Title: MEASUREMENT OF THE CIRCULATING RED CELL VOLUME (CRCV) - A NOVEL, NON-RADIOACTIVE, EXACT AND INEXPENSIVE METHODOLOGY

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Introduction: The accurate assessment of the circulating red cell volume (CRCV) is of critical importance in the clinical management of a wide spectrum of disease states, e.g. in the perioperative period, and in monitoring fluid imbalances, e.g. during septicemia. Indirect estimation of the CRCV by plasma volume determination as evaluated by the dye methods (Evan's blue) or radioactively labeled albumin is known to be inaccurate (4,7). Although the 51-Cr-labeling of the red blood cells has been widely used as a routine method, continuing desire for reduction of radioactivity has been proposed (3). Therefore the present investigation was performed to develop a novel and non-radioactive method for evaluation of the CRCV.

Methods: Fluorescein-isothiocyanate (FITC), a compound used to covalently couple fluorescein to protein, was used as red cell marker: rat, swine and human erythrocytes were suspended in FITC-containing NaCl-solution under physiological conditions (pH 7,35; 280 mosmol; 37° C). Concentrations of 10, 40, 100 and 400 ug were added to 1 ml red cell suspension (hematocrit: 50 %) and incubated for 10, 15, 30 and 60 min. Fluorescence intensities of the red cells were controlled by flow cytometry (FACS) and fluorescence microscopy, whereas supernatant fluorescence was quantitated spectrofluorometrically.
CRCV was measured in 42 adult female Wistar rats according to the following procedure: Blood sampling - separation of the red cell fraction - incubation with FITC solution - elimination of free FITC in the supernatant by centrifugation - injection of the fluorescence labeled erythrocytes i.v. (volume 0,25 ml) - sampling of blood after 10 min up to 24 h - cell counting of the labeled and unlabeled cell fraction using a fluorescence microscope - calculation of CRCV - determination of systemic hematocrit and calculation of the circulating blood volume.
In addition double labeling using 51-Cr and different concentrations of FITC (40, 100, 400 ug) in rats of the same strain (n=20) was compared to 51-Cr-labeling. Organ distribution of the radioactivity of the 51-Cr-labeled cells and of the double labeled cells were studied in the lung, liver and spleen.
Furthermore CRCV was determined in swine (n=6) and simultaneuously ECG, heart rate, arterial - , pulmonary artery - , left atrial - and left ventricular pressure and cardiac output were registrated continously for 2 h.

Results: FACS-analysis after the labeling reaction demonstrated labeling of 75 % of the red cells after 10 min, 90 % after 15 min and a range between 92-96 % at 30 and 60 min. Following in-vitro labeling all the FITC-red cells were visible by fluorescence microscopy at any concentration of FITC.
Cellular fluorescence evaluated 10 min after i.v. application of the FITC labeled cells could be detected by fluorescence microscopy when using 40, 100 and 400 ug FITC/ml red cells for the labeling reaction. FACS analysis showed a reduction of the individual red cell fluorescence intensity to 30 % within 2 hours and to 25 % within 8 hours and a constant number of recirculating FITC-cells for at least 24 h. Fluorescent cells were visible by microscopy until 8 hours with 40 ug FITC and 24 h with 100 and 400 ug FITC. Thus for CRCV determination 15 min incubation and 40 ug FITC were considered being sufficient and therefore utilized for the subsequent series of experiments.
CRCV determined in Wister rats was 3,33 $\pm$ 0.15 ml/100 body weight. The corresponding value with the 51-Cr-method was 3,40 $\pm$ 0.24 ml /100 g b.w.
Organ distribution of the 51-Cr radioactivity in lung, spleen and liver was 1,4 , 1,0 and 4,6 % of the radioactivity injected with each of the different concentrations of FITC and 51-Cr (duoble-labeling procedure) and corresponded to the 51-Cr-labeling values.
Hemodynamic studies in swine revealed no significant alterations as compared to baseline values even when the dose of FITC was increased to 2mg.

Summary and Conclusions: Our data of the CRCV are in good agreement with previous reports (5). Moreover no significant hemodynamic side-effects have been observed although "thiocyanate" has been reported to induce hypotension (1). Additionally our experimental findings of a safe use of FITC are further supported by the successful application of FITC-albumin for microlymphography in more than 1000 patients (2). Thus in comparison to previous methods our new measurement of CRCV shows some relevant advantages: FITC-labeling of red cells is non-radioactive, CRCV is performed within 1 hour, it is reproducible and will not put any clinic or laboratory to expenses, since a fluorescence microscope and centrifuge are the only equipment necessary.
Furthermore we demonstrated that red cell labeling is still present after 24 h, therefore this approach opens out the promising opportunity to replace radioactive measurements of red cell survival by this non-radioactive and simple labeling procedure (6).

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