CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-278

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-278
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	10/4/2007
DRUG NAME:	MembraneBlue™ 0.15% trypan blue ophthalmic solution
INDICATION:	Ophthalmic posterior membrane staining
SPONSOR:	Dutch Ophthalmic Research Center International, b.v., Scheijdelveweg 2, NL-3214 VN Zuidland, The Netherland Tel: 31-181-45-80-80; Fax: 31-181-45-80-90 Dutch Ophthalmic USA, One Little River Road, Kingston, NH 03484 Tel: 603-642-8486; Fax: 603-642-8465
REVIEW DIVISION:	Division of Anti-Infective and Ophthalmic Products
PHARM/TOX REVIEWER:	Zhou Chen, MD, PhD
PHARM/TOX SUPERVISOR:	Wendelyn Schmidt, PhD (pharmacology/toxicology team leader)
DIVISION DIRECTOR:	Wiley Chambers, MD
PROJECT MANAGER:	Mike Puglisi

Date of review submission to Division File System (DFS): June 25, 2008

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Approval is recommended.

B. Recommendation for nonclinical studies

No recommendation is necessary.

C. Recommendations on labeling

No recommendation is necessary.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Only one cytotoxicity study was submitted. Trypan blue produced slight cytotoxicity at concentrations of up to 0.15% in this *in vitro* cytotoxicity study using the MTT test system.

B. Pharmacologic activity

Trypan blue is widely used to selectively stain dead tissues or cells. The drug is not absorbed in a viable cell. For the indication proposed in this NDA, only the membranes are stained in contrast to the retina. The stained membranes will be removed from the eye.

C. Nonclinical safety issues relevant to clinical use

There are no drug-related safety issues relevant to clinical use.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

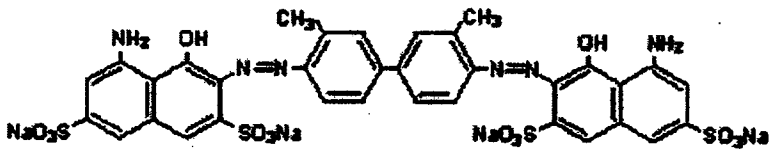
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 22-278
Review number: 001
Sequence number/date/type of submission: 0000/October 1, 2007/Commercial
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Dutch Ophthalmic Research Center International, b.v., Scheijdelveweg 2, NL-3214, VN Zuidland, The Netherland
Dutch Ophthalmic USA, One Little River Road, Kingston, NH 03484
Manufacturer for drug substance: ——— b(4)

Reviewer name: Zhou Chen
Division name: Division of Anti-Infective and Ophthalmic Products
Review completion date: June 25, 2008

Drug:

Trade name: MembraneBlue™
Generic name: Trypan blue ophthalmic solution 0.15%
Code name: Not provided
Chemical name: 3,3-[(3,3-dimethyl-4,4-biphenylylene) bis (azo)] bis(5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid) tetra sodium salt
CAS registry number: 72-57-1
Molecular formula/molecular weight: $C_{34}H_{24}N_6O_{14}S_4Na_4$, MW: 960.8
Structure:



Relevant INDs/NDAs/DMFs: NDA 21-670, DMF ——— DMF ——— and DMF ——— b(4)

Drug class: Biological staining

Indication: MembraneBlue™ is indicated for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures.

Clinical formulation: Each mL of MembraneBlue™ 0.15% contains 1.5 mg trypan blue; 1.9 mg sodium mono-hydrogen orthophosphate ($Na_2HPO_4 \cdot 2H_2O$); 0.3 mg sodium di-hydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$); 8.2 mg sodium chloride; and water for injection. The pH is 7.3 to 7.6.

Route of administration: 0.5 mL of MembraneBlue™ is packaged in a 2.25 mL syringe to which a thin blunt cannula is attached. During vitrectomy surgery, when the entire vitreous cavity is filled with air, MembraneBlue™ is carefully applied to the retinal membrane using a blunt cannula.

Proposed use: MembraneBlue™ is developed to provide contrast between the membranes on the inner surface of the retina and the retina when performing the vitrectomy surgery. ——————————————— . The proposed clinical dose is 0.75 mg/person (0.5 mL of 0.15% trypan blue). b(4)

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Toxicology
——— Accelerated aging test
——— /MTT test system 24 hr (GLP)

2.6.2 PHARMACOLOGY

No pharmacology studies were conducted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

No PK studies were conducted.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Only one cytotoxicity study was included in this submission. Trypan blue produced slight cytotoxicity at concentrations of up to 0.15% over 24 hr of exposure in this *in vitro* cytotoxicity study using the MTT test system.

Several literature reports related to the toxicity of trypan blue were submitted. Below is a summary of the publications.

In an ocular toxicity study using the rabbit vitrectomy model, animals were treated by intravitreal injection with 0.1 mL of 0.06% or 0.2% trypan blue solution and were observed for 4 weeks. No toxicity

was seen in the 0.06% group. At 0.2%, light and electron microscopic examinations of the inferior retina showed damaged photoreceptors and marked disorganization. Immunohistochemical staining for rhodopsin was strongly reduced corresponding with the morphological loss of photoreceptor outer segments. The staining for proliferation with Ki-67 was positive, possibly indicating a reactive proliferation in Muller cells as a response to photoreceptor damage.

In a study to analyze the effect of trypan blue on the retina on an experimental *ex vivo* model of macular surgery, porcine eyes were used within 3 hr after death. Trypan blue (0.15%) was applied to the retina for one min, followed by 10 min of illumination. There was no acute morphologically detectable toxic effect reported.

In several *in vitro* studies, trypan blue at different concentrations (0.0075-0.30%) incubated with cultured human retinal pigment epithelium cells (and Muller cells in one study) for 3 min to 72 hr showed no toxic effect.

In an *in vitro* study to evaluate the retinal toxicity of trypan blue, indocyanine green (ICG) and triamcinolone acetonide using isolated rat retina incubated in artificial aqueous humors, treatment with trypan blue (0.06%, one min) showed no retinal damage.

In an *in vitro* study to evaluate the retinal toxicity of trypan blue using cultured human retinal pigment epithelium cells (ARPE-19) and rat neurosensory retinal cells (R28), treatment with trypan blue at concentrations of 0.0125% to 0.1% for 2 min in combination with illumination (0, 5 or 15 min) showed no toxicity in ARPE-19 cells as evidenced by cell viability, mitochondrial dehydrogenase activity, and ^{3}H-thymide incorporation assay. However, at 0.1% in R28 cells, trypan blue caused a significant decrease in mitochondrial dehydrogenase activity. This study suggested that R28 cells may be more sensitive to trypan blue than ARPE-19 cells.

In an *in vitro* study to evaluate the acute (5 min) and long term (6 days) toxicity of trypan blue, indocyanine green and infracyanine using cultured human retinal pigment epithelium cells, treatment with trypan blue at concentrations of 0.05%, 0.06%, 0.1%, 0.15% and 0.5% for 5 min showed no toxicity to cultured RPE cells. However, cytotoxicity was noted with 6-day treatment for all concentrations evidenced by decreased esterase activity, decreased cell number, and morphological changes.

In one *in vitro* study, trypan blue at concentrations of 0.006%, 0.06% and 0.4% was applied to human retinal pigment epithelium cells for one min. Cell viability was measured using the MTT assay for 6 consecutive days. Trypan blue caused a dose-dependent suppression effect on cell viability with high reduction at 0.06% and 0.4%. An increase in p53 (an apoptosis associated gene) expression was found in 0.4% group. A significant increase in p21 expression was seen in both 0.06% and 0.4% groups, implying the arrest of cell cycle progression or induction of transient arrest in the G1 phase.

In one *in vitro* study to measure the effect of indocyanine green 0.05%, trypan blue 0.15% and patent blue 0.48% on bovine retinal function, bovine retinal preparations were perfused with one of the dye solutions for 10 sec to 2 min, and ERG was performed. Decreases of b-wave amplitude were found in all dyes. Trypan blue produced a loss of b-wave when the retina was exposed to trypan blue for 10 sec or longer.

In a study to examine the intracellular uptake and extracellular binding of indocyanine green and trypan blue by human RPE (ARPE-19) cells, human RPE cells were exposed to trypan blue at several concentrations (0.06%, 0.15% or 0.25%) for 15 sec, 30 sec, and 5 min. The results showed that trypan blue was not taken up or bound to RPE cells.

In a study to evaluate the effect of subretinal injections of indocyanine green, trypan blue, glucose, and BSS in Dutch-belted rabbits, 10 animals underwent vitrectomy and subretinal injection of 20 μL of 0.15% trypan blue, 0.05% ICG, 5% glucose, or BSS. Ten additional animals underwent subretinal injection of 20 μL of 0.13% trypan blue, 0.046% ICG, 4.6% glucose, or BSS. Animals were examined 6, 12, 24 hr and 14 days after the injection. Fluorescein angiography showed window defects where trypan blue had been injected. Subretinal injection of trypan blue resulted in edema of the photoreceptor outer segments and the photoreceptor inner segments, and pyknosis of the outer nuclear layer 6 and 12 hr after surgery. The retinal pigment epithelium was also affected 24 hr and 14 days after surgery.

In submitted publications related to reproductive toxicity studies, trypan blue caused teratogenic effects in Swiss albino mice and Wistar rats. The findings included exencephaly, hematomata, spina bifida, microcephaly, crooked neural tubes, failure of neural folds to close, and fusion of somities. Trypan blue also induced ocular defects (microphthalmia and anophthalmia with abnormal changes in the retina and lens) in 16-day rat fetuses from the dams exposed to trypan blue at 100 mg/kg (IP). The author indicated that trypan blue caused development arrest in this eye model, and the lack of lens differentiation was attributed to absence of the retina.

In a study to evaluate trypan blue-induced lymphomas in Wistar/Lewis and Wistar/Furth rats, treatment Regimen I (50 mg/kg, SC injection once every other week for 52 weeks) and Regimen II (50 mg/kg, SC injection once a week for 6 weeks) produced a similar incidence of tumors of identical cell type, suggesting that a short but intense exposure to trypan blue is sufficient to trigger the chains of events necessary for neoplastic transformation of the target cells.

2.6.6.2 Single-dose toxicity

No single-dose toxicity studies were provided.

2.6.6.3 Repeated-dose toxicity

No repeated-dose toxicity studies were provided.

6.6.6.4 Genetic toxicology

No genotoxicity studies were submitted.

2.6.6.5 Carcinogenicity

No carcinogenicity studies were submitted.

2.6.6.6 Reproductive and developmental toxicology

No reproductive studies were submitted.

2.6.6.7 Local tolerance

No local tolerance studies were submitted.

2.6.6.8 Special toxicology studies

——— Accelerated aging test and ——— MTT test system 24 hr (GLP)

This GLP study was performed by ——————————————— The purpose of this study was to determine the cytotoxic effects of the test articles before and after aging on the growth, morphology and metabolism of fibroblasts in the MTT test. b(4)

Test articles: 160-05A: Trypan blue 0.3%. The test concentrations were 0.15% (160-05A1), 0.075% (160-05A2), 0.0375% (160-05A3), and 0.01875% (160-05A4).

160-05B: Trypan blue 0.2%/indocyanine green 0.1%. The test concentrations were 0.1%/0.05% (160-05B1), 0.05%/0.025% (160-05B2), 0.025%/0.0125% (160-05B3), 0.0125%/0.00625% (160-05B4)

160-05C: Trypan blue 0.3%, after aging [Trypan blue 0.3% (160-05A) samples were conditioned at 73 °C and > 40% rH (relative humidity) for a maximum of 6 weeks, mimicking a shelf life of 3 years.]. The test concentrations were 0.15% (160-05C1), 0.075% (160-05C2), 0.0375% (160-05C3), and 0.01875% (160-05C4).

Procedure: A monolayer of mouse lung fibroblasts (L929) was challenged with the test articles at different concentrations, positive or negative controls or medium (n=6/concentration). After exposure to the test samples for 24 hr at 37 °C, the cells were examined microscopically for cytotoxic effects: confluence of the monolayer and changes of cellular morphology. MTT, a water soluble tetrazolium salt yielding a yellowish solution, was added to each culture. MTT was converted into an insoluble purple formazan dye by active mitochondrial dehydrogenases of living cells. After 3-hr incubation at 37 °C, cells were processed and formazan concentrations were measured by the optic density (OD) at 570 nm. The mean OD value for the negative control was standardized as 0% growth inhibition, the positive control as 100% inhibition. Finally, cytotoxicity was regarded by addition of the mean score for microscopic changes and the mean score for growth inhibition.

Toxic grade	0	1	2	3	4
Confluency of monolayer (%)	100	90-100	60-90	30-60	0-30
Change of cell morphology	No difference from negative control	Slight changes, few cells different from negative control	Mild changes, some cells different	Moderate changes, many cells rounded	Several changes, about all cells with morphologic changes
Inhibition of cell metabolism (%)	0-10	10-30	30-50	50-70	70-100

Interpretation of results: 0-1.0: none; 1.1-3.0: slight; 3.1-5.0: mild; 5.1-7.0: moderate; 7.1-8.0: severe.

Results: 160-05A3 and 4, 160-05B3 and 4, and 160-05C3 and 4 showed no cytotoxicity (score = 0.5-1.0).

160-05A1 and 2, 160-05B2 and 160-05C2 showed slight cytotoxic reactivity (score = 1.5-2.5).

160-05B1 and C1 showed mild cytotoxic reactivity (5.0 and 3.5, respectively).

In conclusion, trypan blue produced slight cytotoxicity at concentrations of up to 0.15% in this *in vitro* cytotoxicity study using the MTT test system.

2.6.6.9 Discussion and Conclusions

In this NDA submission, the sponsor wants to develop trypan blue ophthalmic solution for the selective staining of membranes on the inner surface of the retina in vitrectomy surgery. Staining the membrane will facilitate the surgical treatment and _____ b(4)
_____.

Trypan blue has been investigated for staining the membranes in posterior surgery since 2001 with concentrations of up to 0.30%. MembraneBlue™ obtained CE approval as a medical device Class IIa in 2002. According to the sponsor, over —— units have been marketed in European and Asian countries, and no complaint or information about adverse events has been reported. b(4)

Trypan blue has been approved for ocular staining in cataract surgery (VisionBlue™ 0.06%) under NDA 21-670. NDA 21,670 is cross-referenced for this NDA. The toxicological profile for carcinogenicity, teratogenicity, and mutagenicity has already been established. It is reported that trypan blue was teratogenic in rats, mice, rabbits, hamsters, dogs, guinea pigs, pigs, and chickens. It caused external, skeletal, and internal malformations. Trypan blue was mutagenic in Ames test. Trypan blue is carcinogenic in rats. Chronic intermittent exposure by subcutaneous injection of trypan blue in Wistar/Lewis rats induced a reticuloendothelial neoplasm, predominantly in the liver. The aforementioned information has been listed in the proposed labeling for MembraneBlue™.

In this NDA submission, only one cytotoxicity study was included. Trypan blue produced slight cytotoxicity at concentrations of up to 0.15% over 24 hr of exposure in this *in vitro* study using the MTT test system. The sponsor also cited other toxicology information from the literature. Mostly, the papers selected are concentrated on the toxic effects of trypan blue in the retina using *in vitro* cultured human and animal retinal pigment epithelium cells or *in vivo* animal models. Some studies indicated that trypan blue was safe. Other studies showed positive findings of trypan blue including damaged photoreceptors and disorganization, decrease in mitochondrial dehydrogenase activity, morphological changes of the RPE cells, lowed ERG b-wave in bovine retina, and increased p53 and p21 expression. The toxicity was usually seen at higher concentrations and longer treatment duration.

Trypan blue is a vital stain widely used to selectively stain dead cells. The drug is not absorbed in a viable cell. Therefore, only the epiretinal membranes are stained in contrast to the retina. Clinically, only 0.75 mg of trypan blue will be administered to the eye, and almost all dye will be immediately irrigated out from the eye, leaving less than — to mark the membrane that too will be removed from the eye. Therefore the amount of the drug left in the patient after the surgery will be very low. The sponsor indicated that the final dose used in vitreoretinal surgery is less than — mg. It appears that possible systemic and ocular toxic effect of MembraneBlue™ is small. b(4)

Considering the drug history and clinical experience, nonclinical study results, proposed indication, and dosage, the reviewing pharmacologist believes that, from the nonclinical standpoint, the data are adequate for the approval of the drug. The labeling of MembraneBlue™, which is based on the approved labeling of VisionBlue, is considered acceptable.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Considering the drug history and clinical experience, nonclinical study results, proposed indication, and dosage, the reviewing pharmacologist believes that, from the nonclinical standpoint, the data are adequate for the approval of the drug.

Unresolved toxicology issues (if any): No

Recommendations:

Approval is recommended.

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/s/

Zhou Chen
6/25/2008 09:50:42 AM
PHARMACOLOGIST

Wendelyn Schmidt
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