Original articles

Bactericidal effect of trypan blue and fluorescein sodium in ophthalmic practice

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Abstract

Objective: Trypan blue and fluorescein sodium are two commonly used dyes in ophthalmic practice. The staining of anterior lens capsule is achieved using trypan blue while fluorescein finds its application in fluorescent angiography. We assessed the 'in vitro' bactericidal effect of these two agents.

Materials and methods: The cidal properties of these two dyes were tested in vitro against thirty representative strains of four commonly isolated bacteria from the eye. The concentration of dyes used were 0.6 mg/ml, 0.1 mg/ml, and 0.05 mg/ml for trypan blue and 20 mg/ml, 10 mg/ml and 5 mg/ml for fluorescein sodium.

Result: We found that trypan blue and fluorescein sodium at an optimum concentration of 0.6 mg/ml and 20 mg/ml respectively could produce a significant fall ($p < .05$) in the bacterial count of representative Gram positive and Gram negative bacteria.

Conclusion: The use of prophylactic antibiotics/antiseptic eye drops can be avoided if these two dyes are used at an optimum bactericidal concentration but side effects of the same at these concentrations need to be kept in mind.

Key-words: bactericidal effect, fluorescein sodium, trypan blue

Introduction

Trypan blue is a vital dye used in diagnostic laboratories for tissue and fungal staining. In cataract surgery, it is used to enhance visualization of the anterior lens capsule of the lens during removal of cataract. It provides a clear visible staining at low amounts without diffusing into or through said tissues and facilitates controlled opening of anterior capsule thus reducing the risk of inadvertent damage to the capsule. The optimal staining effect is achieved in the range of 0.01 % and 0.1 % solution.

Fluorescein sodium is a fluorescent dye which is commonly used in fluorescent microscopy. It is also used in angiography because it demarcates the vascular area under observation to distinguish it from adjacent area by producing yellowish green fluorescence. In eye it is used in diagnostic fluorescent angiography or angioscopy of the fundus and iris vasculature in concentration of 10 % and 20 %.

The current study was undertaken to determine the in vitro bactericidal effect, if any, of these two commonly used dyes in ophthalmology.

Patients and methods

The study was conducted in the Microbiology Lab of Guru Nanak Eye Centre, New Delhi, India which is a tertiary care centre exclusively for Ophthalmology and attached to Maulana Azad Medical Col-

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lege, New Delhi. The test strains of bacteria, ie S aureus, CNS, E.coli and Pseudomonas aeroginosa were from our own Microbiology Lab. These strains were obtained from routine cases of conjunctivitis, keratitis, corneal ulcers, post operative endopthalmitis, stye etc. These strains were identified by standard microbiological techniques based on colony morphology and biochemical reactions (Collee et al, 1996)

Dyes: Trypan blue solution (0.6mg in 1ml ampoules manufactured by SuNayan Pharmaceuticals) was used in three concentrations i.e. 0.6mg/ml; 0.1mg/ml; and 0.5mg/ml. Fluorescein sodium (60mg in 3ml ampoule, manufactured by SuNayan Pharmaceuticals) was used in a concentration of 20mg/ml, 10mg/ml and 5mg/ml.

Bacterial strains: Thirty strains each of Staphylococcus aureus (S aureus), Coagulase negative staphylococcus (CNS), Escherichia coli (E coli) and Pseudomonas aeruginosa (P aeruginosa) were tested against all three concentrations of the above mentioned dyes). The counts performed were semiquantative counts. The bactericidal suspensions were made at a concentration of 1.5 x10^8 cfu /ml (0.5 Mc Farland standard) which was diluted 1000 times to give a concentration of 1.5 x10^5 cfu/ml (Collee et al, 1996), because this count can easily be counted on a standard blood agar plate (90 mm).

Bactericidal effect: 100µl of 1.5x 10^5 cfu/ml bacterial suspension prepared in nutrient broth was added to 1 ml each of trypan blue (0.6 mg/ml, 0.1 mg/ml, 0.05mg/ml) dilutions prepared in distilled water. These inoculated suspensions were then incubated at 37^{0}c for 24 hours and 10µl each of these was subcultured on 5 % sheep blood agar at 0, 15, 30, 60 minutes and finally at 24 hours incubation & plates incubated at 37^{0}c for 18 – 24 hours.A control subculture without the dye to obtain a colony count of 1.5 x 10^5 cfu/ml was incorporated with each batch of test for individual bacteria. Colony count was performed the next day which was then compared with the count in the control plates to calculate % fall in bacterial count (Reller, 1986).

Statistical analysis: Statistical analysis was performed using Fisher exact T test and chi square test of significance and p value of 0.05 or less than was considered as significant.

Results

A total of one hundred twenty bacterial strains were tested against three concentrations of trypan blue fluorescein sodium. Trypan blue at a concentration of 0.6mg/ml could produce a more-than-100 fold inhibition of 100(83.3 %) bacterial isolates and fluorescein sodium also showed more than 100 fall for 100 (83.3 %) bacteria at a concentration of 20mg/ml. The cidal effect was optimal at 15 minutes of incubation & hence all results have been analyzed at this time of incubation. At further lower concentrations of these two dyes, cidal effect was present but it was much lower than the results obtained at higher concentrations.

Fig 1: Trypan blue: S aureus

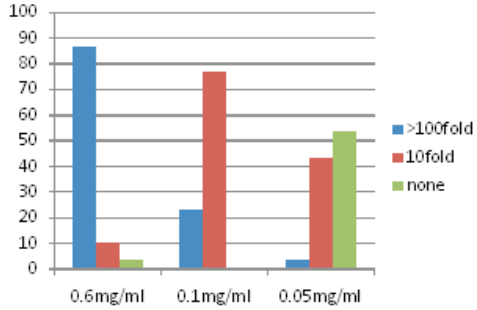


Fig 2: Fluorescein: S aureus

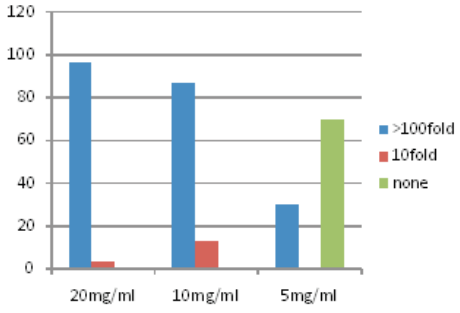


Fig 3: Trypan blue cons

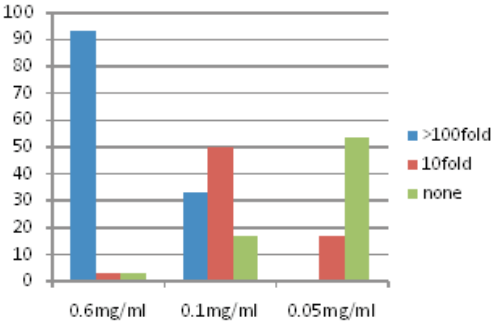


Fig 4: Fluorescein: Cons

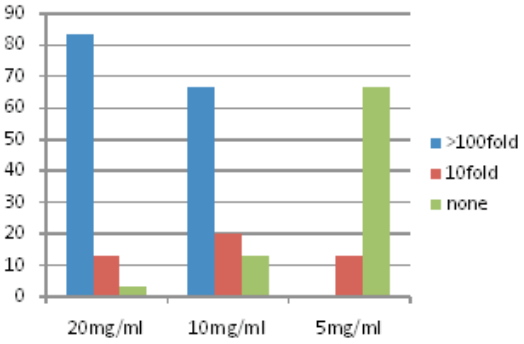


Fig 5: Trypan blue: E coli

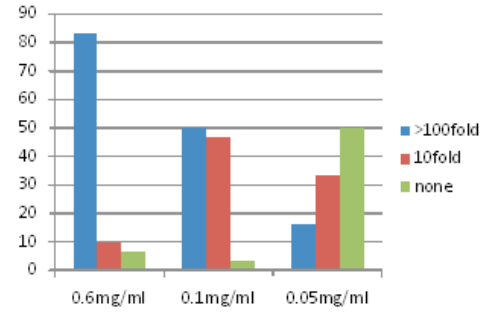


Fig 6: Fluorescein: E coli

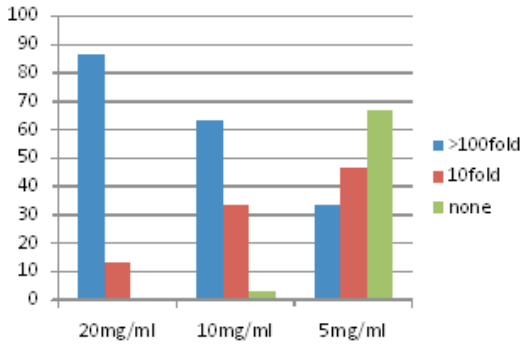


Fig 7: Trypan blue: P aeruginosa

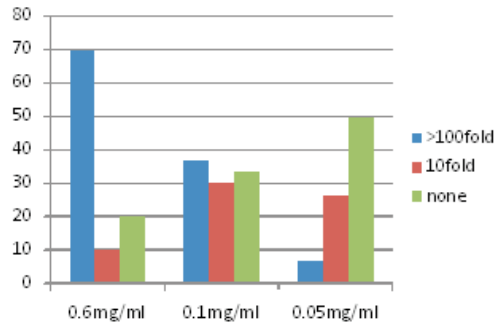
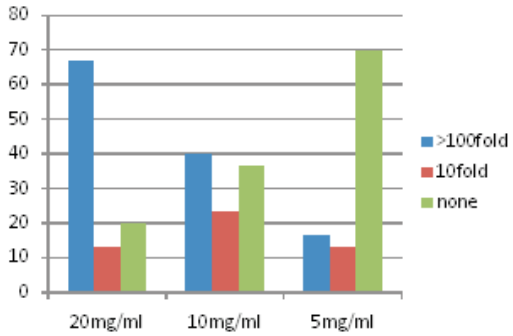


Fig 8: Fluorescein: P aeruginosa



At optimal concentration, trypan blue inhibited maximum number of CNS (43.4 %) followed by S aureus (86.7 %), E coli (33.4 %) and P aeruginosa (70 %). However fluorescein sodium (20mg/ml) inhibited 96.7 % of S aureus followed by E coli (86.7 %) CNS (83.4 %) and Pseudomonas aeruginosa (66.7 %) (Figures 1 to 8). At a lower concentration of 0.1mg/ml, Trypan blue showed a 100 fold fall in 50 % E coli followed by 36.7 % P aeruginosa, 33.3 % CNS and only 23.3 % S aureus. Fluorescein (10mg/ml) on the other hand produced a more than 100 fold fall in 86.7 % S.aureus followed by CNS(66.7 %), E coli (63.45) and P aeruginosa (40 %).

Discussion

Trypan blue and fluorescein sodium are widely used dyes in ophthalmology. Trypan blue is used for staining of anterior lens capsule during cataract surgery while fluorescein sodium is used in fluorescent angiography of fundus and iris vasculature. Because of widespread use of these dyes, we performed in vitro bactericidal effect of these dyes on commonly

isolated Gram positive and Gram negative ocular pathogens.

Trypan blue was found to be the most effective at 0.6 % concentration at which it produced a significant ($p < .05$, more than 100 fold) fall in bacterial counts. Amongst Gram positive bacteria it was more effective against CNS (93.4 % isolates inhibited) as compared to S aureus (86.7 %).

Amongst Gram negative rods, it could inhibit 83.4 % E. coli and 70 % P aeruginosa. The stain was found to be more effective against Gram positive cocci than Gram negative bacilli.

Fluorescein at an optimal concentration of 20mg/ml was more effective against S. aureus (96.7 %) than CNS (83.4 %). It produced a more than 100 fold fall in 86.7 % E coli and 66.7 % P aeruginosa strains.

There are studies which indirectly relate to the cidal properties of trypan blue. The dye has been reported to be toxic to cultured retinal pigment cells (Kwok et al, 2004). It has also been reported to enhance bactericidal effect of laser irradiation on carcinogenic bacteria (Streptococcus mutans) when used as photosensitizer (Taketo & Taketo 1982). It inhibits hemolytic activity of oligonucleotide streptolysin S (SLS) complex, an exotoxin produced by Streptococcus pyogens (Taketo & Taketo 1982). Its inhibitory action on complement mediated phagocytosis by interaction with C3 receptors has also been analyzed in details in various studies (Harpers et al, 1981; Ouckian et al, 1978).

Fluorescein on the other hand has been tried experimentally in designing surfaces that kill bacteria on contact (Tiller et al 2001). No other study has been performed earlier on bactericidal effect of this dye to the best of our knowledge and search on net.

We found that incubation of trypan blue and fluorescein dye with bacteria for an optimum time period of 15 minutes produced a significant ($p < .05$) fall in bacterial count from 10^5 cfu/ml to 10^3 cfu/ml.

Conclusion

Trypan blue and fluorescein dye do have bactericidal effect and they should be further tested at higher concentration in vitro and correlated in vivo studies. If a positive correlation can be established keeping in mind the side effects of excess concentration of both the dyes in eye, these can be used in ocular procedures without using any other prophylactic antibiotic / antiseptic eye drops.

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