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Role of Cytochrome Modulators in Altering the Occurrence of Cataract in Rats

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ABSTRACT

Background: Cataract is one of the primary causes of blindness all over the world. It indicates the onset of secondary complications of diabetes. The only treatment available is surgery as there are no satisfactory drugs which can prevent or retard the initiation and maturation of cataract. It was hypothesized that cytochrome P 450 (CYP) inducers or inhibitors can modify the cataract occurrence by accelerating or delaying the occurrence of cataract respectively.

Objective: To study the effect of two commonly used drugs, phenytoin (CYP inducer) and ciprofloxacin (CYP inhibitor) on the initiation and maturation of cataract with the galactose-induced cataract model.

Materials and Methods: The experiment was conducted in 24 new born male Wistar rats. Cataract formation was induced with a 50% galactose diet. The rats were randomized into four groups of 6 rats each: Group 1 rats received a normal diet; Group 2, 3 and 4 rats received 50% galactose diet day 23 onwards. In addition, Group 3 rats were pre-treated with ciprofloxacin (20mg/kg) and Group 4 rats were pre-treated with phenytoin (50mg/kg) day 18 onwards once a day orally. The appearance of cataract was checked daily with an ophthalmoscope. The maturation pattern was examined using Fundus Fluorsen Angiographer (FFA). The cataract was graded according to Sippel's classification. The experimental and control groups were compared by chi square test and the results were considered significant at $p< 0.05$.

Results: The initiation of cataract was significantly delayed with ciprofloxacin as compared to galactose; however, there was no difference in the maturation pattern of cataract in both the groups. In spite of being a CYP inducer, the initiation of cataract was not accelerated in phenytoin group. Rather, it was significantly delayed and the cataract did not progress to stage 5 even on 30th day of galactose administration

Conclusion: CYP450 modulators have a significant effect on the initiation of cataract without significantly altering the maturation pattern. It is not reasonable to extrapolate the results of one enzyme inhibitor or inducer to other CYP modulators. Hence, further studies are needed to identify the pharmacological profile of various CYP modulators on the occurrence of cataractogenesis.

Keywords: Galactose, Microsomal enzyme inducer, Microsomal enzyme inhibitor

INTRODUCTION

Cataract is defined as the clouding of the lens that affects vision. It is one of the primary causes of blindness all over the world [1]. The various risk factors for cataract include aging, smoking, diabetes, female gender and use of corticosteroids. Cataract development heralds the onset of secondary complications of diabetes. At present, the only treatment option available for cataract is surgery as there are no satisfactory drugs available which can prevent or retard the initiation and maturation of cataract. The three major mechanisms that may be involved in the development of diabetic cataract are nonenzymatic glycation of eye lens proteins, oxidative stress and activated polyol pathway [2].Various animal studies have shown that diabetic cataracts occur through polyol osmotic mechanism in which intercellular accumulation of polyol via aldose reductase contributes to lenticular opacity [3,4]. Enzyme aldose reductase helps in the conversion of excess glucose in sorbitol using NADPH as cofactor. Electron transfer from NADPH further depends upon cytochrome P 450 enzyme system [5]. Hence, it was hypothesized that cytochrome P 450 inducers or inhibitors can modify the activity of aldose reductase and thus the synthesis of sorbitol and the cataract occurrence. Galactose induced cataract in rats corresponds to lenticular polyol accumulation in humans.

Patients who have cataract might be epileptics as well. Hence, they might be on one the anti-epileptics including phenytoin (CYP1A2 microsomal enzyme inducer) [6]. Moreover, cataract patients might develop an infection for which ciprofloxacin (CYP1A2 microsomal enzyme inhibitor) [7] might be indicated. Hence, the use of these drugs may have an impact on the occurrence of cataract. In this study, the effects of these two commonly used drugs, phenytoin (CYP inducer) and ciprofloxacin (CYP inhibitor) was studied on the initiation and maturation of cataract with the galactose- induced cataract model.

MATERIALS AND METHODS

This prospective interventional study was conducted in the Department of Pharmacology at Dayanand Medical College & Hospital, Ludhiana, Punjab in February and March, 2014.

Experimental Design: The experiment was conducted in 24 new born male Wistar rats weighing between 20 and 40gm. Sample size was calculated using Resource Equation Method in which E (degree of freedom of ANOVA) is calculated as E = Total number of animals – Total number of groups [8]. In this case, E = 24 – 4=20. Cataract formation was induced in the experimental groups by feeding them with a 50% galactose diet along with the normal diet. Galactose was purchased from HiMedia Laboratories Pvt Ltd, India. The rats were randomized into four groups of 6 rats each: Group 1 rats received a normal diet; Group 2 rats received 50% galactose diet (50% w/w with normal diet) day 23 onwards; Group 3 and Group 4 rats were also fed with 50%galactose diet day 23 onwards. In addition, Group 3 rats were pre-treated with ciprofloxacin (20mg/

kg) and Group 4 rats were pre-treated with phenytoin (50mg/kg) day 18 onwards once a day by oral route. The experiment was conducted till either all the lenses had been affected with cataract or up to day 60, whichever was earlier. Body weights were recorded daily throughout the study. Control group was fed with standard diet obtained from Aashirwad Industries, Mohali.

Animal care: The project was approved by the Institutional Animal Ethics Committee. Animal care was in accordance with ethical committee guidelines of the institution. The animals were kept at an ambient temperature (25 ± 10°C) and 12 hours light/dark cycle was maintained. All animals had free access to water.

Eye examination and cataract scoring: The eyes of all animals were checked daily for the cataract appearance with an ophthalmoscope (WelchAllyn). The maturation pattern was examined using Fundus Fluorsen Angiographer (FFA) machine (Zeiss FF 450 plus) by a masked observer on alternate days after the appearance of cataract. The stages of cataract were graded according to Sippel's classification [9]. Stage 1 - Clear lens, Stage 2 - Peripheral vacuoles, Stage 3 - Irregular peripheral vacuoles with the involvement of lens cortex, Stage 4 - Irregular opacity of lens and Stage 5 - Pronounced opacity.

STATISTICAL ANALYSIS

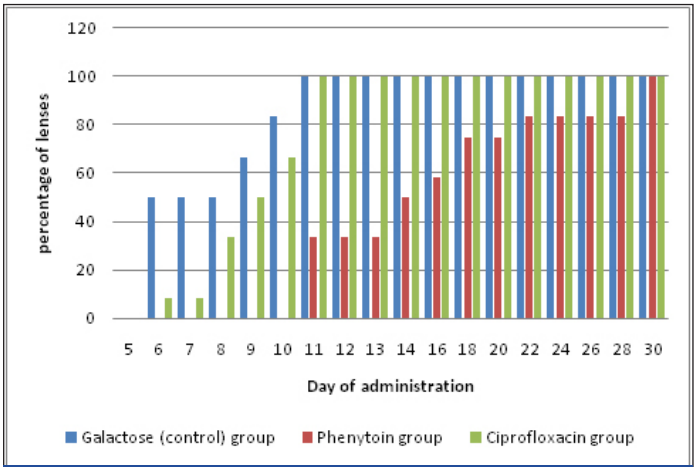
The incidence of cataract appearance was calculated as the percentage of total lenses affected in each group. The experimental and control groups were compared by chi square test and the results were considered significant at $p< 0.05$.

RESULTS

The effect of phenytoin (enzyme inducer) and ciprofloxacin (enzyme inhibitor) on the progression of cataract is shown in [Table/Fig-1] and the percentage of lenses affected by cataract in different groups is shown in [Table/Fig-2]. Cataract appeared both in galactose and ciprofloxacin group but not phenytoin group on 6th day of galactose administration as seen with ophthalmoscope. On the same day (6th day), however, cataract was observed in 50% lenses in galactose group as compared to 8.3% lenses in ciprofloxacin group depicting a significant delay in the appearance of cataract ($p<0.05$). In phenytoin group, cataract appeared on 11th day of galactose administration in 33% of lenses implying a highly significant delay in the appearance of cataract in phenytoin group ($p<0.001$) Cataract did not appear in normal control group. Cataract was observed in 100% of lenses in both galactose and ciprofloxacin group on 11th day of galactose administration implying that there is no difference in the maturation pattern of cataract development in both the groups. However, cataract appeared in all the animals in phenytoin group on 30th day. The animals were examined with FFA and the cataract was classified according to Sippel's grading. The grading of cataract in various stages along with the number of lenses affected is shown in [Table/Fig-3]. As can be observed, complete cataract i.e. stage 5 occurred in all the animals in both galactose and ciprofloxacin group on 20th day but in phenytoin group, even on 30th day, the cataract did not progress to stage 5.

Day of galactose administration	No of lenses affected in Galactose control group	No of lenses affected in Phenytoin pre-treated group	No of lenses affected in Ciprofloxacin pre-treated group
5	0	0	0
6	6	0*	1*
7	6	0*	1*
8	6	0*	4
9	8	0$	6
10	10	0$	8
11	12	4$	12
12	12	4$	12
13	12	4$	12
14	12	6#	12
16	12	7*	12
18	12	9	12
20	12	9	12
22	12	10	12
24	12	10	12
26	12	10	12
28	12	10	12
30	12	12	12

[Table/Fig-1]: Effect of phenytoin and ciprofloxacin on the progression of cataract as seen by an ophthalmoscope
Statistically significant from galactose control at $p<0.05$*, $p<0.01$#, $p<0.001$$



[Table/Fig-2]: Percentage of lenses affected by cataract in different groups

Day of galactose administration	Stage (No. of lenses affected) in Galactose control group	Stage (No. of lenses affected) in Phenytoin pre-treated group	Stage (No. of lenses affected) in Ciprofloxacin pre-treated group
5	0 (12)	0(12)	0(12)
6	1 (6)	0(12)	1(1)
7	1(6)	0(12)	1(1)
8	1(4), 2(2)	0(12)	1(2),2(2)
9	1(6), 2 (2)	0(12)	1(4),2(2)
10	1(6), 2(4)	0(12)	1(3),2(5)
11	1(5), 2(7)	1(4)	2(12)
12	1(1), 2(11)	1(4)	2(12)
13	2(12)	1(4)	2(12)
14	2(12)	1(5),2(1)	2(9),3(3)
16	3(4), 4(8)	1(4),2(2),3(1)	4(12)
18	3(1), 4(11)	1(3), 2(5),3(1)	4(12)
20	5(12)	1(2), 2(6),3(1)	5(12)
22	5(12)	1(2), 2(7),3(1)	5(12)
24	5(12)	1(2), 2(7),3(1)	5(12)
26	5(12)	1(2), 2(8), 3(2)	5(12)
28	5(12)	1(2), 2(8), 3(2)	5(12)
30	5(12)	1(2), 2(7), 3(3)	5(12)

[Table/Fig-3]: Grading of cataract in phenytoin and ciprofloxacin pre-treated galactosemic rats

DISCUSSION

Cataract is one of major causes of visual disability and blindness all over the world [1]. The chances of occurrence of cataract are 2–5 times more in diabetic patients as compared to non-diabetics [5]. With the increasing incidence of diabetes in developing countries, it is expected that the enormity of blindness due to cataract will also

increase and pose a burden to the society. Once cataract develops, the only treatment is surgical removal of the cataractous lens. During the last two decades, a lot of research is going on in search of drugs which can delay the onset and slow down the progression of cataract. Sadly, despite serious efforts, no breakthrough results have been achieved in this regard.

Cataract is a multi-factorial disease associated with a number of risk factors and multiple mechanisms [2]. One of the major mechanisms is activation of the polyol pathway. Galactose is converted to the corresponding polyols by the enzyme aldose reductase which requires NADPH as a co-factor. The polyols are not able to penetrate the cell membrane and are not metabolized further effectively. Hence, their accumulation within the lens creates an osmotic stress leading to collapse and liquefaction of lens fibres. This ultimately results in the formation of lenticular opacities [10-12]. Galactosemic cataractogenesis in rats is an important model used to observe the role of the aldose reductase pathway in diabetic complications.

In the previous studies, there was delayed occurrence of cataract with CYP450 inhibitors (diltiazem, nifedipine) and early occurrence with CYP450 inducer (pioglitazone) [5,13]. However, in another study, there was a significant delay in cataract occurrence with a CYP inhibitor (erythromycin) [14] but CYP inducer (Rifampicin) did not alter the initiation of cataract significantly. Similarly, in our study, the occurrence of cataract was significantly delayed with CYP inhibitor (ciprofloxacin) but the results obtained with CYP inducer (phenytoin) are quite contradictory. Instead of accelerating the process of occurrence of cataract, it delayed the process significantly. Thus, it is not reasonable to extrapolate the results of one enzyme inhibitor or inducer to other CYP modulators.

The paradoxical results seen with Phenytoin are not well understood. Probably, phenytoin is a spirohydantoin and spirohydantoins are aldose reductase inhibitors [15,16]. Since aldose reductase is clearly implicated as an important factor that lead to cataract formation, inhibitors of the enzyme were developed to prevent or at least to delay the cataractous process. In the phenytoin group, the nuclear opacity did not appear till 10th day of galactose administration. However, partial opacity ultimately developed in all rats which did not progress to complete cataract even on 30th day. Thus, the aldose reductase inhibitor delays the onset of cataract, but does not prevent it. Many established aldose reductase inhibitors (sorbinil, sulindac, naproxen, aspirin, tolrestat etc) have been shown to delay the galactose-induced cataract in different animal models [17,18]. Hence, the effect of phenytoin needs to be explored further.

As compared to the initiation pattern, the maturation pattern of the cataract observed microscopically in all groups was similar. The CYP450 modulators did not bring about any alteration in the maturation pattern of the cataract. But, the time required for maturation was altered. The extent of cytochrome enzyme induction or inhibition will vary significantly with change in dose and duration of drug treatment. Thus, further studies are needed to evaluate the efficacy of these drugs in different doses, as this is only a single dose study. The drawbacks of our study include small sample size and inability to assess the effects of different doses of CYP modulators in cataract.

If the results obtained in these experimental conditions could be tested and proved in clinical scenario, it may help in decreasing the incidence of diabetic cataract by simply modifying our prescription preferring a cytochrome inhibitor instead of inducer for a diabetic patient.

CONCLUSION

It can be concluded that with ciprofloxacin (CYP inhibitor), the initiation of cataract was delayed significantly but phenytoin (CYP inducer), instead of accelerating the process of initiation of cataract, delayed it significantly. Thus, the results may be drug specific and not group specific. Moreover, it has been seen that CYP450 modulators may have an effect on the initiation of cataract without significantly altering the maturation pattern.

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