Effect of rifampicin and erythromycin on the initiation of galactose induced cataract in rats

Sir,

Cataract is the leading cause of blindness with a number of risk factors such as aging, genetic, steroids, nitric oxide, diabetes, ultraviolet radiation and smoking.[1] An increased incidence of cataract is associated with diabetes and it is one of the major cause of visual impairment in diabetics.[2]Cataractogenesis is one of the earliest secondary complications of diabetes mellitus.[3]

The mechanism involved in the development of diabetic cataract is different from senile cataract. The accumulation of polyols within the lens is an important contributing factor in diabetic cataractogenesis.[3] The enzyme aldose reductase within the lens converts glucose to sorbitol or galactose to galactitol using NADPH as a co-factor. Electron transfer from NADPH further depends on cytochrome P450 (CYP) system. These polyols thus formed, accumulate and lead to the formation of cataract. It has thus been hypothesized that by using enzyme inducer, pioglitazone, and inhibitor, diltiazem and nifedipine, the activity of aldose reductase and formation of cataract can be altered.[4,5] However Jaykaran[6] and Mahajan *et al*.[7] have questioned the inclusion of pioglitazone, a weak inducer of CYP enzyme.

The present study was undertaken to test the role of rifampicin, known cytochrome inducer[8] and erythromycin, known cytochrome inhibitor[9] on the development of cataract based on the above hypothesis.

Experimentally naive 18 day old male Sprague Dawley albino rats were used. The rats were maintained under standard conditions of temperature (25°C ± 5°C), relative humidity (55 ± 10%) and a 12/12 h light/dark cycle. The rats were fed with commercial rat pellet diet manufactured by Pranav Agro Food, Pune and water ad libitum. The study was approved by the Institutional Animal Ethics Committee.

These rats were screened for any abnormality of lens and randomly allotted to four groups:

Group I - Lab diet control group **(L)**: Rats were fed normal laboratory chow from day 23 after parturition (no galactose and no drug added).

Group II- Galactose control group **(G)**: Rats were fed with galactose - rich diet from day 23 after parturition with no

additional drug.

Group III- Rifampicin pretreated group **(G + R)**: Rats were pre-treated with rifampicin (40 mg/kg once daily PO) from day 18 after parturition and fed with galactose-rich diet from day 23, after parturition.

Group IV- Erythromycin pretreated group **(G + E)**: Rats were pre-treated with erythromycin (90 mg/kg once daily PO) from day 18 after parturition and fed with galactose-rich diet from day 23, after parturition.

Cataract was induced in group II, III and IV by feeding galactose (30%).

The 4 rats in each of the above mentioned groups were taken (8 lenses in each group) and the lenses were examined using ophthalmoscope from day 23 of life (the first day from galactose feeding in group II, III and IV). The examination was done daily in the evening by a trained blind evaluator. The day of initiation of cataract was noted in each of the lens. Morphological changes in lens were compared in all groups of rats to evaluate the effects of the drugs on the initiation of cataract. The incidence of occurrence of cataract on a given day of galactose feeding was recorded and the data was tabulated. The experiment was continued until cataract developed in all the lenses.

The *P* value for mean day of initiation of cataract among the groups was calculated using ANOVA. The incidence of cataract (total number of lenses affected with cataract) was calculated as a percentage to the total number of lenses and the statistical significance was performed using Fischer exact test/Chi square test. *P* value ≤ 0.05 was considered statistically significant.

It was observed that the weight gain of rats was similar across all the four study groups during the entire study period. Cataract did not develop in any lens in lab diet control group (L) throughout the study period.

The cataract development was delayed in erythromycin pretreated group compared to galactose control group and rifampicin pretreated group, and this was highly significant statistically with a *P* value = 0.001. The mean day of occurrence of cataract was earlier in rifampicin pretreated group compared to galactose control group; however this was not statistically significant. [Table 1]

The incidence of cataract was studied in all four groups from day 1 to 19 of galactose feeding. The development of cataract was not seen in any lens in lab diet control group throughout the study period. In galactose control group, no lens developed cataract till 9th day. In this group 7 out of 8 lenses developed cataract on day 10 and remained so till the 12th day. Therefore the incidence was 87.5% from 10th to the 12th day. On 13th day all the lenses were affected and hence the incidence was 100%. In rifampicin pretreated group, 2 out of 8 lenses developed cataract on the 9th day itself (25% incidence). Further, 6 out of 8 and 7 out of 8 lenses were affected by day 10 and 11 respectively. On these days the incidence was 75% and 87.5% respectively. All the lenses developed cataract by the 12th day, therefore the incidence was 100%. In erythromycin pretreated group, the cataract was seen only in 2 lenses out of 8 i.e. 25% incidence on day 10. Four more lenses developed cataract on day 14 with 75% incidence. 7 out of 8 lenses developed cataract by day 15 (incidence 87.5%) and remained so till day 18. All the lenses developed cataract by day 19 (incidence 100%). [Table 2, Figure 1]

The incidence of cataract in rifampicin pretreated group was not significantly higher compared to galactose control group. The incidence of cataract was significantly lower in erythromycin pretreated group on day 10, 11, 12 and 13 compared to the galactose control group.

Diabetes mellitus is one of the leading causes of morbidity and mortality worldwide.[10] Cataract is one of the common causes of visual impairment in these patients.[2] Cataracts occur

Table 1: Mean day of initiation of cataract

Groups	Mean day of initiation of cataract
G	10.38±1.06
G+R	10.13±0.99
G+E	13.75±2.87*

*P = 0.001

Table 2: Incidence of cataract

N	G		G+R		G+E	
	No	%	No	%	No	%
9	0	0.0	2	25.0	0	0.0
10	7	87.5	6	75.0	2	25.0*
11	7	87.5	7	87.5	2	25.0*
12	7	87.5	8	100.0	2	25.0*
13	8	100.0	8	100.0	2	25.0*
14	8	100.0	8	100.0	6	75.0
15	8	100.0	8	10.00	7	87.5
16	8	100.0	8	100.0	7	87.5
17	8	100.0	8	100.0	7	87.5
18	8	100.0	8	100.0	7	87.5
19	8	100.0	8	100.0	8	100.0

Outcome = number of lenses affected and percentage of lenses affected
Total number of lenses in each group = 8 (4 rats) N = nth of galactose feeding*P< 0.05 ANOVA/Fischer exact test/Chi square test applied

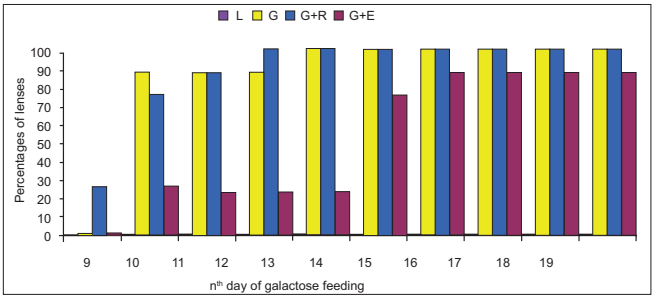

Figure 1: Incidence of cataract on nth day

2–5 times more frequently in patients with diabetes.[10] Various pharmacological strategies have been proposed for prevention and treatment of cataract. The agents tested range from aldose reductase inhibitors, NSAIDS, vitamins to herbal drugs.[1] It is hypothesized that by inducing or inhibiting CYP one can alter the activity of aldose reductase and may delay the formation of cataract. In the polyol pathway enzyme aldose reductase within the lens converts glucose to sorbitol or galactose to galactitol using NADPH as a co factor. Electron transfer from NADPH further depends on cytochrome P450 (CYP) system. Further, the accumulated polyols lead to the formation of cataract.[4,5,11]

In the study by Nair *et al*.,[4] pioglitazone, a cytochrome inducer and diltiazem, a cytochrome inhibitor were used. Similarly, in the study by Patel *et al*.,[5] pioglitazone was used, but the CYP inhibitor used was nifedipine. However, the choice of the test drug pioglitazone is questionable, as it is a weak inducer of CYP.[6,7] Therefore in the present study, effect of modulation of polyol pathway has been studied using known CYP inducer[8] rifampicin and known CYP inhibitor[9] erythromycin.

In this present study, CYP inducer rifampicin did not have a significant effect on either reducing or increasing the mean day of initiation of cataract. However CYP inhibitor erythromycin significantly delayed the mean day of initiation of cataract. In erythromycin pretreated group the incidence of occurrence of cataract was significantly lower compared to rifampicin pretreated group and galactose control group on day 10, 11, 12, and 13. Although not significant, the overall rank order of initiation of cataract based on the incidence of the lenses affected in the study groups followed the following trend: Rifampicin pretreated group > galactose control group > erythromycin pretreated group.

The method of examination used in the study by Nair *et al*.[4] was ophthalmoscopic examination and there is no mention on staging. Their comment on maturation pattern is made based on the cumulative incidence of cataract on the last day of galactose administration. Maturation of cataract is its progression to higher stage. The present study comments only on the initiation of cataract and incidence of cataract on a particular day or days of galactose feeding. We have a study ongoing testing the effect of rifampicin and erythromycin on the progression of cataract.

Small sample size, inability to directly extrapolate the results to humans and lack of population data implicating CYP modulation and incidence of cataract in human subjects are some drawbacks of the present study.

Previous studies[4,5] quote that the inducers have significantly caused early initiation of cataract, but in the present study the inducer rifampicin did not. But the inhibitors have significantly delayed the occurrence of cataract in the present study which is consistent with the results of the previous studies.[4,5] It is therefore, not possible to extrapolate results of one enzyme inhibitor or inducer to other CYP modulators. Therefore further investigations using a variety of CYP modulators are needed to identify the pharmacological profile of CYP modulators on cataractogenesis in large scale. Also if this hypothesis is tested in a clinical setting then it may help reduce the burden of diabetic cataract by simply altering the choice of the medicines preferring a CYP inhibitor instead of inducer for a diabetic patient.

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