IN VIVO KINETICS IN AUTOLOGOUS TRANSFUSION OF RED CELLS PRESERVED 42 AND 49 DAYS AT + 4°C IN PAGGSS AND IN ADSOL-AS1

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Extending the storage period of red blood cells at +4°C will help to improve the supply in some countries and will simplify the development of autologous transfusion programmes. Transfused red blood cells (RBC) are expected to act as efficient substitutes; this implies the same capacity to bring oxygen to the tissues, and the same life span as normal red blood cells. The control of these properties is mandatory to the validation of new red cell preservation procedures. As the oxygen dissociation curve is a function of the 2,3 DPG level which will be regenerated if needed after 12 to 48 hours in the circulation [1], the RBC ability to actually deliver oxygen to the tissues depends on the red cells capacity to survive in the circulation. We studied the in-vivo kinetics of RBC stored at +4°C in ADSOL-AS1 (Fenwal Laboratories) and PAGGSS (Biotest Laboratories) for 42 and 49 days in autologous transfusion. PAGGSS and ADSOL-AS1 are two optional additive solutions according to the concept pioneered by Lovric and Högman [2,3]. ADSOL-AS1 is a variant of Högman’s SAG-Mannitol [4] and PAGGSS contains phosphate adenine guanosine glucose saline and sorbitol according to Spielman and Seidl [5].

Measurement of the in vivo recirculation of preserved RBC has been a controversial subject over the last few years [6,7]. In order to give an accurate 24 hour recirculation percentage, the time zero amount of injected labelled cells in the circulation must be determined. If one relies on a log linear back-extrapolation at time zero from samples collected between 5 and 20 minutes after reinjection, assuming a simple log linear kinetics within this period, an immediate exaggerated destruction of stored RBC during the very first minutes might be overlooked (fig. 1). In order to take into account such a possibility we used a double isotope technique according to Mollison [8]: an aliquot of stored RBC labelled with 51Chromium (51Cr) was reinjected simultaneously with an aliquot of fresh autologous RBC labelled with 99mPertechnetate (99mTc). Thus an independant measure of the RBC volume could be obtained from the dilution of the 99mTc.

The ratio Rm, derived from the RBC volume as measured with stored cells over the RBC volume measured with fresh cells, is used to correct an eventually underestimated 51Cr time 0 activity.

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Figure 1. Diagram showing how an exaggerated destruction of transfused stored RBC within the first five minutes could be ignored if a single label is used. The Rm ratio is the ratio of the RBC volume as measured with the labelled stored cells ($^{51}$Cr) to an independent measure of the actual RBC volume (fresh cells, $^{99m}$Tc).

Material and methods

450 ml of whole blood was collected in the primary bag of a closed triple bag system from 29 healthy male volunteers duly informed of the means and objectives of the study. The primary bag contained 63 ml of anticoagulant citrate phosphate dextrose solution, one of the satellite bags 100 ml of ADSOL-AS1 solution ($n = 12$) or PAGSS solution ($n = 17$). Within 6 hours, RBC concentrates and platelet poor plasmas were prepared after centrifugation at 6000 g for 10 minutes at 20°C (RC-3B, Sorvall Instruments) and RBC concentrates resuspended in ADSOL-AS1 or in PAGSS. Resuspended packed red cells with a mean hematocrit of 0.62 were immediately stored at $+4^\circ $C with a daily agitation, reproducing standard blood bank conditions for 42 or 49 days.

At the end of the storage period, just before labelling, preserved red cell concentrates were filtered through a standard 170 μ filter as we had previously noticed macroaggregates or small clots potentially harmful to the recipient [9]. Furthermore this filtration reproduces actual transfusion conditions. After thorough mixing, an aliquot of approximately 8 ml was labelled with 1295 KBq (35 μCi) of $^{51}$Cr. The method used was according to the guidelines developed by the International Committee on Standardization in Hematology [10]. Meanwhile the volunteers were drawn 8 ml of blood on a trace of heparin and the fresh RBC were labelled with 925 kBq (25 μCi) of $^{99m}$Tc according to Bardy [11], using a commercial stannous pyrophosphate kit (TCK 11 International-CIS, CEA). Both red cell suspensions were mixed together and injected with the same syringe. A single dilution of the mixtures was counted in order to limit errors.

Samples were collected from the opposite arm at 5, 10, 15 and 20 minutes