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Hypericin-enhanced argon laser photocoagulation for subfoveal choroidal neovascular membrane in age-related macular degeneration: A pilot study

G. SOBACI[1], M.Z. BAYRAKTAR[1], Y. KARSLIOĞLU[2], A.H. DURUKAN[1], V. HÜRMERIÇ[1], S. AYKAŞ[1]

[1]Department of Ophthalmology
[2]Department of Pathology, Gülhane Military Medical Academy and Medical School (GMMA-MS), Ankara - Turkey

PURPOSE. *To evaluate the efficacy and safety of hypericin-enhanced argon laser photocoagulation (H-ALP) in the treatment of subfoveal choroidal neovascular membrane (CNM) secondary to age-related macular degeneration (ARMD).*
METHODS. *After preliminary studies for definition of parameters, argon-green laser was administered 4 hours after single dose of oral 1800 mg hypericin (Saint-John's wort tablets, 0.3%, 300 mg) with a subthreshold light fluence, 24 J/cm^2 in 34 eyes (20 with subfoveal classical and 14 with subfoveal occult CNM). Additionally, histopathologic examination was done in two eyes destined for enucleation and exenteration. Maintenance therapy (one tablet, twice a day) was performed for the following 6 months. Anatomic (complete closure of CNM) and functional success (improvement of final visual acuity in three or more Snellen lines) were analyzed with minimum 6-month follow-ups.*
RESULTS. *Histopathologic examinations revealed photothrombosed choriocapillaries together with minimal retinal pigment epithelial disruption in H-ALP exposed areas. One to four (mean 1.88±0.91) treatment sessions were applied in 6 to 29 months (mean 12.2±5.1 months) follow-up period. Twenty-three (67.6%) eyes had 12 months follow-up. Two eyes in each group had functional success (20% in subfoveal classical and 14.3% in subfoveal occult CNM), which had a minimum 12-month follow-up. Anatomic success was achieved in 16 of 20 (80%) eyes with subfoveal classical and 10 of 14 (71.4%) eyes with subfoveal occult CNM. Severe gastric irritation was noted in 1 (2.9%) and pigment epithelial rupture in 2 (5.9%) patients.*
CONCLUSIONS. *H-ALP is a novel and low-cost treatment for subfoveal CNM secondary to ARMD. It seems its efficacy depends on the photodynamic and antiproliferative properties of hypericin. Comparative studies are required to apply this new technique in ophthalmic practice. (Eur J Ophthalmol 2006; 16: 119-28)*

KEY WORDS. *Hypericin, Argon laser photocoagulation, Choroidal neovascular membrane, Age-related macular degeneration*

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INTRODUCTION

Age-related macular degeneration (ARMD), especially with choroidal neovascular membrane (CNM), is the leading cause of blindness in the elderly population. Among many treatment options available for CNM, photodynamic therapy (PDT) has gained recent interest, because of its selective closure of CNM without any harm to the neu-

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rosensory retina in many of the cases. Today, the main drawback of ocular PDT is the requirement of multiple treatment sessions for complete closure of CNM, which causes socioeconomic burden for people living in developing countries (1).

Hypericin, a compound of Hypericum perforatum, known as Saint John's wort (SJW), is probably the most efficient photoactive natural pigment (2, 3). This herbal drug has been used for depression therapy in the elderly for a decade with proven safety (4, 5). Currently, hypericin has been used for PDT of recalcitrant cancers and cutaneous lesions, which revealed that photodynamic effect of hypericin on tumor mass is mostly due to the occurrence of photothrombosis in the vascular lumen, resulting in ischemic necrosis (2, 6). In addition, it has been demonstrated that hypericin has protein kinase C inhibitory activity, and inhibits the proliferation of choroidal endothelial cells in a dose-dependent manner, which is augmented when hypericin is photoactivated (7, 8). Irrespective of photoactivation, potent antiproliferative activities of hypericin have been shown *in vivo* (8, 9). Argon-green wavelength, commonly used for ophthalmic practice, is in turn within the absorption spectrum of hypericin (10).

We supposed that the photoactivation of hypericin in CNM by argon-green laser fluence within the subthreshold level of photocoagulation could cause photothrombosis and would result in closure of CNM. In addition, reproliferations of CNM can be prevented by oral maintenance therapy. Therefore, this pilot study aimed to evaluate the efficacy and safety of hypericin-enhanced argon laser photocoagulation (H-ALP) of subfoveal CNM in light of reported pharmacokinetic and photodynamic properties of hypericin.

METHODS

The study was performed in the Department of Ophthalmology, Gülhane Military Medical Academy and Medical School between September 2000 and January 2003. After a thorough ophthalmic examination, the patients were informed about the treatment options available for this type of CNM and the interventional nature of the treatment was explained. Informed consents, which were approved by the institutional review board (IRB) for both single dose and maintenance therapy, were obtained in all patients.

A two-stage procedure, composed of preliminaies and clinical study, was adopted.

Preliminary studies

The preliminary studies were performed to define the H-ALP parameters.

Definition of drug dosage and time interval for laser administration: The reported single dose of oral hypericin, which is known to be tolerable with no adverse effect, is 1800 mg (11, 12). After oral intake, standardized hypericin tablet reaches its peak blood concentration level in 4 h, the time interval that was chosen for laser administration (12).

Definition of laser parameters for subthreshold argon laser photocoagulation (ALP): This was defined in six eyes of three patients with peripheral lattice degeneration requiring prophylactic laser photocoagulation. They were selected from those having brown irides with moderately pigmented retina. Informed consent was obtained from each patient.

Argon-green laser (Coherent-Novus) was applied at the largest spot size of 1000 microns. Arbitrarily chosen laser power of 100, 150, or 200 mW was tested in three patients, respectively. Likewise, arbitrarily chosen laser application time of 2, 3, or 4 minutes was tested in three laser spots placed in three quadrants of each eye, respectively. So, each eye contained three separate 1000 μm spot sizes of test burn, which consisted of 2, 3, or 4 minutes duration of laser application in the patients treated with laser power of 100, 150, or 200 mW. A visible color change on the retina occurring immediately after ALP was defined as threshold burn. The fluence of ALP was calculated from the following formula:

$$\text{Fluence} = \frac{\text{Power (Watt)} \times \text{Time (second)}}{\text{Area (cm}^2\text{)}} = \text{J/cm}^2$$

The parameters of ALP with the highest fluence associated with subthreshold burn were selected for H-ALP application.

Definition of influence of hypericin sensitization on subthreshold laser photocoagulation for CNM closure: three patients were included.

Each had subfoveal occult CNM associated with well-defined plaque less than 1000 μm at the greatest linear dimension. Four hours after oral intake of six tablets of hypericin (Saint John's wort tablets, 0.3%, 300 mg) after breakfast, a Goldmann three-mirror lens was placed, and predetermined laser parameters (defined in previous study) for subthreshold (1000 μm, 4 min, and 100 mW)

and threshold (1000 µm, 4 min, 150 mW; 1000 µm, 4 min, and 200 mW) photocoagulation were applied to the patients, respectively. In order to verify the closure of CNM funduscopic examination and fluorescein angiography (FA) together with indocyanine green angiography (ICGA; Kowa, Pro-III fundus camera) were performed on the 10th day of laser application.

Histopathologic changes following H-ALP in human eye: To identify histopathologic changes induced by H-ALP, two eyes were studied.

An eye of a 70-year-old man with a large conjunctival squamous cell carcinoma destined for exenteration and a 50-year-old man with choroidal malign melanoma destined for enucleation received two subthreshold burns (H-ALP and ALP) according to the light dose found effective in previous studies and threshold ALP burn to guide histopathologic preparation in unaffected areas. FA and ICGA were performed to localize and quantify the vascular effects before and 1 week after H-ALP.

The globes were fixed in 10% neutral buffered formaldehyde for 5 days and processed for routine paraffin embedding. Standard light microscopic examinations were done in untreated and treated areas with H-ALP and ALP.

Clinical study

Eligibility criteria for this study are listed in Table I.

Treatments were randomly administered by two retina specialists (G.S., M.Z.B.). All measurements for visual acuity (VA) were conducted by the same examiner (S.A.) in the same environment and the best-corrected VA was recorded at each visit. In eyes with CNM larger than one spot-size area, the first spot inside the foveal avascular zone was followed by overlapping spots (two spots at the same area otherwise indicated) covering the entire lesion defined angiographically.

The patients were encouraged to wear gloves and hats and apply sunscreen protection when outside and to avoid excessive exposure to the sun for 3 days following the treatment, and to restrict tyramin-containing food when drug loaded.

They were also informed about the possible skin sensitivity and adverse reactions (paresthesias and darkened coloration of exposed skin) that are likely to occur in hypericin maintenance therapy at 6-month follow-up. Follow-up examinations were done 1, 1.5, 3, 6, 9, 12, and 18 months after the initial treatment session.

The patients were instructed to inform us when they noticed distortion on Amsler grid.

In all visits, VA was measured and FA and ICGA (if needed) were performed; drug usage was monitored, and toxicity to the lens and the retina and photosensitivity reactions were searched.

Toxicity to the lens was evaluated by the Lens Opacification Classification System-II (LOCS-II) by the same examiner (G.S.). For current analysis, lenses were classified as having cataract if their opacity (nuclear, cortical, or posterior subcapsular) developed a grade higher than baseline during at least 6 months of follow-up. Toxicity to the retina was evaluated by funduscopic and angiographic examinations. Any unexpected finding in the surrounding retina was accepted as positive for drug toxicity. In the event of drug intolerance, phototoxicity (more than indicated), or sunburn, the drug was permanently discontinued.

Retreatments were deferred if the slit-lamp or FA appearance of the lesion was unchanged or showed minimal leakage, especially when there is no subretinal fluid or fluorescein leakage from CNM underlying the center of the foveal avascular zone.

TABLE I - ELIGIBILITY CRITERIA FOR HYPERICIN-ENHANCED ARGON LASER PHOTOCOAGULATION FOR SUBFOVEAL CHOROIDAL NEOVASCULAR MEMBRANE (CNM) SECONDARY TO AGE-RELATED MACULAR DEGENERATION (ARMD)

Inclusion criteria

1. CNM secondary to ARMD
2. Age 50 years or more
3. Subfoveal CNM
4. Classical, predominantly classical, or minimally classical, and occult CNM with some classical component, and well-demarcated boundaries
5. Lesion size of 6 disc areas or less
6. Active CNM component comprising 50% or more of lesion
7. Visual acuity from 0.5 to counting fingers at 1 meter
8. No other ocular or systemic illnesses affecting visualization of the fundus and visual outcome
9. Signing an informed consent
10. Ability and willingness to attend to follow-up visit at least 6 months

Exclusion criteria

1. Hemorrhage obscuring CNM details
2. Presence of pigment epithelial detachment
3. Use of other antidepressives, MAO inhibitors, anticoagulants, cyclosporin, indinavir, digoxin, protease inhibitors, sulfonamides, and oral contraceptives

Main outcome measures

The changes in VA and occlusion rates of the CNM were recorded. VA was recorded with decimal notation. Decimal visual acuities were converted to fractional values in order to compare with the data in the Macular Photocoagulation Study (MPS) and other reports. The change in lines was determined according to the line classification of the Snellen chart. Anatomic and functional outcomes were defined as follows:

- Anatomic success: Complete closure of CNM at the final visit.
- Anatomic failure: Incomplete or lack of closure of CNM at the final visit.
- Functional success: Increase in VA of three or more lines at the final visit.
- Functional failure: Decrease in VA of three or more lines at the final visit.

Persistence was defined as having anatomic failure within 1.5 months. Recurrence was defined as having anatomic failure after 1.5 months. Persistence or recurrence was defined if two specialists reached a consensus for each patient and treated with the same parameters of H-ALP at that visit.

Analyzed variables were age of patient, size of CNM, type of CNM (subfoveal classical and subfoveal occult), number of treatment sessions, occlusion, persistence, recurrence, initial VA, final VA, and follow-up period.

Statistical evaluation

Mann-Whitney U test, chi-square, or Fisher exact probability test was used to evaluate the results as indicated. A p value of less than 0.05 was considered significant.

RESULTS

Preliminary study

None of the patients with peripheral lattice degeneration developed complications during or after ALP in dose-escalation study (definition of laser parameters for subthreshold argon laser photocoagulation).

Presenting visual acuities were finger counting at 2 m, 2 m, and 1 m in the patients included in H-ALP dose-escalation study (definition of influence of hypericin sensitization on subthreshold laser photocoagulation for CNM closure). Each had similar demographic characteristics (male, 55, 56, and 60 years of age) and fundus appearance (moderately pigmented), with the same systemic disease (hypertension). Contrary to two eyes with threshold burn, an eye with subthreshold burn had no visible alteration on the retina immediately after H-ALP. Closure of CNM was confirmed angiographically in these eyes on the 10th-day visit. So, the fluence of subthreshold burn, 24 J/cm^2, consisting of 100 mW, 4 minutes, and 1000 µm, was found to be effective for CNM closure.

In eyes evaluated histopathologically, FA and ICGA demonstrated normal findings in ALP-exposed areas, and homogeneous hypofluorescence with occlusion of the choriocapillaries in H-ALP-exposed areas (Fig. 1). Although histopathologic preparation was associated with some artifacts such as detachments of the retinal and choroidal layers, light microscopy revealed thrombosed vascular lumina consisting of red blood cells, white blood cells, and fibrin. No damage was observed in deeper choroidal layers of medium to large choroidal vessels and retinal layers except some retinal pigment epithelium (RPE) changes in H-ALP-exposed areas (Fig. 2).

Clinical study

Patient demography, CNM characteristics, and the treatment results are shown in Table II.

Immediately after H-ALP application, no color change in H-ALP-treated areas was observed. However, all H-ALP-treated eyes showed pale gray discoloration in the H-ALP-treated area at the first month visit.

There was subfoveal classical CNM in 20 eyes (58.8%) and subfoveal occult CNM in 14 eyes (41.2%). The age distribution (70.9±9.4 and 71.3±5.2; p=0.83), mean follow-up period (12.6±6.0 and 11.7±3.7 months; p=0.91), and mean size of CNM (4.9±1.5 and 5.0±1.7; p=0.74) between the groups were similar. Initial VA (0.08±0.08 and 0.08±0.11) and final VA (0.09±0.09 and 0.14±0.18) values in subfoveal classical and subfoveal occult group were not different (p=0.32 and p=0.81), and the mean gain of visual acuity in the groups (0.037±0.129 and 0.114±0.088) was similar (p=0.20). All patients (34 patients) had a minimum 6 months follow-up. Twelve patients in subfoveal classical CNM and 11 patients in subfoveal occult CNM group had a minimum 12-month follow-up. Two eyes in each group had functional success (20% in subfoveal classical and 14.3% in subfoveal occult CNM), which had a minimum 12-month follow-up (Fig. 3). The number of

treatment sessions in the groups (1.9±1.0 and 1.8±0.7) was similar (p=0.76). The anatomic success rates (80% and 71.4%; p=0.68), like the persistence (50% and 57.1%; p=0.68) and the recurrence rates (20% and 14.2%; p=1.00), in these groups were also similar.

None of the study eyes developed functional failure. Functional success was achieved in two eyes in each group (10% in subfoveal classical and 14.3% in subfoveal occult) (p=0.55). All eyes with initial VA of 0.1 or more showed no recurrence throughout the study period. They maintained that level of VA (within two lines of initial VA) in the final visit. Additionally, distribution of other variables, age (69.3±9.0 and 73.7±6.8), follow-up time (11.9±6.5 and 12.0±3.6), the amount of lesion size (4.76±1.6 disc areas and 5.35±1.2 disc areas), the number of treatment sessions (2.11±1.0 and 1.64±0.7), and persistence rate (52.9% in each), showed no statistical significance for predicting the final VA of 0.1 or more (for each, p>0.05).

Three eyes (8.8%) had increase in LOCS score (first with increase in one grade within 6 months, second with increase in one grade within 14 months, third with increase in two grades within 18 months) in the follow-ups.

TABLE II - ANATOMIC AND FUNCTIONAL OUTCOMES OF HYPERICIN-ENHANCED ARGON LASER PHOTOCOAGULATION FOR SUBFOVEAL CHOROIDAL NEOVASCULAR MEMBRANE (CNM) SECONDARY TO AGE-RELATED MACULAR DEGENERATION

Patient	Age yr	CNM	Follow-up (months)	Session	Size disc area	Occlusion	Persist.	Recurr.	Initial VA	Final VA
1	72	SFC	9	4	6	+	+	+	0.02	0.02
2	77	SFC	12	2	4	+	+	-	0.10	0.10
3	65	SFC	15	1	4	+	-	-	0.02	0.02
4	68	SFC	14	3	3	-	+	+	0.04	0.02
5	70	SFC	6	1	6	+	-	-	0.10	0.10
6	84	SFC	12	1	6	+	-	-	0.02	0.02
7	68	SFC	12	2	2	+	+	-	0.10	0.20
8	82	SFC	6	2	6	+	+	-	0.30	0.20
9	66	SFC	9	1	6	-	-	-	0.02	0.02
10	75	SFC	6	1	6	+	-	-	0.10	0.10
11	72	SFC	15	4	6	+	+	+	0.02	0.04
12	77	SFC	24	3	4	+	+	-	0.10	0.10
13	65	SFC	29	1	4	+	-	-	0.02	0.10
14	50	SFC	15	3	3	-	+	+	0.04	0.04
15	70	SFC	14	1	6	+	-	-	0.10	0.10
16	84	SFC	18	1	6	+	-	-	0.02	0.04
17	50	SFC	12	3	2	+	+	-	0.10	0.40
18	82	SFC	9	2	6	+	+	-	0.30	0.20
19	66	SFC	9	1	6	-	-	-	0.02	0.02
20	75	SFC	6	1	6	+	-	-	0.10	0.10
21	69	SFO	6	2	6	-	+	-	0.20	0.40
22	73	SFO	10	3	6	+	-	+	0.02	0.02
23	64	SFO	18	2	6	-	+	-	0.02	0.01
24	70	SFO	12	1	3	+	-	-	0.02	0.05
25	80	SFO	12	2	6	+	+	-	0.02	0.02
26	76	SFO	14	2	6	+	+	-	0.02	0.02
27	67	SFO	12	1	2	+	-	-	0.30	0.50
28	69	SFO	12	2	6	-	+	-	0.20	0.30
29	73	SFO	6	3	6	+	-	+	0.02	0.02
30	64	SFO	18	2	6	-	+	-	0.02	0.02
31	70	SFO	12	1	3	+	-	-	0.02	0.10
32	80	SFO	12	2	6	+	+	-	0.02	0.02
33	76	SFO	14	2	6	+	+	-	0.02	0.02
34	67	SFO	7	1	2	+	-	-	0.30	0.50

VA= Visual acuity; Persist= Persistence; Recurr= Recurrence; SFC= Subfoveal classical; SFO= Subfoveal occult

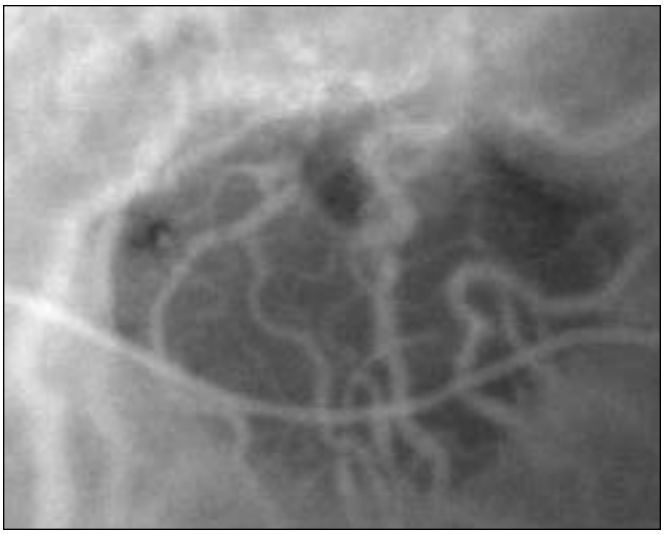

Fig. 1 - *Indocyanine green angiography (ICGA) in hypericin-enhanced argon laser photocoagulation (H-ALP) applied eye, which is studied histopathologically. Early phase of ICGA shows absence of the choriocapillary background fluorescence but maintenance of the vascular pattern of larger caliber vessels 7 days after H-ALP.*

Funduscopy and FA showed no noticeable change in the surrounding retina or RPE throughout the study in all eyes. Some RPE changes in the margins of treated areas were observed in some patients. Retrobulbar anesthesia for laser application was not required in any patient. No intolerance except 1 (2.9%) patient with severe gastric irritation at the second month was noted. RPE rupture was observed in 2 (5.9%) eyes with occult CNM at the third month of the study. No new CNM developed in the study eye and in the other eye with ARMD during the follow-up period.

DISCUSSION

The advantage of PDT over laser photocoagulation is the relatively low light intensities required to initiate the process that produces nonthermal occlusion of CNM, thus minimizing thermal damage to surrounding tissues. A clinically approved PDT modality, Visudyne, has been used successfully; however, the socioeconomic burden of Visudyne PDT engenders considerable debate on the routine use of this treatment modality (13). This is especially true for developing or underdeveloped countries. In recent years, many potential PDT agents including ICG were suggested to fulfill the requirements of PDT for CNM (14, 15).

Since the used laser wavelength was a thermal one, we

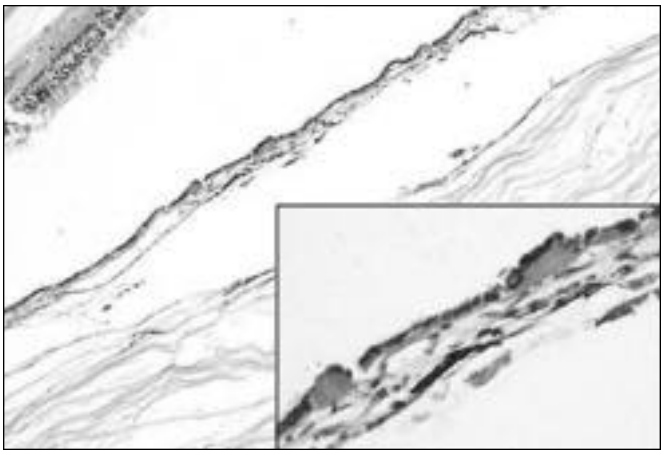

Fig. 2 - *Histopathologic changes following hypericin-enhanced argon laser photocoagulation (H-ALP). Light microscopy of the retinochoroidal section following H-ALP to the area shown in Figure 1. While medium to large vessels remains patent (original x50), superficial portion of the choroid appears condensed, with obliterated vascular lumens in the capillary layer beneath minimally destructed retinal pigment epithelial layer (inset; original x200).*

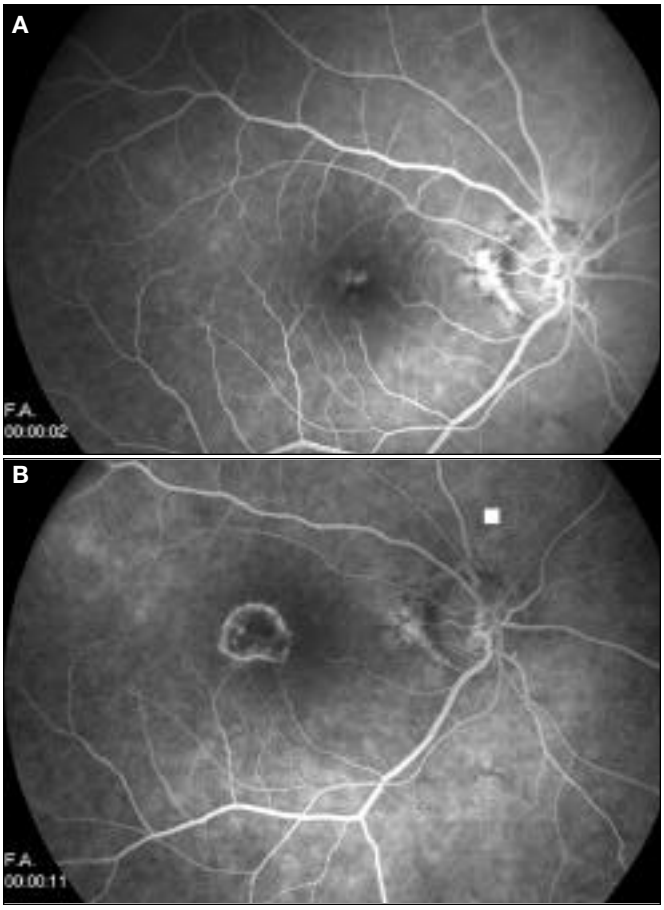

Fig. 3 - *Fluorescein angiography appearance of choroidal neovascular membrane (CNM) before hypericin-enhanced argon laser photocoagulation (H-ALP)* **(A)***, and closure of CNM after H-ALP* **(B)**.

considered H-ALP as a photocoagulation modality in this study. We included criteria somewhat similar to those of MPS for subfoveal CNM. In MPS, the visual benefit after laser photocoagulation for subfoveal CNM has been reported to be significant only in the long-term period and has been dependent upon the lesion size and initial VA (16). Likewise, about 50% of argon laser treated eyes in MPS experienced recurrence within 3 years of treatment. These recurrences usually led to further deterioration of the VA, most of which occurred within 6 months of the treatment. To avoid foveal scarring after subfoveal laser photocoagulation in the MPS study, the foveal-sparing laser technique, perifoveal laser photocoagulation, has been suggested for selected cases with 0.5 to 2.5 disc diameters, without detectable fibrous tissue and visual acuity from 20/100 to 20/1000 (17). All H-ALP-treated eyes including those with larger diameters and higher level of VA achieved stabilization of vision without macular scarring in this study. The persistence (52.9%) and recurrence (17.6%) rates in the eyes with subfoveal CNM in this study were lower than those in the MPS study. Interestingly, the functional outcomes in subfoveal classical and subfoveal occult CNM groups were similar. These eyes were also not associated with further deterioration of vision in a 12-month follow-up. However, we are cautious to suggest H-ALP as a substitute for ALP in the treatment of subfoveal occult and subfoveal classical CNM, since this is an interventional study with a limited number of patients and follow-up time. In addition, our criterion for functional success is limited only to the usual parameter: VA. Our findings suggest that H-ALP induces an insignificant amount of inflammation within the target and its surrounding tissue, or the drug itself, in maintenance therapy, inhibited the inflammation. However, occurrence of RPE rupture in 5.9% of cases suggested the thermal effect and inflammation. Our results need to be confirmed by multicenter studies with longer follow-up.

In the present study, there were no differences between the groups (types of CNM) with respect to anatomic and functional outcome. Recurrence and persistence rates were similar. None of the patients had severe visual loss in a mean 12-month follow-up (ranging from 6 to 29 months). Four eyes (11.7%, 2 eyes in each group) were associated with functional success. In addition, preoperative VA of 0.1 or more was found to be predictive for final VA of 0.1 or more, and the other variables were not effective in this visual outcome. However, we had a lack of results for CNMs with predominantly classical and minimally classical features. Large-scale studies are needed to reach a final decision.

In addition to its antidepressive effect, hypericin has been demonstrated to have anti-inflammatory, antiviral, antitumoral, and scarring effects (18-20). Its antiretroviral potential has led to its use in the ex vivo treatment of blood components (21). Pharmacokinetic properties of hypericin have been studied extensively in the literature (5). High doses of hypericin either orally or intravenously have been used in the clinic safely (11, 12). However, higher doses (>0.25 mg/kg hypericin) have been reported to be associated with significant phototoxicity (22). None of the patients we studied developed phototoxicity. This finding is consistent with the previous reports that confirm safety of hypericin in doses we used (23). Markowitz et al have found little perturbation of cytochrome P-450 and 3A4 activity in healthy volunteers taking SJW preparations at recommended doses for depression (24). Therefore, some drugs known to have interaction with hypericin were excluded from the study. Hypericin has been reported to be associated with photo-oxidation of the lens alpha crystalline *in vitro* (25). Our study, like large-scale studies in the literature, shows no association with cataract formation. Since these are older individuals with a tendency to lenticular opacification, 8.8% of progress in LOCS score may not be a sign of progression of cataract in eyes with H-ALP, if we consider the criteria for cataract in this study was limited only to increase in one grade. Toxicity to the RPE also has been shown *in vitro* (26). Our clinical and angiographic findings show no toxicity to the retina. In a monkey study, Fox et al showed that hypericin can not pass the blood-brain barrier (27). We do not know the distribution of hypericin in ocular fluids; however, that kind of toxicity was not reported in any clinical study, even in those including thousands of people. Hypericin seems to be a rather safe drug; however, in a multicenter study of 3250 patients, adverse effects have been noted in 2.4% of subjects, including gastric irritation, restlessness, tiredness, and allergic reactions (5). None of the patients in this study developed allergic or adverse reactions except gastric irritation in 1 (2.9%) patient. It is remarkable that they were satisfied with the use of the drug, due probably to its antidepressive effect in the elderly.

The results of the present study are encouraging. Still, it is difficult to apply our results to routine ophthalmic practice. Although the parameters in H-ALP we used seem to be appropriate not only for dose but also for route of hypericin, additional preclinical as well as experimental in-

vestigations are needed, since they were arbitrarily chosen. However, several comments can be raised. First, the used laser spectrum was a thermal one. Second, hypericin may not selectively accumulate in proliferating endothelial cells of CNM. Third, the study design (i.e., patient and lesion characteristics, eligibility, evolution, and exclusion criteria) may not be optimal. Fourth, the sample size and the follow-up time of the study were limited and variable (6 to 29 months). Still, we used argon-green light at a subthreshold fluence for photocoagulation (24 J/cm^2), which was similar to the threshold for minimal closure for CNM (25 J/cm^2) in Visudyne trials (14). Occlusion of choriocapillary layer proven angiographically and histopathologically was evidence of deeper penetration of argon-green light at this fluence. We are not sure whether this may simply relate to higher absorption coefficient of hypericin in this mode of therapy or other factors effective at this stage of the study. In their experimental study, Blank et al reported that tumor necrosis in hypericin PDT is not only determined by photophysical considerations (light penetration of number of absorbed photons) but is also influenced significantly by other mechanisms such as vascular effects (8). We are aware that definition of the actual optimum time in terms of drug loading into endothelial cells for hypericin is an absolute requirement in development of hypericin PDT, which is expected to be more safe and effective. This study is still underway at our institution. In order to treat CNM secondary to ARMD, we aimed to use hypericin with its well-known pharmacokinetic and photodynamic properties, to induce choriocapillary occlusion at subthreshold burn of ALP in this study. In a mean 12-month (6 to 29 months) follow-up, no H-ALP applications including the eyes requiring overlapping treatment spots in the same treatment session for large subfoveal CNM resulted in severe visual loss (more than three lines). The choriocapillary occlusion was limited to H-ALP-exposed area. In clinical examinations, no harmful effect on the retina was noted. These findings together with histopathologic ones have decreased our concerns over thermal damage to the functional retina in H-ALP applications. However, presence of a few RPE disruptions in histopathologic specimens and the clinical picture indicating pigmentary disturbances in perifoveal areas in some of the cases after H-ALP should be evaluated in future studies. The availability of the largest spot size as 1000 µm limited us to use overlapping treatment to cover a lesion greater than 1000 µm in diameter. Therefore, additional times were required for complete coverage of lesion. It is apparent that an argon laser equipped with a delivery system of larger spot size covering the entire lesion would be of great benefit to alleviate the concerns from overlapping burns and patient discomfort.

Histopathologic features of the choriocapillary endothelium in H-ALP-exposed areas were similar to those found in our previous study, and suggested that endothelial damage-initiated thrombosis (photothrombosis) was the primary event in this study (28). Such findings have also been shown in the treatment of cancers with hypericin PDT (2, 6). One main drawback of this study seems to be the variability in systemic drug availability in oral intake, which can be overcome with administration of intravenous hypericin. However, oral administration is beneficial not only for the patient's comfort, but also for the surgeon, enabling a longer application time, which is required when a 1000 µm spot size is used for closure of large CNM. It has been reported that oral bioavailability of hypericin is quite stable around 14%, and single dose pharmacokinetics of hypericin is associated with linearity over 750-µm doses. Likewise, kinetic parameters after intravenous administration of hypericin corresponds to those estimated after an oral dosage like ours (11). Hypericin is known to have a peak absorption spectrum of 596 nm, which is within the orange light spectrum. Since this wavelength has much more affinity for hemoglobin, photoactivation of hypericin with this wavelength is expected to have more effective closure for CNM and ocular vascular tumors, as well. As a second-generation photosensitizer, there are some studies to enhance the target specificity of hypericin by changing its chemical structure, and potentiation of photodynamic action by pH drop, and proton transfer (29, 30). Since hypericin is lipophilic, it has been postulated that the membranes are the principal target of hypericin, where it likely exerts its cytotoxic effect through the locally photogenerated reactive oxygen species (31). The fact that hypericin is mostly bound to low density lipoprotein (LDL) and its photodynamic action targets the neovascular lumen, which is rich with LDL receptors, makes hypericin a drug of choice for ophthalmic PDT (6). Moreover, hypericin was shown to be more stable than most photosensitizers used in PDT, including mTHPC and photofrin (32). In addition, fluorescence properties of hypericin can enable us to scan the PDT procedure *in vivo* (33). In addition to PKC inhibition, photoactivation in longer wavelength with efficient photo-oxidation make this drug a potential PDT agent in ophthalmology. In the current status we used, however, we cannot consider

H-ALP as a PDT option. Therefore, further studies are needed in this respect.

Our study design does not permit us to compare our results with Visudyne PDT with respect to difference in the type of laser wavelength and the inclusion and evaluation criteria in the study, nonetheless, it can be considered in clinical use owing to the lack of clinical worsening, stabilization, and even the improvement in vision after a limited number of treatment sessions were noticeable findings in H-ALP. In addition, the availability of argon-green laser as an ordinary tool in the clinic and the availability of hypericin as an inexpensive herbal drug in the market make this treatment modality attractive for developing countries. Since hypericin was shown to inhibit choroidal endothelial cell proliferation in natural formula without photo-oxidation, it is reasonable to assume that maintenance therapy might have beneficial effect for the absence of new CNM in the other eye of patients throughout the follow-up period. This effect of hypericin can also be explained by the low rate of recurrences observed in this study. If this is the case, both primary H-ALP application and its maintenance therapy can be used as an alternative new approach to PDT with antiproliferatives, which is under development.

Comparative and multicenter studies on the use of H-ALP for subfoveal CNM are necessary.

The authors have no proprietary or commercial interest for the drug mentioned in the manuscript.

Reprint requests to:
Güngör Sobacı, MD
Gülhane Askeri Tıp Fakültesi Göz AD
06018, Etlik-Ankara, Turkey
gsobaci@gata.edu.tr

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