

The effect of sodium fluorescein angiography on erythrocyte properties

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Abstract. Sodium fluorescein angiography is a widely used routine ophthalmological diagnostic procedure which enables the study of chorioretinal microcirculation and consists of the injection of sodium fluorescein into the systemic bloodstream.

The aim of the present study was to evaluate whether or not fluorescein interferes with erythrocyte properties during the angiographic procedure.

In a group of 37 patients, 26 with non-insulin-dependent diabetes mellitus (DM) with and without retinopathy, and 11 without diabetes mellitus (non-DM) although affected by other ophthalmological diseases, all undergoing routine angiography, blood samples were drawn before (T_0) and 30 min (T_{30}) after fluorescein injection. The erythrocyte aggregation index (EAI), membrane lipid fluidity and erythrocyte acetylcholinesterase activity were determined in both groups.

After fluorescein injection there was no statistical change in EAI and erythrocyte membrane fluidity in either group. Erythrocyte acetylcholinesterase activity, a marker of membrane protein integrity, decreased significantly ($p < 0.01$) in the DM group.

Membrane lipid fluidity did not change with fluorescein injection, however, (i) in the DM group erythrocyte membranes became more rigid than in the non-DM (DPH: $p < 0.01$); (ii) EAI and membrane lipid fluidity became significantly correlated ($r = 0.6263$, $p < 0.05$) in non-DM patients at T_{30} .

In conclusion, fluorescein administration for angiographic procedures seems to interact with erythrocyte membrane, namely, in diabetic patients, which may interfere with the blood flow in the microcirculation.

Keywords: Sodium fluorescein angiography, erythrocyte acetylcholinesterase, erythrocyte membrane fluidity, erythrocyte aggregation, diabetes mellitus

1. Introduction

Sodium fluorescein solutions (10 and 20%) are used as contrast dyes to investigate the chorioretinal microcirculation (morphology, dynamics and permeability). Fluorescein angiography is a routine clinical procedure widely used in the study of retinal vascular disorders often associated with systemic and blood abnormalities [1].

The presence of an exogenous compound in the circulation may influence blood flow homeostasis. Several studies *in vivo* and *in vitro* have shown that radiologic contrast media interfere with blood rheologic properties [2] and the endothelial surface [3], probably having the highest effects immediately after endovenous bolus injection, as in angioplasty and selective angiography.

When injected into the bloodstream 50–80% of sodium fluorescein binds to plasma proteins, mainly albumin, and about 10% to erythrocyte membranes [4]. Recently [5], a rise of the retinal circulation

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time with increasing erythrocyte aggregation (adjusted for 40% hematocrit) was reported one hour after fluorescein injection.

The purpose of the present work was to investigate the effects of sodium fluorescein on the erythrocyte membrane during fluorescein angiography in patients with or without diabetes mellitus.

2. Material and methods

2.1. Population

Thirty-seven adult patients (20–62 years old) undergoing routine fluorescein angiography were admitted to the study. The clinical profile of the subjects who completed the full investigation protocol was as follows: 26 patients had non-insulin-dependent diabetes mellitus (DM group), 12 without retinopathy and 14 with retinopathy; 11 patients did not have diabetes mellitus (non-DM group) but were affected by ocular diseases (2 central serous retinopathy, 2 age-related maculopathy and 7 other maculopathies).

2.2. Methodology

In each subject two blood samples of 3 ml each were collected from an antecubital vein with a 21 gauge catheter. The first sample (T_0) was taken just before the injection of a 5 ml fluorescein solution (H. Faure Lab., Paris) and a second sample (T_{30}) 30 min later. In each case the blood was collected in heparin tubes.

2.3. Methods

The erythrocyte aggregation index was determined by the Schmidt-Schönbein method [6] using an MA 1 Myrenne aggregometer. Although EAI is affected by the hematocrit, we did not correct its value because any blood sample manipulation may interfere and significantly decrease EAI [7].

Red blood cell acetylcholinesterase (AChE) is an integral membrane protein which hydrolyzes acetylcholine. Being an erythrocyte outer layer membrane protein its function (catalytic capacity) might be influenced directly by enzymatic effectors and indirectly by membrane phospholipid or cytoskeleton modulation [8]. This enzyme can be used as a marker of membrane integrity and to evaluate a possible modulation of its enzymatic activity. Erythrocyte AChE activity was determined by Kaplan's modified method [9].

Erythrocyte membrane fluidity was assessed by fluorescent polarization of two probes: (i) 1,6-diphenyl-1,3,5-hexatriene (DPH) for the hydrophobic region, and (ii) 1,4-(trimethylamine)-phenyl-6-phenylhexa-1,3,5-triene (TMA-DPH) for the external polar region [10].

2.4. Statistical analysis

The paired *t*-Student's test was used to compare fluorescein action after its endovenous injection, and the group *t*-test for comparison between groups. Pearson's correlation method was used, and *p* values smaller than 0.05 were considered statistically significant.

Table 1
Correlation coefficient (r) and significant level (p) between erythrocyte aggregation index (EAI) and membrane lipid fluidity, when assessed with DPH and TMA-DPH probes, before (T_0) and 30 min after (T_{30}) fluorescein injection; in patients without diabetes mellitus.

	EAI	
	T_0	T_{30}
DPH	$r = -0.6362$ $p = 0.035$	$r = -0.3316$ $p > 0.05$
TMA-DPH	$r = 0.2982$ $p > 0.05$	$r = 0.6263$ $p = 0.039$

3. Results

3.1. Patients without DM

In these patients there was no variation in EAI, in AChE activity or in membrane lipid fluidity 30 min after fluorescein injection.

However, a relationship between EAI and membrane lipid fluidity was determined (Table 1); at T_0 there was an inverse significant correlation ($r = -0.6362$, $p = 0.035$) when assessed with DPH. At T_{30} we observed no significant relation of EAI with DPH, however, TMA-DPH and EAI became positively ($r = 0.6263$, $p = 0.039$) correlated.

3.2. Patients with DM

There was no variation in EAI or in membrane lipid fluidity, but a significant ($p = 0.002$) decrease in AChE activity was observed at T_{30} .

3.3. Comparison between groups (Table 2)

At baseline and after 30 min no statistic difference was observed for EAI and AChE activity between the two groups. For hydrophobic region fluidity (DPH) after 30 min, erythrocyte membrane of the DM group became significantly more rigid ($p = 0.009$) than non-DM erythrocyte membranes. For the external polar region at baseline, erythrocyte membranes of the DM group were more rigid ($p = 0.036$), and after 30 min continued to be more rigid ($p = 0.004$).

4. Discussion

Lipid and protein membrane components can undergo a variety of dynamic behavior [11]. Fluorescence polarization is particularly useful to evaluate membrane lipid dynamic order [11]. Membrane fluidity is associated with the lipid bilayer and especially with the core of the acyl chains [12]. The order of the acyl chains of membrane phospholipid is predicted with accuracy by a DPH probe; meanwhile, membrane surface chains can be measured by a TMA-DPH probe [13]. It is now well recognized that the phospholipid bilayer matrix may form clusters by interaction with a variety of endogenous (e.g., proteins, cholesterol) or exogenous compounds (e.g., drugs and related compounds).

Table 2
Mean \pm standard deviation of all determined parameters in patients with diabetes mellitus (DM) and without (non-DM)

		Non-DM	DM	p^*
AChE	T ₀	319.0 \pm 36.0	325.9 \pm 47.4	NS
	T ₃₀	320.5 \pm 40.3	309.5 \pm 48.0	NS
EAI	T ₀	10.9 \pm 6.0	13.2 \pm 6.0	NS
	T ₃₀	10.3 \pm 5.6	13.0 \pm 6.6	NS
DPH	T ₀	0.287 \pm 0.03	0.297 \pm 0.03	NS
	T ₃₀	0.279 \pm 0.03	0.309 \pm 0.03	$p = 0.009$
TMA	T ₀	0.297 \pm 0.02	0.314 \pm 0.03	$p = 0.036$
	T ₃₀	0.292 \pm 0.02	0.315 \pm 0.02	$p = 0.004$

* p significance level for comparison between groups.

Abbreviations: NS = non-significant; T₀ and T₃₀ = periods of observation; AChE = acetylcholinesterase; EAI = erythrocyte aggregation index; DPH and TMA-DPH = membrane lipid fluidity as assessed by DPH and TMA probes, respectively (see text for more details).

These interactions may be the cause of less mobility of adjacent phospholipid, thus decreasing membrane fluidity [14]. Proteins, as well as cholesterol have been shown to stabilize the fluid lipid bilayer [15]. The lower fluidity of the red cell membrane compared to that of hepatocytes seems to be a consequence of the higher cholesterol phospholipid ratio in erythrocyte membranes [14].

According to the observed results (Table 2), endovenous fluorescein administration interferes with the erythrocyte membrane by decreasing AChE activity and by modifying erythrocyte membrane lipid fluidity. A membrane viscosity change may be associated with changes in membrane-bound enzymes and carrier-mediated transporters [16].

At baseline, hydrophobic region fluidity was similar in both groups. However, after fluorescein injection, erythrocyte membranes of DM patients became more rigid. Erythrocyte membranes of diabetic patients were apparently more susceptible to fluorescein than erythrocyte membranes of non-DM patients.

A membrane fluidity change may be the earliest modification following the interaction of fluorescein with red cells. Loss of membrane integrity or loss of membrane fragments might induce a release of membrane-bound AChE, which could be an explanation for the decrease of enzyme activity. Alternatively, the observed lower AChE activity could be related either to the interference of fluorescein molecules with enzyme molecules or to a chemical modification by inducing physical constraints (e.g., by intercalation). AChE is a sensitive probe of membrane integrity dynamics [8] and is affected by membrane fluidity [18]. Nonenzymatic glycosilation of membrane proteins, by inducing higher membrane rigidity [17], could explain the apparent higher susceptibility to fluorescein of erythrocyte membranes of diabetic patients.

According to our results, the modification of the red cell membrane fluidity, caused indirectly either by fluorescein intercalation or chemical modification of the membrane structure, seems to be a good explanation for the lower AChE activity observed in the more susceptible red cell membranes of diabetic patients.

In contrast to Jung et al. [5], we did not find any change in EAI after fluorescein injection, although we used the same type of aggregometer. However, we did not correct hematocrit to 40%, because any centrifugal manipulation of blood aliquots is able to interfere with EAI [7]. We have no further explanation for the difference between the two studies.

Acknowledgements

We thank Mrs. Teresa Freitas, Mrs. Elvira Sabino and Mr. Chim Win San for their technical support.

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