, Austria), plasmin activator inhibitor (PAI-1, Actibind® Kit; Technoclone, Vienna, Austria) and PAP (PAP micro; DRG International, East Mountainside, NJ, USA) were used as markers for endogenous fibrinolytic capacity. Plasma levels of von Willebrand factor were measured using an enzyme-linked immunosorbent assay (REAADS VWF Ag Test Kit; Corgenix, CO, USA).

The ROTEM® Modified Rotation Thrombelastogram Analyzer

The method and the parameters of TEG and the ROTEM® Coagulation Analyzer (Pentapharm, Munich, Germany) have been described in detail previously [1,18]. Briefly, TEG measures shear elastic modulus during clot formation and subsequent fibrinolysis. The ROTEM® uses a ball-bearing system for power transduction, which makes it less susceptible to mechanical stress, movement and vibration.

Whole blood samples were collected into 3.8% sodium citrate tubes. In the literature, inconsistent references exist on the stability and reproducibility of TEG measurements after sample storage at room temperature. Depending on the references [19–21], TEG measurements produced accurate and reproducible results within 30 min up to 4 h. We performed TEG measurements between 1 and 2 h after blood storage at room temperature. Just before running the assay, citrated blood samples were recalcified with 20 μL of $CaCl_2$ 0.2 M (Start-TEG; Nobis, Endingen, Germany) and the test was started. To adjust as much as possible to physiologic conditions and to quantify the intrinsic changes in TF-triggered coagulation we did not add activators to the test system [so-called non-activated TEM (NATEM)]. The following ROTEM® parameters were analyzed: the clotting time (CT), the clot formation time (CFT), the maximum clot firmness (MCF) and the maximum lysis (ML).

PFA-100 assay

Peripheral venous blood was collected into tubes containing 3.8% sodium citrate. The functional properties of the PFA-100 (Dade Behring, Deerfield, IL, USA) have been described in detail elsewhere [22,23]. In brief, the system consists of a disposable test cartridge where a platelet plug occludes a microscope aperture cut into a membrane coated with collagen and epinephrine (CEPI) or collagen and ADP (CADP). The plug formation occurs under high shear flow conditions produced by a constant vacuum and controlled by a capillary. The time required for occlusion (closure time, CT) is indicative of platelet function and primary haemostasis capacity.

Data analysis

Data are expressed as mean and the 95% confidence intervals for description in the text. Non-parametric statistics were applied. All statistical comparisons were performed with the Friedman ANOVA and the Wilcoxon signed rank test for *post hoc* comparisons. A two-tailed *P*-value of < 0.05 was considered significant. The Spearman ranks correlation test was used for computations of associations. All statistical

calculations were performed using commercially available statistical software (Statistica Vers. 5.0; Stat Soft, Tulsa, OK, USA).

Results

No severe, serious, or unexpected adverse events were observed after LPS infusion. There was no difference in ROTEM® baseline parameters between groups (Table 1).

Effect of endotoxin on ROTEM® CT and CFT in their relation to coagulation activation (F_{1+2})

LPS-infusion shortened CT by 36% (95%CI 0.26–0.46) after 6 h [CT: 441 s (95%CI 373–509)] compared with placebo and baseline ($P < 0.05$ between group and time) (Fig. 1). After 24 h these LPS induced changes diminished and the clotting time returned to baseline values. CFT showed no significant changes in the time course, as well as compared with placebo.

The maximum shortening of CT coincides with the maximum coagulation activation as measured with prothrombin fragments (F_{1+2}), which showed an approximately sevenfold increase compared with baseline values after 4 h [(F_{1+2}: 3.4 nmol L^{-1} [95% CI 2.4–4.4)] ($P < 0.05$ between time). Nadir values of CT showed a strong inverse correlation with peak F_{1+2} levels in the endotoxemia group ($r = -0.83$, $P < 0.05$).

Effect of endotoxin on ROTEM® MCF in relation to CEPI closure time (CEPI-CT) measured with the PFA-100, VWF-antigen levels and platelet counts

LPS-infusion had no obvious effect on the strength of the blood clot, which was measured as MCF, and which is considered a measure of platelet function [24,25] (Fig. 2). There was neither a change in the time course nor compared with placebo.

These results are in contrast to the effects of endotoxemia on the closure time measured with the PFA-100 device [26]. LPS-infusion significantly decreased CEPI-closure time by approximately 50% to reach minimum levels after 4 h [80 s (95%CI 73–88)]; $P < 0.05$ vs. baseline and placebo).

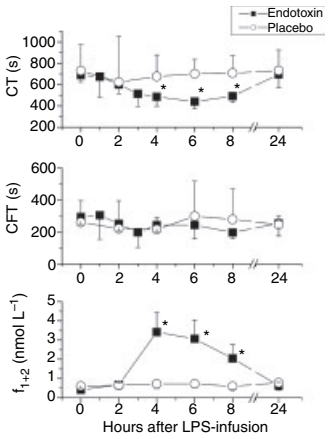

Fig. 1. Effect of endotoxin on ROTEM® clotting time (CT) and clot formation time (CFT) in their relation to coagulation activation (F_{1+2}). Healthy male volunteers received either an endotoxin (LPS) bolus infusion (2 ng kg^{-1}) (■, $n = 16$) or placebo (○, $n = 4$). Endotoxemia led to a significant transient shortening of ROTEM® CT by 36% (CI 0.26–0.46) with an inverse correlation to the peak plasma levels of F_{1+2}. Data represent the mean value ±95% CI; *$P < 0.05$ vs. placebo.

Von Willebrand factor-Ag levels increased from baseline levels almost threefold (229 IU dL^{-1}; 95% CI 187–271) at 4 h after endotoxin challenge. As previously published [26–28], peak VWF levels inversely correlated with nadir values of CEPI-CT ($r = -0.56$, $P < 0.05$).

The LPS-infusion induced a transient decrease in platelet count by 16% to reach minimum levels after 3 h [platelet count: 168×10^9 L^{-1} (95%CI 144–192); $P < 0.05$ vs. baseline]. In the placebo period we could not observe changes in the time course.

Effect of endotoxin on ROTEM® ML in relation to markers of fibrinolysis (t-PA, PAI-1, PAP)

Endotoxin infusion caused an obvious short-term activation of ML with a 3.9-fold (CI: 2.5–5.2) increase to a peak value of

Table 1 Baseline values of ROTEM® parameters and markers of coagulation and fibrinolysis

	Placebo, mean (±95% CI); $n = 4$	Endotoxin, mean (±95% CI); $n = 16$	*P*-value
CT (s)	729 (484–972)	689 (625–753)	ns
CFT (s)	260 (125–396)	293 (245–341)	ns
MCF (mm)	54 (46–61)	53 (50–56)	ns
CT plus CFT (s)	990 (609–1370)	998 (891–1104)	ns
ML (% of MCF)	14 (8–21)	14 (12–16)	ns
Prothrombin fragment 1 + 2 (nmol L^{-1})	0.41 (0.34–0.48)	0.61 (0.42–0.80)	ns
PFA-100, CEPI-CT (s)	165 (130–200)	161 (120–202)	ns
VWF-Ag levels (IU dL^{-1})	81 (68–94)	83 (64–102)	ns
Platelet count (G L^{-1})	200 (177–224)	194 (135–253)	ns
t-PA (ng mL^{-1})	1.7 (−1.0–4.6)	1.1 (−0.5–2.7)	ns
PAI-1 (ng mL^{-1})	8.1 (4.2–12.0)	7.3 (−2.6–16.1)	ns
PAP (μg L^{-1})	476 (338–616)	468 (307–627)	ns

CT, clotting time; CFT, clot formation time; MCF, maximum clot formation; VWF-Ag, von Willebrand factor-antigen; t-PA, tissue plasminogen activator; PAI-1; plasminogen activator inhibitor; PAP, plasmin antiplasmin complexes.

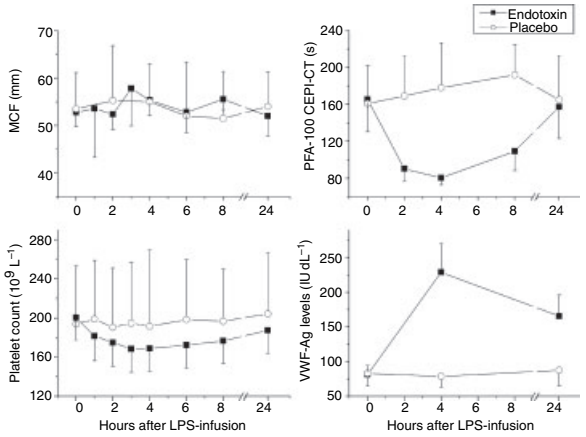

Fig. 2. Effect of endotoxin on ROTEM® maximal clot firmness (MCF) in relation to CEPI closure time (CEPI-CT) measured with the PFA-100, von Willebrand factor (VWF)-antigen levels and platelet counts. Healthy male volunteers received either an endotoxin (LPS) bolus infusion (2 ng kg^{-1}) (■, $n = 16$) or placebo (○, $n = 4$). MCF was not affected by endotoxemia. data represent the mean value ±95%CI; *$P < 0.05$ vs. placebo.

53% (95% CI: 35–72) of MCF compared with placebo after 2 h ($P > 0.05$ vs. baseline and placebo) (Fig. 3). Activation of lysis diminished 2 h later.

These results are consistent with the effects of endotoxemia on t-PA plasma levels, which increased transiently 13-fold compared with baseline after 2 h [t-PA: 23.3 ng mL^{-1} (95% CI 14.0–32.6)]. Peak t-PA levels correlated very well with peak maximal lysis (ML) ($r = 0.82$, $P < 0.05$).

Plasmin-antiplasmin complexes (PAP) showed a similar time course, with peak values and a 4.4-fold increase compared with baseline after 2 h [PAP: 2095 ng mL^{-1} (95% CI 1480–2711)].

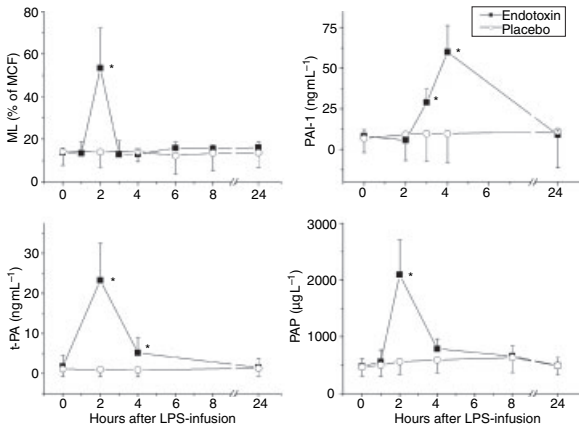

Fig. 3. Effect of endotoxin on ROTEM® maximal lysis (ML) [% of maximum clot formation (MCF)] in relation to markers of fibrinolysis [tissue plasminogen activator (t-PA), plasminogen activator inhibitor (PAI-1), plasmin antiplasmin complexes (PAP)]. Healthy male volunteers received either an endotoxin (LPS) bolus infusion (2 ng kg^{-1}) (■, $n = 16$) or placebo (○, $n = 4$). Endotoxin infusion caused an obvious short-term increase in ML with an 3.9-fold (CI: 2.5–5.2) increase compared with placebo after 2 h. Peak ML and peak t-PA values correlated excellently ($r = 0.82$, $P < 0.05$). Data represent the mean value ±95% CI; *$P < 0.05$ vs. placebo.

Plasminogen activator inhibitor increased with an approximately 2 h delay compared with t-PA and reached peak values after 4 h [PAI: 60 ng mL^{-1} (95% CI 43.5–76.5)] with an sevenfold increase (95%CI 5.3–9.4) vs. baseline.

Discussion

This study demonstrates that rotation TEG (ROTEM®) not only detects *in vivo* changes of LPS induced, TF-triggered coagulation *in vivo* but also changes in fibrinolysis. Furthermore, the ROTEM® parameters CT and ML correlate with markers of coagulation activation (prothrombin fragments, F_{1+2}) and markers of the fibrinolytic system (t-PA, PAP, PAI-1) respectively.

Endotoxemia led to a significant transient shortening of CT (Fig. 1). We observed an excellent correlation between the nadir of CT values and the peak plasma levels of prothrombin fragments (F_{1+2}) ($r = -0.83$), which are a marker of LPS induced coagulation activation. Similarly, Rivard *et al.* [29] recently described an excellent correlation between thrombin/antithrombin (TAT) complex generation and the calculated TEG parameter total thrombus generation (TTG). Additionally, our results confirm and extend the separate observations of two previous studies that validated two different point-of-care devices measuring the TF clotting time and the clotting onset time in the same human endotoxemia model [11,12]. However, TEG has the advantage of measuring all parts of the coagulation process including fibrinolysis.

In contrast to CT, CFT was not affected by endotoxemia. Therefore, the time to onset (CT), i.e. the initiation of coagulation, and not its propagation (CFT) seems to be sensitive to low dose endotoxemia.

Of particular interest, we observed a short lasting activation of lysis (ML) after 2 h in the endotoxin model, which was paralleled by activation of fibrinolysis and consequent inhibition of fibrinolysis as measured by t-PA, PAI-1 and PAP (Fig. 3). Peak ML and peak t-PA values correlated excellently ($r = 0.82$), indicating that ML is a measure of circulating t-PA activity. To our knowledge, this is the first report of monitoring the fibrinolytic system with a global bedside assay in experimental human endotoxemia, and its cross-validation with an established fibrinolysis marker (t-PA). So far, monitoring of hyperfibrinolytic states by TEG has been performed in other clinical settings, e.g. orthotopic liver transplantation [30–32], and TEG has been shown to be useful in the early detection of hyperfibrinolysis [33,34].

Furthermore, MCF, which primarily depends on platelet function and platelet count [24,25], was not affected although platelet counts decreased slightly, and VWF-levels increased substantially. These results are in contrast to the effects of endotoxemia on the PFA-100 closure times (CEPI-CT) [26] (Fig. 2). There are likely explanations for these results. First, the platelet count only slightly decreased compared with baseline (minus 16%), which may not be sufficient to have an impact on MCF. Secondly, the activation of platelets, which

occurs in human endotoxemia [35], might counteract the slight decrease in platelet counts. Finally, as TEG is not a high shear system, VWF-GPIb interactions are unlikely to be measurable. However, a modified TEG method may still be a valuable tool to assess the effects of anti-platelet drugs including non-steroidal anti-inflammatory drugs, clopidogrel or GPIIb/IIIa inhibitors [24,36,37].

One limitation of this study is that the human low grade endotoxemia model is not a sepsis model, as discussed previously [9]. Therefore, it is difficult to extrapolate whether TEG might be a useful tool in monitoring sepsis and disseminated intravascular coagulation in the clinical setting. In addition, we only validated the ROTEM® without any activators for the above described reasons, and use of TF-activated TEG may show lower scattering of data as compared with the non-activated test [20,24].

In conclusion, rotation TEG (ROTEM®) not only detects systemic changes of *in vivo* coagulation activation, but also changes in fibrinolysis in human endotoxemia. *Ex vivo* clotting time correlated very well with prothrombin fragment levels, a marker of *in vivo* thrombin generation, and maximal lysis with the *in vivo* fibrinolysis marker t-PA.

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