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Short Communication

### THE PROTECTIVE EFFECTS OF LIDOCAINE ON HUMAN ERYTHROCYTES STORED FOR SEVEN DAYS AT 04°C

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**Abstract:** Erythrocyte storage may result in cell damage due to an alteration of membrane integrity, which results in potassium efflux and hemolysis. Lidocaine has been shown to protect erythrocytes from oxidative stress by a possible membrane effect. We conducted this study to examine the effects of lidocaine on human erythrocyte storage. Erythrocytes were kept for seven days at 04°C in the absence or in presence of plasma, and of lidocaine at 36.9 and 221.6 µM. Cell damage was assessed by measuring potassium efflux in the supernatant after seven days, and studying potassium efflux and hemolysis induced by oxidative stress. As expected, erythrocyte storage in the presence of plasma was less deleterious. Lidocaine decreased potassium efflux after 7 days' storage. Resistance toward oxidative stress was greater when the erythrocytes had been kept in the presence of plasma. Considering that lidocaine is widely used in various clinical situations, this data may be of clinical relevance.

**Key Words:** Erythrocytes, Preservation, Potassium Efflux, Hemolysis, Lidocaine

### INTRODUCTION

When erythrocytes are stored, many changes and modifications may occur and result in an alteration of their functionality and structure [1]. Many adaptations of the (basic) preservative solution have been proposed to enhance the quality of the erythrocytes and increase their lifespan [2]. Some of these permit erythrocyte' storage for up to 5 weeks without major damage [3]. However, despite these adaptations, (sooner or later) erythrocytes in storage undergo hemolysis and suffer oxygen free radical aggression (storage of erythrocytes may result in their hemolysis and in oxygen free radical aggression)[4].

As lidocaine is known to have antioxidant properties [5, 6], we decided to study the effects of lidocaine on human red blood cells stored for 7 days.

## MATERIALS AND METHODS

After venous puncture, 20 ml of fresh blood were obtained from 5 healthy volunteers (research staff). Blood from each volunteer was studied separately in different experiments. For each experiment, the same protocol was followed.

10 ml of blood were spun in order to separate the erythrocytes from the plasma, and the erythrocytes were washed 5 times in isotonic saline solution. Then, isolated erythrocytes were separated in 3 samples: one with 3 ml of saline (C), one with 3 ml of saline and lidocaine at 36.9  $\mu\text{M}$  (Lido1) and one with 3 ml of saline and lidocaine at 221.6  $\mu\text{M}$  (Lido2). The 10 ml of blood remnant were used to prepare 3 different samples: 3 ml alone (BC), 3 ml with lidocaine at 36.9  $\mu\text{M}$  (BLido1) and 3 ml with lidocaine at 221.6  $\mu\text{M}$  (Blido2). Lidocaine was used as the solution for clinical use (xylocaïne 1%, Astra Zeneca, France). Then, all the samples were stored at 4°C for 7 days.

On day 7, all the samples were studied the same way:

Potassium efflux: Samples were spun and the potassium in the supernatant was measured using flame photometry as described elsewhere. Results were expressed in mmol/l [5].

For the second part of the experiment, the packed erythrocytes were washed 5 times in isotonic saline solution, resuspended in phosphate buffer (1.41 g/L  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  – 1.19 g/L  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  – 1.63 g/L NaCl – pH 7.4) and subjected to oxidative stress using the 2-2'-azobis (2 amidinopropane) hydrochloride (AAPH) at 20 mM as a free radical generator. Samples were then incubated at 37°C for 2 hours.

Potassium efflux was measured in the supernatant at T0 (just before incubation) and every 30 minutes. Total potassium efflux (after hemolysis induced by saponification) was also measured for each sample. The ratio of the measured potassium and total potassium at each time was calculated and expressed as a percentage. Then, the areas under % vs. time curves were calculated and expressed in %min. Hemolysis after 2 hours was measured using the Drabkin method and expressed as a percentage (the ratio between measured hemolysis and total hemolysis obtained by saponification).

All the results were expressed in Mean $\pm$ S.D. Statistical analysis was done using a factorial analysis of variance (ANOVA). A  $p < 0.05$  was considered significant.

## RESULTS AND DISCUSSION

All the results are summarised in Tab. 1. Storing isolated erythrocytes resulted in a greater potassium efflux (C, Lido1 and Lido2) than that observed when erythrocytes were stored with plasma (BC, BLido1 and Blido2). Lidocaine at both concentrations (Lido1 and Lido2) lowered the potassium efflux significantly compared to the efflux for the isolated erythrocytes group (C).

When the remnant erythrocytes were washed and submitted to oxidative stress, the potassium efflux was still greater in C than in BC. The protective effects of lidocaine could only be observed in the Lido1 and Lido2 groups. Considering hemolysis at T120, no significative difference was noted between all the groups.

Tab. 1. Potassium efflux after 7 days' storage, and potassium efflux and hemolysis induced by an oxidative stress. All results are expressed as mean±Sd. a: p<0.05 versus group C.

Groups	Supernatant		Oxidative challenge
	K <sup>+</sup> (mmol/l)	K <sup>+</sup> efflux (%.min)	Hemolysis at T120 (%)
C	630±184	7638±1851	33.1±5.9
Lido1	408±317 <sup>a</sup>	4920±32 <sup>a</sup>	34.9±6.1
Lido2	416±220 <sup>a</sup>	4979±763 <sup>a</sup>	31.6±20.1
BC	244±23 <sup>a</sup>	4881±770	28.5±10.1
BLido1	233±26	4670±603	28.1±11.2
BLido2	225±38	3877±859	31.8±23.7

When erythrocytes are stored, cell damage is observed; its extent depends on the conditions and the time of storage [1]. Generally, changes in shape, deformability and membrane functionality are observed and may result in hemolysis and potassium efflux [2, 7]. These modifications may involve a decrease in ATP availability. Because plasma provides the red blood cells with all the nutrients and energy needed for the preservation of its integrity, modifications occurring during storage should be greater in the absence of plasma. This is well demonstrated in our study: potassium efflux is significantly lowered after 7 days of erythrocyte storage in the presence of the plasma. In addition, when stored with plasma, erythrocyte membrane integrity seemed to be less altered, as shown by a better resistance toward oxidative stress.

In our study, the protective effects of lidocaine were only observed in those groups of erythrocytes stored in the absence of plasma. No lidocaine dosage was performed. However, it can be suggested that, because a large proportion of lidocaine binds to plasmatic proteins and is washed away during the erythrocyte preparation, in the group of erythrocytes stored with plasma, the concentration of lidocaine bound to erythrocytes was inefficient to protect the erythrocytes from oxidative stress. Nevertheless, even if not significant, a small effect of lidocaine seemed to be observed in the groups of erythrocytes stored with plasma. It can be assumed that the effects of lidocaine become dominant in the absence of plasma. Lidocaine is a widely used local anaesthetic agent, exerting antioxidant properties, and can act as a free radical scavenger [6], but can also modify cell membrane fluidity or erythrocyte shape and deformability [5, 8]. It may also block many different ion channels, and it could be suggested that such a property might be responsible for the decrease in the potassium efflux. However, previous

data, reported in another study, suggested that such a property could not be used to explain the protective effects of local anesthetic agents toward an oxidative stress [5].

The level of hemolysis at 2 hours of incubation in the presence of an oxidant generator was similar in all the groups. The integrity of the red cells in all the groups should be considered altered, even if this alteration is thought to be less important when the erythrocytes were stored with the plasma.

Such data may be interesting in various clinical situations in which red blood cells are submitted to oxidative stress and in which lidocaine is used for a clinical purpose. Determination of the extent of this clinical relevance needs to be further studied.

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