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# Correlation between human platelet cytoplasmic membrane outer leaflet fluidity, $Na^+/H^+$ exchanger activity and aging

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**Abstract** A cross-sectional survey was undertaken to study the aging effect on platelet cytoplasmic membrane outer leaflet fluidity and $Na^+/H^+$ antiporter activity, and to investigate the relative influence of membrane order and age on the $Na^+/H^+$ exchanger. The study population consisted of 19 healthy subjects (age range 22–83 years, mean age 48.5 ± 16.5 years; 12 females and 7 males). Washed platelets were used as neuronal models and as eventually contributing to brain pathology by aberrant secretion of their granule content. A significant positive correlation ($r = 0.48$, $p < 0.05$) was found between age and the membrane structural order, according to a second-degree equation. Sewall-Wright path coefficient analysis revealed that the age influence on $Na^+/H^+$ exchanger activity was unimportant (0.18), but those of cytoplasmic membrane outer leaflet fluidity and mean platelet volume were strong and positive (0.51 and 0.54, respectively). Women presented a marked standard deviation in the countertransporter results. In conclusion, decreased membrane outer leaflet fluidity and unchanged $Na^+/H^+$ antiporter activity may contribute to the process of normal brain aging.

**Key words** Aging · Human platelet · Neuronal model · Membrane fluidity · $Na^+/H^+$ antiporter

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## Introduction

Aging is known to be associated with changes in membrane composition, structure and function (Igbavboa et al. 1996). Models have been developed to analyse the central nervous system (CNS). Platelets have been used with this purpose (Zubenko 1992). Moreover, platelets can contribute to brain pathology by aberrant secretion of their granule content (Davies et al. 1993).

$Na^+/H^+$ antiporter is an ubiquitous plasma membrane integral protein exchanging system which participates in several important cellular processes, namely cytosolic pH ($pH_i$) homeostasis. Evidence suggests that $Na^+/H^+$ exchanger and/or $pH_i$ may be involved in the aging process (Sugidachi et al. 1993) and neurological disorders (Davies et al. 1993). It is recognized that the physical state of membrane lipids can influence relevant functions of biological membranes and that alterations of membrane organization have been associated with brain aging (Igbavboa et al. 1996) and brain impairment (Zubenko 1992).

There is little information concerning the age influence on the fluidity of the outer leaflet of intact human platelet plasma membrane (Sarmento et al. 1991; Hasan et al. 1995) and $Na^+/H^+$ antiporter activity (Marinho et al. 1990; Rosskopf et al. 1992; Davies et al. 1993). Information is lacking in what concerns the relative influences of age and cytoplasmic membrane fluidity on the human platelet $Na^+/H^+$ countertransporter activity.

The present cross-sectional study was designed to further analyse the influence of aging on cytoplasmic membrane outer leaflet fluidity and $Na^+/H^+$ antiporter activity of human platelets and to investigate how this exchanger is influenced by membrane outer leaflet order and age.

## Subjects and methods

The study population consisted of 19 healthy Caucasians aged between 22 and 83 years (mean age 48.5 ± 16.5 years; 12 females and 7 males). The subjects were recruited from the University of Coimbra staff and from the local community. The study was approved by the Ethics Committee of the University of Coimbra

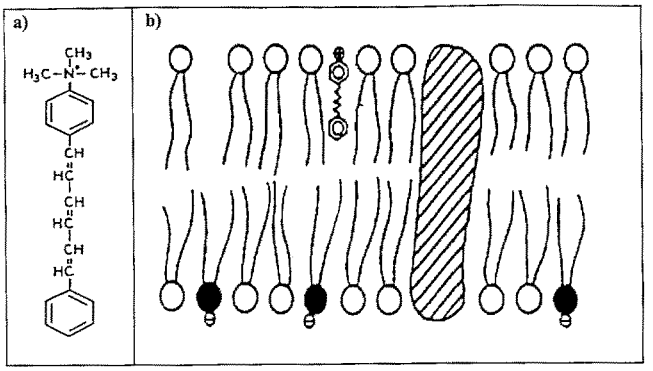


**Fig. 1** **a** Chemical structure of TMA-DPH; **b** localization of TMA-DPH in the outer leaflet of the plasma membrane

Hospital. After written informed consent was obtained from all research candidates, a complete medical evaluation was performed as specified elsewhere (Marinho et al. 1990).

### Platelet isolation

Blood collection and platelet isolation were carried out as previously specified (Marinho et al. 1990).

### Determination of $Na^+/H^+$ antiporter activity

$Na^+/H^+$ exchanger was evaluated as described in detail elsewhere (Marinho et al. 1990).

### Measurement of trimethylamino-diphenylhexatriene (TMA-DPH) fluorescence anisotropy

Assessment of cytoplasmic membrane fluidity with TMA-DPH (Fig. 1) was performed on the basis of the methodology described by Kubina and coworkers (1987).

### Statistical analysis

The results are expressed as mean values ± standard deviation (SD). Correlation between age and membrane fluidity was determined in relation to a second-degree equation. We used Sewall-Wright path coefficient analysis (multivariate regression after normalization of the variables) to look for the relative influence of some parameters on $Na^+/H^+$ antiporter activity. A $p$-value $< 0.05$ was regarded as significant. ANOVA was used to determine intra-individual coefficient of variation (CV).

## Results

The results of TMA-DPH fluorescence anisotropy in the cytoplasmic membrane of intact platelets, mean platelet

**Table 1** Platelet parameters determined in the study population (expressed as means ± SD)

| TMA-DPH fluorescence anisotropy (platelet plasma membrane) | Mean platelet volume (fl) | $Na^+/H^+$ exchanger activity (nmol $H^+$/min/ $2 \times 10^8$ platelets) |
|---|---|---|
| 0.19 ± 0.02 | 7.5 ± 0.96 | 232.8 ± 45.2 |

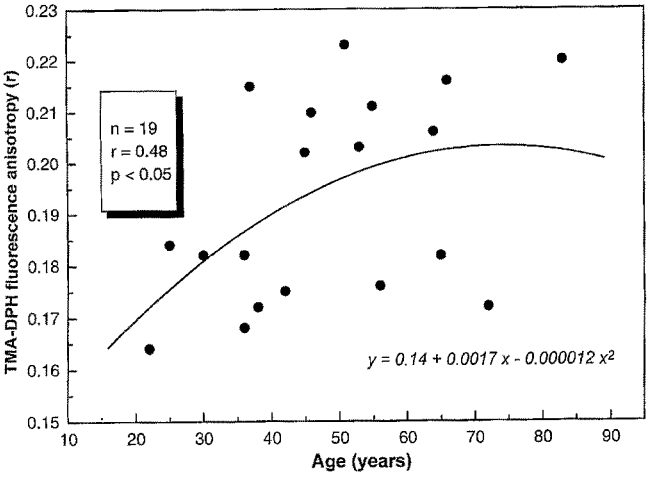


**Fig. 2** Relation between age and the fluorescence anisotropy ($r$) of TMA-DPH inserted into the cytoplasmic membrane outer leaflet of washed intact human platelets. The study population consisted of 19 healthy subjects (age range 22–83 years; mean age 48.5 ± 16.5 years; 12 females and 7 males). A high degree of anisotropy represents a high structural order or low fluidity. A significant positive correlation ($r = 0.48$, $p < 0.05$) was found between age and membrane outer leaflet structural order, according to a second-degree equation. Each point represents the mean of three determinations

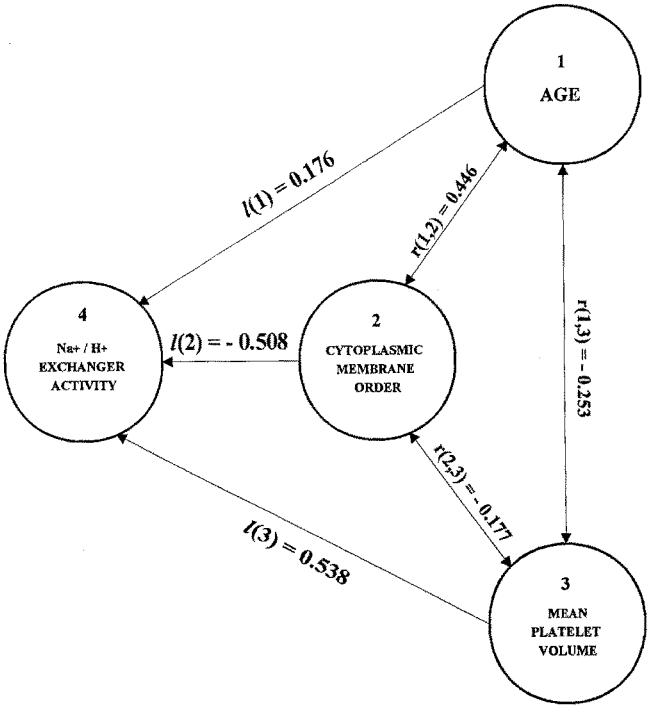


**Fig. 3** Sewall-Wright path coefficient analysis (multivariate regression after normalization of variables) of the relative influence of age, cytoplasmic membrane outer leaflet order and mean platelet volume on the $Na^+/H^+$ exchanger activity of human platelets. The age influence on $Na^+/H^+$ antiporter activity was unimportant ($l = 0.18$), but those of cytoplasmic membrane outer leaflet fluidity and mean platelet volume were strong and positive ($l = 0.51$ and 0.54, respectively). $l$ Sewall-Wright path coefficient; $R$ correlation coefficient

volume (MPV) and $Na^+/H^+$ exchanger activity are presented in Table 1. A significant positive correlation of 0.48 ($p < 0.05$) was found between age and cytoplasmic membrane order of human platelets in relation to a second-degree equation (Fig. 2). Analysis by Sewall-Wright path coefficients with all measured parameters relative to the activity of $Na^+/H^+$antiporter showed that only membrane order and MPV are important in the variation of the countertransporter activity. The influence of age on the platelet $Na^+/H^+$ exchanger was not important (0.18), but those of membrane organization and MPV were strong (–0.51 and 0.54, respectively; Fig. 3). A marked SD in the results concerning platelet $Na^+/H^+$ antiporter activity in women could be found (mean female and male activity were 236.1 ± 56.4 and 227.3 ± 15.7 nmol $H^+$/min/2 × $10^8$ cells, respectively). Intra-individual CV was 6.4% for $Na^+/H^+$ exchanger activity and 4.8% for the fluorescence anisotropy of TMA-DPH.

## Discussion

This study confirms and extends the scarce previous reports of aging not affecting human platelet $Na^+/H^+$ antiporter activity (Marinho et al. 1990; Rosskopf et al. 1992; Davies et al. 1993) and of age being correlated with a decrease in outer leaflet fluidity of platelet plasma membrane (Sarmento et al. 1991; Hasan et al. 1995). Moreover, the positive influence of cytoplasmic membrane outer leaflet fluidity on platelet $Na^+/H^+$ exchanger activity that we now report constitutes new information.

We stress that both, the activity of the countertransporter and the degree of membrane organization, were evaluated in the same platelet sample of each volunteer. We have noticed a bigger SD of the female antiporter activity. We wonder whether this feminine "versatility" can be a biological advantage. The present finding of a positive correlation between MPV and the $Na^+/H^+$ exchanger activity, previously described by Marinho et al. (1990), can be interpreted on the basis of bigger platelets having a larger number of antiporter molecules per cell and/or of the turnover rate of exchange per countertransporter being faster. The observation that human platelet $Na^+/H^+$ exchanger activity is not dependent on age, despite the decrease in cytoplasmic membrane fluidity, raises the possibility that other factors which also influence the countertransporter are setting off the negative effect of the increased membrane order. Our fluidity data reflect similar phenomena occurring in CNS (Igbavboa et al. 1996).

Zubenko's report (1992) of platelet and brain membranes of Alzheimer's disease patients being more fluid than the ones of controls supports the utility of platelet membrane fluidity studies in providing pertinent molecular insights into the CNS aging process both in health and disease. Analysis of concomitant data on membrane protein function should also be performed.

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