Hexadecylphosphocholine may produce reversible functional defects of the retinal pigment epithelium

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Abstract. Hexadecylphosphocholine is a synthetic phospholipid derivative that has antitumor activity due to its interference with membrane functions. Animal experiments have shown photoreceptor and retinal pigment epithelium (RPE) degeneration after its systemic application. In a phase II trial of hexadecylphosphocholine therapy in 23 patients with advanced metastatic colorectal or lung cancer, visual acuity and color vision testing, slit-lamp examination, tonometry, fundoscopy (including photodocumentation), visual field testing, electrooculography (EOG), and electroretinography (ERG) were performed. A baseline examination was carried out prior to therapy. Patients were reexamined after 2 months and after 5 months. In all, 71% of the patients had a reduced light peak in the EOG during therapy. After the end of therapy the light peak improved again and became normal in most patients. The statistical analysis showed that the light-peak values during therapy were significantly lower than the baseline and posttreatment values. This indicates that hexadecylphosphocholine might be capable of producing a partly reversible functional defect of the RPE. Apart from this finding, no other functional or morphological ocular side effect was detected. The functional changes observed in our patients as well as the morphological changes found in animal experiments are similar to those reported for known diseases of the RPE-photoreceptor complex. Therefore, hexadecylphosphocholine-induced retinopathy might become useful as an animal model for such diseases.

Zusammenfassung. Hexadecylphosphocholin ist ein synthetisches Phospholipidderivat mit einer antitumorösen Aktivität aufgrund seiner toxischen Wirkung auf Zellmembranen. Im Tierexperiment wurden Degenerationen des RPE und der Photorezeptoren nach systemischer Gabe beobachtet. In der Phase II der klinischen Erprobung von Hexadecylphosphocholin führten wir die opthalmologischen Untersuchungen durch. Bei 23 Patienten mit metastasierenden kolorektalen und bronchialen Karzinomen untersuchten wir den Visus, den intraokularen Druck, das Farbensehen, die Gesichtsfelder, das EOG und das ERG. Außerdem wurde eine Spaltlampenuntersuchung und eine Ophthalmoskopie inkl. Photodokumentation durchgeführt. Die Patienten wurden vor Therapiebeginn, nach 2 und nach 5 Monaten untersucht. 71% der Patienten hatten einen erniedrigten Hellanstieg im EOG unter der Therapie. Nach dem Ende der Therapie erholte sich der Hellanstieg bei den meisten Patienten wieder. Der Hellanstieg während der Therapie war statistisch signifikant niedriger als der prä- und der posttherapeutische Wert. Hieraus kann man schließen, daß Hexadecylphosphocholin eine reversible Funktionsstörung des RPE hervorrufen kann. Außer diesen Befunden wurden keine anderen funktionellen oder morphologischen Veränderungen gefunden. Die funktionellen Veränderungen, die wir bei unseren Patienten beobachteten, und die morphologischen Befunde aus den Tierexperimenten sind vergleichbar mit bekannten Erkrankungen des RPE-Photorezeptor-Komplexes. Daher scheint es prinzipiell möglich zu sein, mit Hilfe dieser Substanz ein Tiermodell für solche Erkrankungen aufzustellen.

Introduction

Hexadecylphosphocholine is a synthetic phospholipid derivative with similarity to lecithin [8] (Fig. 1). Antitumor activity has been found for this substance in rats and nude mice with experimentally induced mammary carcinoma [7, 9, 11]. Its presumed mode of action is selective interference with cellular membrane functions. Hexadecylphosphocholine is tranformed to lecithin in the liver [4].

In a 27-days study, albino (Wistar) and pigmented (Brown Norway/Lewis) rats of either sex were treated with 34.8 mg/kg hexadecylphosphocholine daily. Electron microscopy of the retinal pigment epithelium (RPE) and the retina showed that the basal infoldings and the apical microvilli of the RPE cells decreased in size and number. The space between the RPE and the outer seg-

ments of the photoreceptors as well as the space between the RPE and Bruch's membrane increased. After 10 days, initial signs of necrosis of the photoreceptor outer segments were observed. After 27 days the membranes of the RPE cells were totally flattened, but they showed no sign of necrosis. These RPE changes were accompanied by an almost complete cell loss in the photoreceptor layer, whereas the ganglion cells showed only a slight swelling. All of these changes were detectable over the whole RPE and retinal surface. Other side effects encountered were hair loss, weight loss, and reversible leukocytosis.

Because of these findings, careful ophthalmological monitoring was important during the phase II trial of hexadecylphosphocholine therapy. The results of the current investigation are presented in this paper.

Patients and methods

In a phase II trial of hexadecylphosphocholine therapy, 23 patients with metastasizing colorectal cancer or metastasizing adenocarcinoma of the lung were treated with 100 mg/day for the first 7 days followed by a maintenance therapy of 150 mg/day. This dose was about 16 times lower than the toxic dose (34.8 mg/kg daily) used in the animal experiment. The duration of the treatment was influenced by the course of the disease and the patients' tolerance of the medication (median, 8 weeks).

The opthalmological examination included visual acuity testing, slit-lamp examination, tonometry, ophthalmoscopy (including fundus photography), static perimetry of the central and the peripheral visual field (TAP program 6), color vision testing (panel D-15 desat-

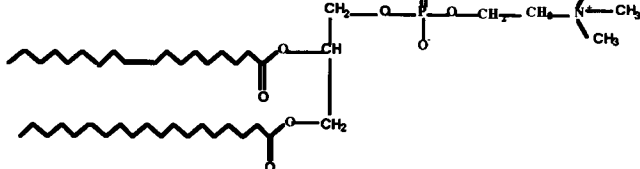## HEXADECYLPHOSPHOCHOLINE
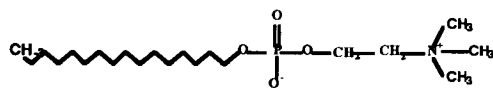
Fig. 1. Chemical structure of hexadecylphosphocholine as compared with that of lecithin

urated and Nagel's anomaloscope), electrooculography (EOG), and electroretinography (ERG).

The light peak of the EOG was elicited according to the method of Rohde et al. [14].During a dark-adaptation period of 30 min, the light was continuously reduced from 10 to 0.1 asb. The light peak was recorded after the intensity of the light had been increased to 1 000 asb. The light peak:dark baseline ratio was calculated (normal value, $\geq 151\%$). The ERG was performed according to the ISCEV standard except that no oscillatory potentials were elicited [12]. After a dark-adaptation period of 40 min, the scotopic ERG was recorded in maximal possible mydriasis. The white light was supplied by a xenon lamp. It was uniformly scattered onto the retina by a + 100-D contact lens. Six different light intensities (increasing by 1 log unit) were used. The lowest light intensity corresponded to the b-wave threshold of the normal eye; the maximal light intensity was 780 cd/m^2. The stimulus duration was 10 ms. The photopic ERG was performed under a white-light adaptation of 4.5 cd/m^2 using stimuli 4–6. Stimulus 5 was used for the 30-Hz flicker response.

The patients were examined prior to therapy as well as after 2 an 5 months. Statistical evaluation was done using the paired t-test.

In 21/23 patients a baseline examination was performed prior to therapy. Due to the advanced stage of disease and the high mortality rate in this population, the first and the second follow-up examination could be performed in only 14 and 8 patients, respectively.

Results

Neither the visual acuity, the intraocular pressure, the clarity of the cornea or lens, nor the color vision of our patients was altered by the treatment. Scotopic and photopic ERG recordings as well as the 30-Hz flicker response were within the normal range before, during, and after treatment. No visual field defect occurred during therapy. No retinal change was detected by fundoscopy during or after treatment (Table 1).

At the baseline examination the mean light peak:dark baseline ratio of the EOG was within the normal range. During therapy, however, it decreased significantly ($P = 0.01$) from 169.9% to 139.6%. After the end of the treatment the light peak increased again and became normal in most patients (mean value, 160.3%; $P = 0.04$). In some patients the recovery began already before to the end of the treatment.

Furthermore, we found a negative correlation between the total amount of hexadecylphosphocholine applied and the light peak:dark baseline ratio obtained during treatment ($P = 0.03$).

Table 1. Clinical results of the current phase II trial of hexadecylphosphocholine

	Baseline (n = 42 eyes)		P^a	First control (n = 28 eyes)		P^b	Second control (n = 16 eyes)		Normal value
Visual acuity	0.88 ±	0.18	>0.1	0.97 ±	0.16	>0.1	0.96 ±	0.2	
IOP (mmHg)	14.0 ±	2.5	>0.1	13.00 ±	2.2	>0.1	13.20 ±	3.2	
Anomalous quotient	1.05 ±	0.20	>0.1	1.13 ±	0.2	>0.1	1.18 ±	0.2	
EOG: light peak/dark baseline (%)	169.9	±25.0	=0.01	139.6	±21.6	=0.04	160.3	±28.3	>151
ERG A-max. scotopic (μV)	300	±40	>0.1	279	±24	>0.1	290	±39	>238
ERG A-max. photopic (μV)	120	±19	>0.1	115	±25	>0.1	110	±19	> 34
ERG B-max. scotopic (μV)	362	±56	>0.1	335	±44	>0.1	347	±88	>288
ERG B-max. photopic (μV)	67	±15	>0.1	71	±20	>0.1	60	±11	> 27
30-Hz flicker response (μV)	106	±20	>0.1	96	±20	>0.1	102	±21	> 50

[a] Comparison of the baseline with the first control
[b] Comparison of the first control with the second control

Discussion

Hexadecylphosphocholine disturbs the function of RPE cells in rats, probably by altering the normal function of plasma membranes, which leads to destruction of the photoreceptor layer. In a phase II trial of hexadecylphosphocholine therapy, we monitored the ocular side effects of this substance in humans. None of our patients complained of visual problems. There was no evidence of acquired defects in any of the psychophysical tests performed. No sign of retinopathy was detectable on the ERG.

The EOG, however, demonstrated clearly that during treatment, the function of the RPE-photoreceptor complex was disturbed. This finding is in perfect coincidence with the morphological changes observed in rats. These changes are due to alterations in membrane functions in the RPE-photoreceptor complex.

In the literature there are numerous descriptions of retinal degenerations that are due to changes in cellular membranes. Lipid peroxidation, for instance, leads to a decrease in amplitude in the ERG and shortens the survival time of the isolated rat retina [1, 5, 10]. The RCS rat is a widespread model for retinal degeneration. The pigment epithelium of these animals cannot effectively phagocytize the photoreceptor outer segments. Characterization of the plasma membranes and the lipids of the RPE in RCS dystrophic and normal rats has revealed significant biochemical differences, especially in the phospholipid composition [2, 3]. Lecithin, which is very similar to hexadecylphosphocholine, not only is an important component of the plasma membrane but also plays an important part in the metabolism of retinol [15]. Changes in the metabolism of neutral lipids and phospholipids in the RPE and in Bruch's membrane have also been described in age related macular degeneration [13]. Finally, a certain number of cases of autosomal dominant retinitis pigmentosa are due to a defect in the membrane protein, rhodopsin [6].

In conclusion, many cases of primary and secondary retinal degeneration are due to changes in cellular membranes. Hexadecylphosphocholine is capable of disturbing the function of these membranes in the RPE-photoreceptor complex. This causes functional defects of the RPE in humans as well as in rats. The morphology of the changes observed in rats is comparable to that of known types of retinal degeneration. Hexadecylphosphocholine-induced retinopathy could therefore become an experimental model for diseases of the RPE and the retina that might enable us to learn more about the normal functions and the pathophysiology of these membrane systems.

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