Otto et al. Critical Care 2012, 16:426
http://ccforum.com/content/16/3/426

CRITICAL CARE

LETTER

A method for detection and quantification of hydroxyethyl starch in plasma

Gordon P Otto[1,2,*], Bianka Wissuwa[1,3], Anne Mehnert[1], Sascha Marx[1], Michael Raessler[4], Wolfgang Lösche[1,3], Ralf A Claus[1,3] and Maik Sossdorf[1,3]

The use of hydroxyethylstarch (HES) is a controversial issue due to increasing evidence that HES accumulates in plasma and various tissues and therefore leads to unfavourable outcome in critically ill patients [1,2]. No simple methods are available for monitoring HES plasma levels; present technologies to measure HES are based on gas chromatography-mass spectrometry, which are time-consuming and need advanced equipment [3]. Here, we applied Lugol's iodine solution (LUGOL; Sigma-Aldrich, Steinheim, Germany) to determinate HES concentrations in plasma and compare the results with those obtained by high-performance liquid chromatography (HPLC).

The study was approved by the institutional ethical committee (Friedrich-Schiller-University, Jena) as well as by the animal welfare committee (Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz, Germany). Blood samples from healthy volunteers were diluted with balanced 6% HES 130/0.4 (Fresenius Kabi, Bad Homburg, Germany) to get concentrations up to 30 mg/ml. Samples were centrifuged (10 minutes, 4,700×g, 4°C), and plasma aliquots were mixed with 10% trichloroacetic acid and re-centrifuged (5 minutes, 4,700×g, 4°C). The supernatant was mixed (4:1) with LUGOL and optical density was measured at 530 nm. For *in vivo* experiments, 5 ml or 10 ml of 6% HES 130/0.4 was infused into wistar rats over 1 hour via a central venous catheter. Blood samples were obtained prior and up to 24 hours after infusion. Plasma samples were prepared as described. For HPLC analysis, plasma samples were diluted 1:10 with water, then mixed with 60% perchloric acid, heated at 90°C for 60 minutes, diluted again (1:50) and subjected to HPLC [4]. For correlation analysis the Pearson correlation coefficient was calculated.

Using the LUGOL method, we found a linear correlation ($r^2 > 0.99$) between calculated and measured plasma HES concentrations after serial dilutions of HES 130/0.4 in human whole blood (Figure 1a). At a concentration of 6 mg/ml the half life of HES 130/0.4 was estimated to be 1.8 hours (Figure 1b). In rats, plasma

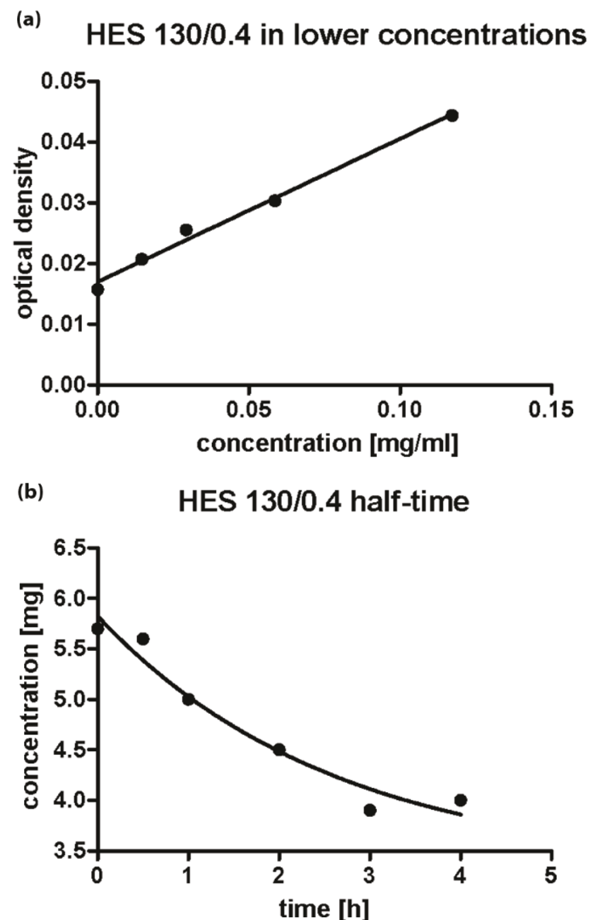

Figure 1. Quantification of HES by Lugol's iodine solution. **(a)** Measurement of HES concentration using LUGOL's iodine solution in samples of human whole blood incubated with different HES 130/0.4 concentrations. **(b)** Time course of HES concentration in human whole blood after incubating with HES 130/0.4 at a final concentration of 6 mg/ml.

*Correspondence: gordon.otto@med.uni-jena.de
[1]Clinic for Anesthesiology and Intensive Care, Jena University Hospital, Erlanger Allee 101, 07747 Jena, Germany
Full list of author information is available at the end of the article

BioMed Central © 2012 BioMed Central Ltd

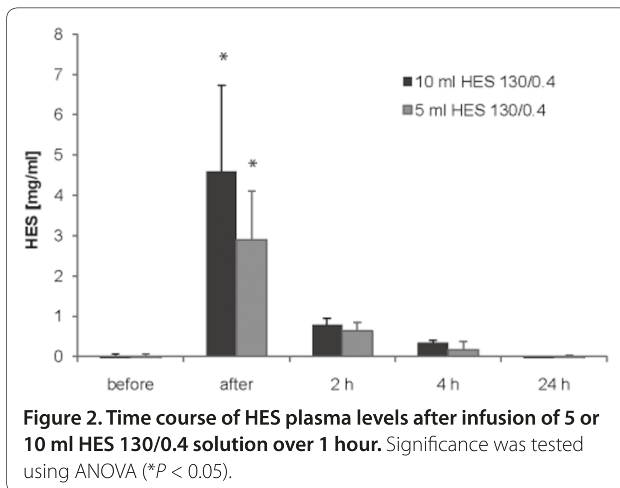

Figure 2. Time course of HES plasma levels after infusion of 5 or 10 ml HES 130/0.4 solution over 1 hour. Significance was tested using ANOVA (*$P < 0.05$).

levels of 2.9 mg/ml and 4.6 mg/ml were measured immediately after infusion of 5 or 10 ml HES 130/0.4. Two hours after infusion the measured levels were only 10% of the initial levels (Figure 2). Comparing the plasma concentrations measured by LUGOL and HPLC revealed a correlation coefficient of $r^2 > 0.90$ ($P < 0.001$).

The new method described here allows the rapid and simple detection of HES in plasma samples. LUGOL is known to stain polysaccharides, including amylose, amylopectin and glycogen. Amylose and amylopectin, due to their herbal origin, should not be found in blood. However, glycogen might interfere with the measurements, but it is present in only negligible amounts in plasma [5].

Measurement of HES plasma levels in patients could help to gain new insights into HES degradation and plasma accumulation after infusion.

Abbreviations

HES, hydroxyethyl starch; HPLC, high-performance liquid chromatography; LUGOL, Lugol's iodine staining solution.

Competing interests

All authors declare that they have no competing interests.

Authors' contributions

GPO and MS designed the study and wrote the first draft of the manuscript. AM and SM were involved in data analysis and interpretation. BW and MR performed HPLC analyses. WL and RAC were involved in supervision data analysis and interpretation. All authors read and approved the final draft of the manuscript.

Acknowledgements

The authors would like to thank Barbara Schmidt and Brigitte Specht for her excellent technical support.

Author details

[1]Clinic for Anesthesiology and Intensive Care, Jena University Hospital, Erlanger Allee 101, 07747 Jena, Germany. [2]Clinic for Internal Medicine (KIM III) - Nephrology, 07747 Jena University Hospital, Jena, Germany. [3]Center for Sepsis Control and Care (CSCC), Jena University Hospital, 07747 Jena, Germany. [4]Max-Planck-Institut fuer Biogeochemie, Hans-Knöll-Str. 10, 07745 Jena, Germany.

Published: 8 May 2012

References

1. Brunkhorst FM, Engel C, Bloos F, Meier-Hellmann A, Ragaller M, Weiler N, Moerer O, Gruendling M, Oppert M, Grond S, Olthoff D, Jaschinski U, John S, Rossaint R, Welte T, Schaefer M, Kern P, Kuhnt E, Kiehntopf M, Hartog C, Natanson C, Loeffler M, Reinhart K; German Competence Network Sepsis (SepNet): Intensive insulin therapy and pentastarch resuscitation in severe sepsis. *N Engl J Med* 2008, **358**:125-139.
2. Hartog CS, Kohl M, Reinhart K: A systematic review of third-generation hydroxyethyl starch (HES 130/0.4) in resuscitation: safety not adequately addressed. *Anesth Analg* 2011, **112**:635-645.
3. Deventer K, Van Eenoo P, Delbeke FT: Detection of hydroxyethylstarch (HES) in human urine by liquid chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006, **834**:217-220.
4. Raessler M, Wissuwa B, Breul A, Unger W, Grimm T: Determination of water-extractable nonstructural carbohydrates, including inulin, in grass samples with high-performance anion exchange chromatography and pulsed amperometric detection. *J Agric Food Chem* 2008, **56**:7649-7654.
5. Lutkic A, Cepo M, Dodikovic V, Zjacic-Rotkvic V, Goldoni V, Mazuranic V, Cabrijan T, Cizmic A, Jurilj N: Concentration of glycogen in blood of diabetics. *Clin Investig* 1992, **70**:707.

doi:10.1186/cc11302
Cite this article as: Otto GP, *et al.*: A method for detection and quantification of hydroxyethyl starch in plasma. *Critical Care* 2012, **16**:426.