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ORIGINAL ARTICLE

The function of the blood aqueous barrier in eyes with emulsified silicone oil

Petr Soucek[1], Jaroslav Havlícek[2], Marcela Tkadlecová[2], Ivan Karel[3], Karel Volka[2], Ilona Cihelková[1]

[1] The Eye Department, University Hospital, Srobárova 50, 100 34 Prague, CZECH REPUBLIC, address for correspondence: soucek@fnkv.cz
[2] Department of Analytical Chemistry, Institute of Chemical Technology Prague, CZECH REPUBLIC.
[3] Department of Ophtalmology of the 1. Medical Faculty, Charles University, Prague, CZECH REPUBLIC.

Correspondence to: Dr. Petr Soucek
The Eye Department, University Hospital,
Srobárova 50, 100 34 Prague,
CZECH REPUBLIC
EMAIL: soucek@fnkv.cz

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Abstract

PURPOSE: To evaluate the function of the blood aqueous barrier(BAB) in the eyes with silicone oil emulsification (SOE).
METHODS: Protein concentrations, expressed in albumin equivalents,were determined in aqueous humor of the eyes with SOE in 11 consecutive patients by means of proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. Correlations with various clinical factors were
studied.
RESULTS: Normal function of the BAB (albumin equivalents 1mg/ml and less was found in 8 eyes (73%) independently on underlying disease, early postoperative reaction after pars plana vitrectomy with SO
implantation, degree of SOE and late postoperative complications. Increased permeability of the BAB (albumin equivalents equal 2, 3 and 6.5 mg/ml) was found in 3 eyes (27%) with recent acute complication (retinal detachment after SO removal in 2 eyes, and secondary angle closure glaucoma in 1 eye).
CONCLUSION: SOE *in vivo* was associated with increased permeability of the BAB in the minority of the eyes. Other factors should be studied to explain the variability of SOE. ^{1}H NMR spectroscopy might be a valuable method for the study of SOE.

Introduction

Emulsification of the silicone oil (SO), which is used for the inner tamponade of the retina after pars plana vitrectomy, is a frequent late postoperative complication, threatening the result of the operation by secondary glaucoma and keratopathy. That is why the process of silicone oil emulsification (SOE) is topical in many studies [1,2,3]. In SOE a number of factors play an important role, these include mechanical and physico-chemical. Continuous, though minimal movements of the silicone oil bubble in the vitreous cavity represent the principal mechanical factor. The physico-chemical factors

are given partly by the properties of SO itself, partly by the interactions of SO with substances from different eye tissues and blood. Some of these substances can act as surfactants, decreasing the interfacial tension between SO and intraocular fluids and participate in the formation of micells of the emulsified SO. Typical surfactants are lipoproteins and phospholipids [4]. Different plasma proteins and blood constituents were proved as potential emulsifiers [5,6,7].

High resolution proton nuclear magnetic resonance (^{1}H NMR)spectroscopy is an excellent method not only for the qualitative analysis, i.e. the determination of the structure of organic compounds, but also for the quantitative analysis.

Recently this method was applied *in vitro* for analysis of the human [8] and rabbit aqueous humours [9,10]and of the vitreous in experimental endophtalmitis [11]. Different types of phospholipids were detected and quantified in the crystalline lens and in the meibomian gland secretion of humans by ^{31} P NMR spectroscopy [12,13].

The goal of this study was to evaluate the function of the blood-aqueous barrier (BAB) in the eyes with SOE by means of ^{1}H NMR.

Material and Methods

The study was undertaken on a consecutive series of 11 patients, 7 males and 4 females between the ages of 19 and 73 years, on average 38.6 years (Tab.1). All patients had undergone extreme vitreoretinal surgery with silicone oil implantation (SOI), and now manifested SOE, and were indicated for various intraocular surgery. Two grades of SOE were distinguished: grade I of SOE, droplets of emulsified SO in the upper half of the anterior chamber angle, was found in 7 eyes (63.6%); grade II of SOE, a layer of emulsified SO masking the upper half of the peripheral retina and of the anterior chamber angle, in 4 eyes (36.4%). The aqueous humour was aspirated from the anterior chamber at the beginning of the operation by a single surgeon (I.K.). The tenets of the World Medical Association Declaration of Helsinki were followed and prior informed consent was obtained from all patients. After the aspiration, the samples were kept at –78^{o}C and lyophilised. The initial volume of the aqueous humour was determined from the difference of masses of the sample before and after lyophilisation. These volumes varied from 60–970 µl, on average 261 µl.. The samples of the aqueous humour were taken during SO removal (SOR) or at various operations which followed 6 days to 25 months after SOR. The operations were: SOR in 6 eyes (55%), SO reimplantation (SOreI) in 2 eyes (18%), exchange of SO (ExSO), implantation of anterior chamber intraocular lens after SOR and glaucoma operation (trabeculectomy) in 1 eye (9%) each.

^{1}H NMR measurement of the aqueous humour

The lyophilised samples of the aqueous humour were redissolved in the defined volume (typically 0.5ml) of deuterium oxide (D_2O, 99.9%, Aldrich) in 5-mm NMR tubes. Varian Gemini 300 HC NMR spectrometer operating at 300.075 MHz for protons, field-frequency lock (D_2O) and temperature 303 K were used. The 1D proton NMR spectra were acquired using S2PUL pulse sequence (45 degree pulse, spectral

Table 1. Clinical data: patient (Pat.), sex, age (in years), SO viscosity (in centistokes), time of the presence of SO in the eye (in months), indication for the SOI (ind.), early postoperative reaction in the anterior chamber (react.), degree of SO emulsification (SOE), complications at time of sampling (compl.), type of operation (oper.), total protein concentration from NMR spectra ρ_{prot} expressed in albumin equivalents, albumin concentration from nephelometry ρ_{alb}.

Pat.	sex	age [yr]	SO vis.	time [mn]	ind.	react.	SOE	compl.	oper.	ρ_{prot} [mg/ml]	ρ_{alb} [mg/ml]
A	f	20	5000	32	GT	fib.	II	ACG	SOR	6.8	1.65
B	m	71	5000	3	PVR	fib.	I	G	SOR	~1	α
C	m	65	5000	10	PVR	cel.	I		IOL	<1	α
D	m	62	5000	25	PVR	cel.	I	G	SOR	~1	α
E	m	24	1000	21	PVR	fib.	I	D	ExSO	<1	0.04
F	f	28	5000	70	PVR	fib.	I	GRD	SoreI	~3	α
G	f	35	5000	2	PVR	fib.	I	D	SoreI	~2	α
H	f	54	5000	8	PVR	fib.	II	G	SOR	<1	0.10
I	m	19	5000	3	MH	cel.	II		SOR	<1	α
J	m	44	1000	25	PVR	phyp.	II	g	TE	<1	0.11
K	m	73	5000	8	MH	cel.	I	G	SOR	<1	0.19

α not measured, PVR-proliferative vitreoretinopathy, GT-giant tear, MH-macular hole cel.-cellular inflammatory reaction, fib.-fibrinous exudate, phyp.-pseudohypopyon, I-droplets of the emulsified silicone oil in the upper half of the anterior chamber angle, II-a layer of emulsified SO masking the upper half of the peripheral retina and of the anterior chamber angle, G-open-angle glaucoma, ACG-angle-closure glaucoma, R-rubeosis, D-retinal datachment, SOR-silicone oil removal, SOreI-SO reimplantation, ExSO-exchange of SO, IOL-anterior chamber intraocular lens, TE-trabeculectomy.

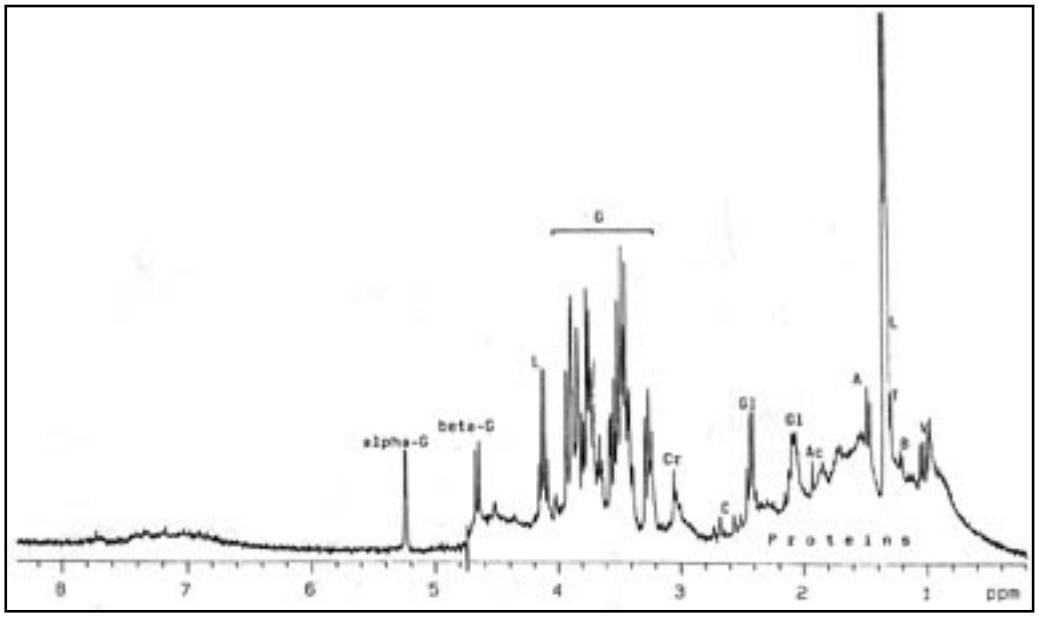

Figure 1.
Identification of signals in sample G:lactate (L), glucose (G; α, β- anomer), citrate (C), alanine (A), acetate (Ac), glutamine (Gl), creatin (Cr), threonine (T), valine (V), 3-hydroxybutyrate (B).

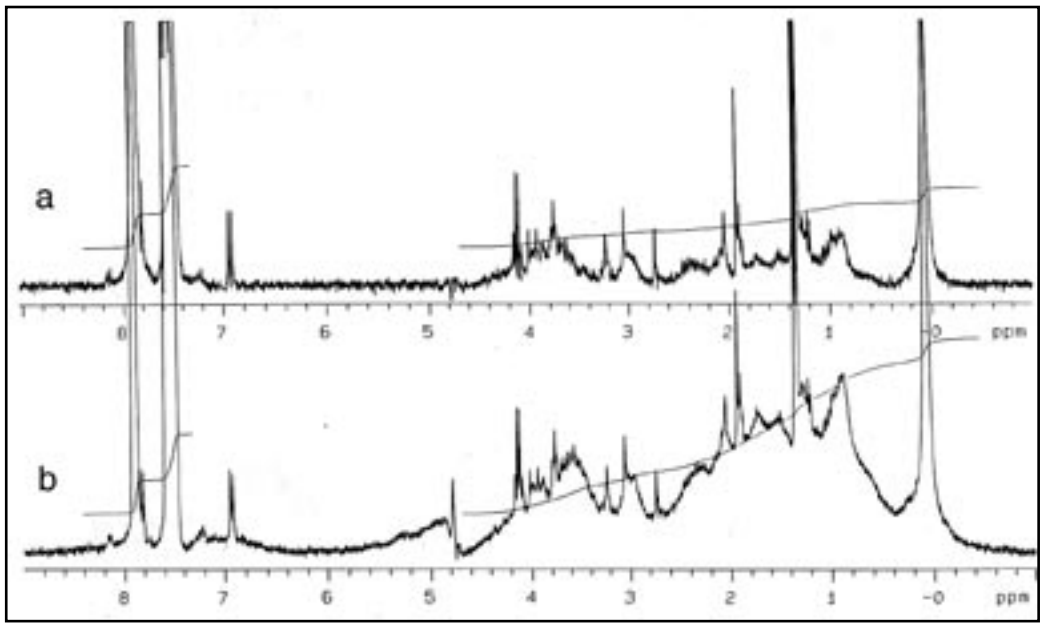

Figure 2.
Quantification of high amounts of proteins using an internal standard by comparing the spectra measured by CPMG (a) and S2PUL (b) pulse-sequences for sample A.

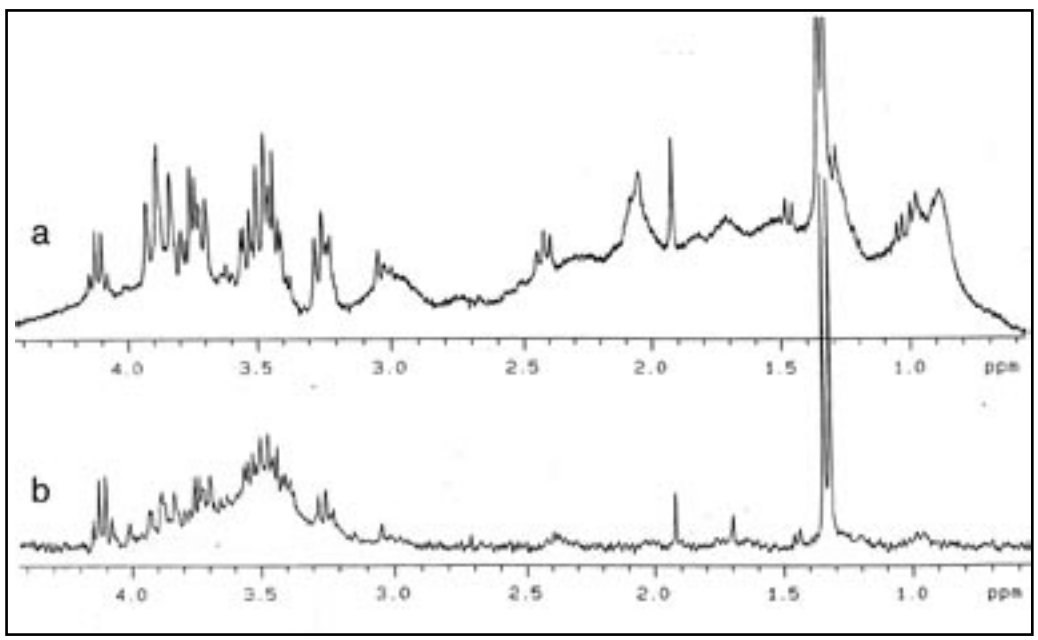

Figure 3.
Comparison of protein content. Aliphatic region of the ^{1}H NMR spectra of samples F(a), J(b) - with and without protein signals, respectively.

width 4.5 kHz, acquisition time 1.778 s, 1024 accumulations and repetition delay of 10 s). For suppression of the water signal, the gated decoupling was used. For suppression of proteins, the CPMG pulse sequence with total spin-spin relaxation delay of 16 ms and total delay between pulse cycles of 10 s was used. In order to determine the concentrations of substances present in aqueous humour natrium benzoate (purity of 99.5%, Lachema, Czech Republic) was added to samples A,B,C,D,E as an internal standard.

Different substances, such as proteins, glucose, lactate, acetate, glutamine, alanine, threonine, 3-hydroxybutyrate, valine, citrate and creatine-creatinine were identified in the samples (Fig.1) like in the previous ^{1}H NMR study of human aqueous humour [8]. The averaged concentrations calculated from samples A,B,C,D,E were 5.44±0.61 mmol/l for glucose, 4.94±0.76 mmol/l for lactate and 0.75±0.31 mmol/l for acetate. The lactate concentration agrees well with the values of 4.28±1.30 mmol/l and 4.69±0.90 mmol/l determined in [14]. The protein content in the samples was determined semiquantitavely supposing the constant concentration of lactate.

The intensities of the protein signals in ^{1}H NMR spectra were related to the constant intensity of the lactate signal at 1.33 ppm and compared together and with the spectra measured for modeled aqueous humour solutions.

For preparation of modeled aqueous humour solutions sodium lactate (L-lactic acid, sodium salt, 99%, Fluka), D-glucose (min. 99.5%, Sigma), albumin (human, 99%, Sigma) were used. Prepared modeled aqueous humour solutions had constant lactate and glucose concentrations (5 mmol/l) and variable albumin concentration (0.1, 0.3, 0.5, 1, 2, 5 mg/ml). The total protein content of the sample A was determined quantitatively using the following method: ^{1}H NMR spectra (S2PUL) and CPMG spectra were measured and the integrals of the internal standard and of the aliphatic region (0–4.5ppm) were determined in both spectra. The difference between integrals of aliphatic region in both spectra corresponds to the contribution of proteins suppressed by the CPMG pulse sequence (Fig.2). The minimal concentration for which the protein signals were observed in ^{1}H NMR spectrum of the aqueous humour was about 1 mg/ml. The suggested quantification of proteins based on subtracting of two values provided reproducible results only for relatively high amounts of proteins.

Assuming the glucose and lactate concentration to be about 5 mmol/l, total protein content under 1 mg/ml was impossible to quantify by this way. For this purpose all proteins were assumed to have the same mass content of aliphatic protons like albumin, for which the exact composition and therefore also mass content of aliphatic protons is known [15]. The quantification was proved for model albumin solution. The total protein content was expressed in albumin equivalents which also serves for laser flare measurements [16,17].

Nephelometry

Albumin concentrations in the samples after ^{1}H NMR measurement were determined using the nephelometer TURBOX and kits for microalbuminuria, both from Orion Diagnostica.

Results

Normal function of the BAB, characterized by albumin equivalents equal or less than 1 mg/ml) was found in 8 patients B,C,D,E,I,J,K,L (Tab.1, Fig. 3b).

Primary indication for SOI had been rhegmatogenous retinal detachment with advanced proliferative vitreoretinopathy in 6 eyes (75%) and retinal detachment due to a macular hole in 2 eyes(25%). SO 5000 centistokes(Adatomed, Germany) had been used in 6 eyes (75%) and SO 1000 centistokes (Adatomed Germany) in 2 eyes (25%). A pronounced cellular reaction in the aqueous after the SOI had been observed in 4 eyes (50%), a marked fibrinous exudate on the anterior surface of the SO in 3 eyes (37.5%), and an abundant fibrinous exudate as an hypopyon in 1 eye (12.5%). SO filled the eye for 3–25 months, on average 13 months. The grade I of SOE was observed in 5 eyes (62.5%), the grade II in 3 eyes (37.5%). The retina was detached in the lower periphery in the patient E, in whom exchange of SO 1000 cs for the SO 5000 cs was performed. Five patients B,D,I,K,L (62.5%) suffered from a secondary open-angle glaucoma.

The increased permeability of the BAB, characterized by albumine quivalents 2, 3 and 6.8 mg/ml, was found in 3 female patients A,F,G (Tab.1, Fig.3a).

Primary indication for SOI had been rhegmatogenous retinal detachment with advanced proliferative vitreoretinopathy in 2 eyes (67%) and giant tear in 1 eye (33%). SO 5000 cs had been used and the early postoperative course of SOI had been complicated by fibrinous exudate in the anterior chamber in all 3 eyes. The SO was present in the eye for 2–70 months, on average 35 months, the grade I of SOE was found in 2 eyes (67%), the grade II of SO in 1 eye (33%). In the patient A, a secondary angle closure glaucoma was diagnosed 30 months after SOI, 2 months before sampling. In the patients F and G the retina redetached after SOR 6 days and 1 month before sampling.

In the patient F, the redetachment was associated with rubeosis of the iris.

In 5 patients A,E,I,K,L albumin concentrations in the aqueous were determined by means of nephelometry, as well. The albumin concentration was 1.65 mg/ml in the sample A and 0.10–0.19 mg/ml in the samples I,K,L and under the range of sensitivity in the sample E. These values corresponded qualitatively to the intensities of protein signals in ^{1}H NMR spectroscopy.

Dependence of the function of the BAB on various factors

Increased permeability of the BAB was found in 2 out of 8 eyes with proliferative vitreoretinopathy (25%), in 3 out of 7 eyes with pronounced exudate in the anterior chamber after SOI (36%), in 2 out of 7 eyes with the grade I of SOE (28%), in 1 out of 4 eyes with the grade II of SOE (25%), in 1 out of 5 eyes suffering from secondary open-angle glaucoma (20%) and in 2 out of 3 eyes (66%) with the detached retina. Increased permeability of the BAB was revealed in all 3 eyes (100%) with acute postoperative complications, secondary angle closure glaucoma or recent retinal redetachment.

Discussion

Breakdown of the BAB makes it possible for some blood components to penetrate into the anterior chamber and vitreous cavity, where they can reach significant levels. Among them, phospholipids, lipoproteins and plasma proteins were proved to be surfactant substances, participating in the process of SOE *in vitro*. Different concentrations of these substances in the aqueous and different lengths of their contact with SO, in the vitreous cavity, are thought to be responsible for the interindividual variability of the SOE.

Our results demonstrated a higher plasma protein content in the aqueous due to the breakdown of the BAB only in a few eyes, suffering from recent and acute complications after SOI or SOR, such as secondary angle closure glaucoma or redetachment of the retina.

SOE represents a complex multicausal process in which sometimes mechanical, other times physicochemical factors can play the decisive role. By means of ^{1}H NMR spectroscopy, momentary concentrations of the plasma proteins in the aqueous were determined. Previous though transitory breakdowns of the BAB could not be excluded. This fact has to be taken into account, when the concentrations of the plasma proteins in the aqueous are correlated with the process of SOE.

In the present study, SOE was associated with enhanced concentrations of the plasma proteins in the aqueous only in a few eyes. Other factors should be studied to understand the complexity and interindividual variability of SOE. ^{1}H NMR spectroscopy might be a valuable method for this purpose as recent study shows [18].

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