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Ocular Hypotensive Effect of Topical Verapamil and Diltiazem in Steroid Induced Glaucoma Model of Rabbits

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Authors' contributions

Authors PA and HSS designed the study, performed the statistical analysis, wrote the protocol, and the first draft of the manuscript. Authors CGG, AA, MNK and RS managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Objectives: To evaluate possible ocular hypotensive effect of 0.5% diltiazem and 0.1% verapamil eye drops on intraocular pressure in steroid induced glaucoma model of rabbits. And compare with 0.5% timolol eye drops.
Methodology: Glaucoma was induced in rabbits (N=18) by bilateral topical instillation of 1% prednisolone eye drop (10 µl) twice a day for a period of 40 days. Before the induction of glaucoma, baseline intraocular pressure (IOP) in both the eyes of all rabbits was measured under sedation (i.v midazolam) by Schiotz tonometer. At the end of 40 days induced IOP was measured for all rabbits and rabbits were divided into three groups of six rabbits in each. Right eyes of group A, B and C rabbits received 0.5% diltiazem, 0.1%

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verapamil, and 0.5% timolol eye drops twice daily for 12 days respectively. Whereas, left eyes of all rabbits received distilled water hence represented as control. IOP was measured in all rabbits on every 4th day till 12 days of treatment period.
Results: Intra-group comparisons of IOP changes were made by paired't' test. And unpaired't' test for inter group comparisons. One way ANOVA was used for multiple group comparisons followed by post-hoc Tukey's test for group wise comparisons. In 0.5% diltiazem treated eyes, the mean IOP significantly reduced from 22.9±1.9 mmHg (10%) on 4th day to 16.9±1.1 mmHg(S, *P*<.001) on 12th day (34%). Similarly, mean IOP in 0.1% verapamil treated eyes significantly reduced from 22.7±1.3 mmHg (7%) on 4th day to 15.5±1.4 mmHg(S, *P*<.001) on 12th day (37%). Whereas, mean IOP significantly reduced from 22.4±1.9 mmHg (14%) on 4th day to 16.4±1.4 mmHg (S, *P*=.001) on 12th day (36%) in 0.5% timolol treated eyes.
Conclusion: Topical 0.5% diltiazem and 0.1% verapamil significantly reduced the IOP in steroid induced glaucoma model of rabbits. However, Further research has to be carried out both in experimental and clinical subjects to reveal its efficacy and safety profile.

Keywords: Diltiazem; intraocular pressure; timolol; verapamil.

1. INTRODUCTION

Glaucoma refers to a group of diseases that differ in their clinical presentation, pathophysiology, and treatment. These diseases are grouped together because they share certain common features, including cupping and atrophy of the optic nerve head, which has attendant visual field loss and is frequently related to the level of intraocular pressure (IOP). Thus, glaucoma is defined as a disturbance of the structural or functional integrity of the optic nerve that can usually be arrested or diminished by adequate lowering of IOP [1]. Normal intraocular pressure (IOP) of 11-21mmHg is necessary for the physiological functioning of the eye which is created by continuous secretion and drainage of aqueous humour. Any imbalance in the secretion and drainage of aqueous humour can increase the IOP, causing poor blood circulation to optic nerve fibers. Sustained increase in IOP can also result in progressive degeneration of retinal ganglion cells (RGCs) and optic nerve fibers, leading to gradual deterioration of visual field. Glaucoma is the second leading cause of blindness in worldwide next to cataract [2]. In India, prevalence of glaucoma is estimated to be 4% in the population above 30 years of age. The term "ocular hypertension" is used for people with consistently increased IOP without any associated optic nerve damage. However, in 'normal tension' or 'low tension' glaucoma there is optic nerve damage and associated visual field loss, even when IOP is in normal range or low.

Considering patient compliance, cost effectiveness and inherent risk of surgical management, medical line of treatment to reduce IOP appears to be the first choice of treatment compared to surgical and laser therapies. Prophylactic medical reduction of IOP in such individuals reduces the risk of progression to glaucoma from ~10 to 5% [3]. The pathogenesis of low-tension glaucoma still remains obscure, mechanical susceptibility of the optic nerve to intraocular pressure and/or inadequate blood perfusion of the optic nerve are the proposed causes [4].

Currently used cholinergic agonists, β-adrenergic blockers, topical carbonic anhydrase inhibitors and prostaglandin analogues, lower IOP either by reducing production of aqueous humour or by increasing outflow from the eye. But these drugs are associated with untoward adverse effects like - blurring of vision, myopia, headache, retinal detachment (miotics), cataract formation (cholinesterase inhibitors), red eye (epinephrine related

compounds), bradycardia, bronchoconstriction (β-receptor antagonists), nephrolithiasis, depression (carbonic anhydrase inhibitors) and blurring of vision, pigmentation of iris, darkening of eyelashes (prostaglandin analogues) [5]. Hence medical need for newer and better tolerated drug remains high.

Calcium channel blockers (CCBs) are commonly used for the treatment of hypertension and coronary vascular disease. The role of topical and systemic CCBs in glaucoma is still controversial. Many studies have shown the beneficial effect of topical and systemic CCBs in the management of glaucoma [6-13]. However, study by Beatty et al. failed to demonstrate ocular hypotensive action of topical CCBs in human and preclinical subjects. Whereas, some previous studies have shown no effect of CCBs in glaucoma [14-17]. So the present study was taken to evaluate the effect of topical CCBs on intraocular pressure in steroid induced glaucoma model of rabbits.

2. MATERIALS AND METHODS

2.1 Animals

Albino rabbits of either sex, weighing 1.5-2.5 kg were included in the present study. The rabbits were inbred in the Central Animal House of the Department of Pharmacology, J.J.M Medical College, Davangere, Karnataka, India under suitable conditions of housing, temperature, ventilation and nutrition.

2.2 Drugs

- 1% Predisolone acetate eye drop was used to induce glaucoma in rabbits.
- 0.5% Diltiazem eye drop was prepared by diluting inj. diltiazem 25 mg/ml with normal saline to give strength of 0.5% eye drop (5mg/ml). It was used as test drug for right eyes (test eyes) of group “A” rabbits.
- 0.1% Verapamil eye drop was prepared by diluting inj. verapamil 2.5 mg/ml with normal saline to give strength of 0.1% eye drop (1.25mg/ml). It was used as test drug for right eyes (test eyes) of group “B” rabbits.
- 0.5% Timolol eye drop was purchased from pharmacy and used as standard drug for right eyes (test eyes) of group “C” rabbits.
- 4% Xylocaine eye drop was used as surface anesthetic before measuring IOP by Schiotz tonometer.
- Inj. Midazolam 5mg/ml was used to sedate rabbits before performing tonometry. It was given in dose of 0.5-1 mg/kg through the marginal ear vein.
- Distilled water was used as control drug for left eyes of all rabbits.

2.5 Study Procedure

Institutional Animal Ethical Committee (IAEC) clearance was obtained before conducting the study. A total of 18 Albino rabbits (n=18) of either sex were used. They were housed in cages containing two rabbits per cage. They were randomly housed at a controlled temperature of 21°± 3°C, with a 12 hour light: 12 hour dark cycle. The rabbits had free access to standard pellet and water. Baseline IOP for the both the eyes of all rabbits was measured before inducing glaucoma. To induce glaucoma in rabbits, steroid model was opted. Rabbits were bilaterally instilled with 10 µl of 1% prednisolone eye drop twice a day for a period of 40 days. Before measuring IOP by Schiotz tonometer, rabbits were

sedated with intravenous (marginal ear vein) midazolam in a dose of 0.5-1.0 mg/kg and cornea was anaesthetized with topical 4% xylocaine drop. Bilateral topical instillation of 1% prednisolone resulted in elevation of IOP in both the eyes of all the rabbits above baseline level. Induced IOP was measured at the end of 40 days by Schiotz tonometer. The corticosteroid induced glaucoma is well known in human and closely resembles the human disease in clinical features as well as in the underlying mechanism[18].

After the induction of glaucoma, rabbits were divided into three groups of six rabbits in each and drugs were instilled as follows,

Group A: Received 0.5% diltiazem into right (test) and distilled water drops into left eyes (control) for 12 days.

Group B: Received 0.1% verapamil into right (test) and distilled water drops into left eyes (control) for 12 days.

Group C: Received 0.5% timolol into right (test) and distilled water drops into left eyes (control) for 12 days.

IOP was measured in both eyes for all the rabbits on every 4^{th} day till the end of 12 days of treatment period. A conversion table (Table 3) was used to derive the IOP in millimeter of mercury (mm Hg) from the scale reading and plunger weight. To avoid diurnal variation of IOP, all tonometries were performed at the same time of the day, preferably in the morning hours around 9 AM.

2.6 Statistical Analysis

Results are expressed as mean ± SD. Intra-group comparisons of IOP changes are made by paired't' test. And unpaired't' test for inter group comparisons. One way ANOVA is used for multiple group comparisons followed by post- hoc Tukey's test for group wise comparisons. A '*P*' value of .05 or less was considered for statistical significance.

3. RESULTS

IOP changes in all the rabbits during study period are mentioned in Table 1. Whereas, Table 2 denotes intergroup comparison as well as percentage reduction of IOP in all three groups. Figs. 1, 2 and 3 show the IOP changes in the right eyes of group A, B and C rabbits respectively during the study period.

Table 1. Intragroup comparison of IOP changes

Groups	Eye	Basal IOP	Induced IOP	IOP on 4TH Day	IOP on 8TH Day	IOP on 12TH Day
A	Left	18.0±2.3	25.9±1.9	25.9±1.9	25.2±1.7	24.8±1.7
	Right	16.4±1.4	25.5±1.6	22.9±1.9	17.9±1.3	16.9±1.1
	Left Vs Right	T=1.41 P=0.20*	T=0.37 P=0.72*	T=3.11 P=0.01**	T=8.27 P<0.001***	T=9.47 P<0.001***
B	Left	15.5±1.4	25.9±1.9	25.9±1.9	25.5±1.6	25.5±1.6
	Right	16.4±1.4	24.5±1.0	22.7±1.3	18.0±2.3	15.5±1.4
	Left Vs Right	T=1.12 P=0.29*	T=1.60 P=0.15*	T=3.41 P=0.009**	T=6.64 P<0.001***	T=11.67 P<0.001***
C	Left	16.4±1.4	26.2±2.1	26.2±2.1	25.5±1.6	25.5±1.6
	Right	16.0±1.5	25.9±2.3	22.4±1.9	19.0±1.8	16.4±1.4
	Left Vs Right	T=054 P=0.64*	T=0.26 P=0.80*	T=3.37 P=0.008**	T=6.70 P<0.001***	T=10.62 P<0.001***

D=difference; t=t test value; P=probable value. (Values are expressed as Mean±SD)
Not significant **Significant * Highly significant*

Table 2. Intergroup comparison of IOP changes (Right eye)

Group		Induced IOP	IOP on 4TH Day	IOP on 8TH Day	IOP on 12TH Day	% Reduction of IOP
A		25.5±1.6	22.9±1.9	17.9±1.3	16.9±1.1	34%
B		24.5±1.0	22.7±1.3	18.0±2.3	15.5±1.4	37%
C		25.9±2.3	22.4±1.9	19.0±1.8	16.4±1.4	36%
Anova	F**	1.12	0.18	0.64	1.67	0.18
	P	0.35*	0.84*	0.54*	0.22*	0.83*
Difference between the groups*** P values	A-B	0.06*	0.96*	0.99*	0.21*	0.97*
	A-C	0.91*	0.82*	0.57*	0.82*	0.82*
	B-C	0.34*	0.96*	0.63*	0.47*	0.92*

Values are expressed as Mean±SD)
NS (Not Significant) ** One way ANOVA * Post hoc Tukey's test*

Table 3. Calibration Scale / Friedenwald, Kronfeld, Ballintine, Trotter-1955

	Tonometer Stifgewicht-Plunger Load			
Scale Reading	**5.5 G**	**7.5 G**	**10.0 G**	**15.0 G**
0.0	41.5	59.1	81.7	127.5
0.5	37.8	54.2	75.1	117.9
1.0	34.5	49.8	69.3	109.3
1.5	31.6	45.8	64.0	101.4
2.0	29.0	42.1	59.1	94.3
2.5	26.6	38.8	54.7	88.0
3.0	24.4	35.8	50.6	81.8
3.5	22.4	33.0	46.9	76.2
4.0	20.6	30.4	43.4	71.0
4.5	18.9	28.0	40.2	66.2
5.0	17.3	25.8	37.2	61.8
5.5	16.6	23.8	34.4	57.6
6.0	15.9	21.9	31.8	53.6
6.5	13.4	20.1	29.4	49.9
7.0	12.2	18.5	27.2	46.5
7.5	11.2	17.0	25.1	43.2
8.0	10.2	15.6	23.1	40.2
8.5	9.4	14.3	21.3	38.1
9.0	8.5	13.1	19.6	34.6
9.5	7.8	12.0	18.0	32.0
10.0	7.1	10.9	16.5	29.6
10.5	6.5	10.0	15.1	27.4
11.0	5.9	9.0	13.8	25.3
11.5	5.3	8.3	12.6	23.3
12.0	4.9	7.5	11.5	21.4
12.5	4.4	6.8	10.5	19.7
13.0	4.0	6.2	9.5	18.1
13.5		5.6	8.6	16.5
14.0		5.0	7.8	15.1
14.5		4.5	7.1	13.7
15.0		4.0	6.4	12.6
15.5			5.8	11.4
16.0			5.2	10.4
16.5			4.7	9.4
17.0			4.2	8.5
17.5				7.7
18.0				6.9
18.5				6.2
19.0				5.6
19.5				4.9

Fig. 1. Graph showing IOP changes in right eyes of 0.5% diltiazem treated rabbits (Group A) during study period.

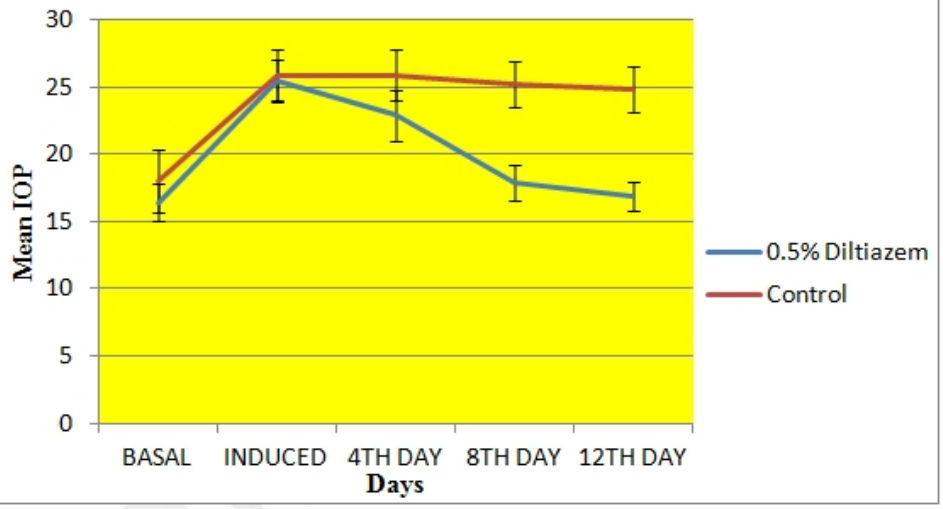
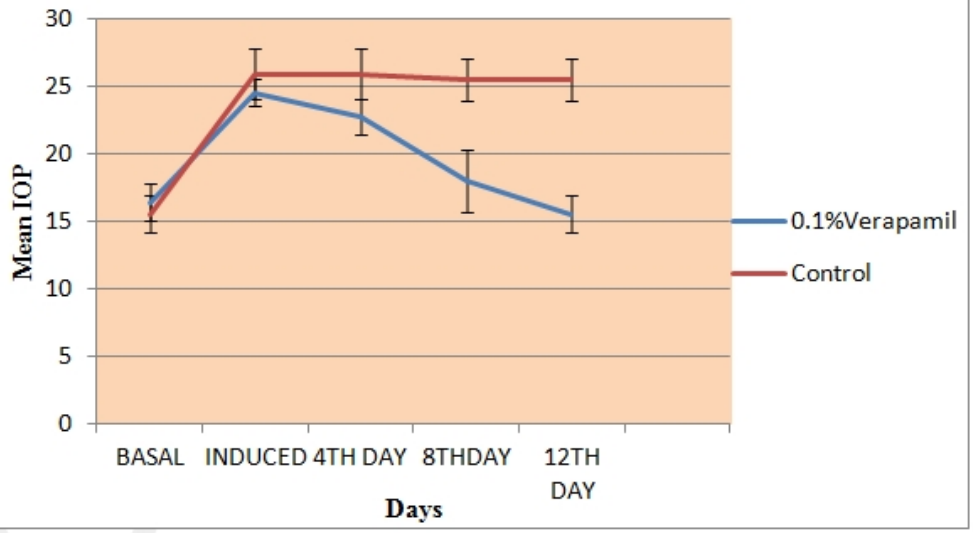
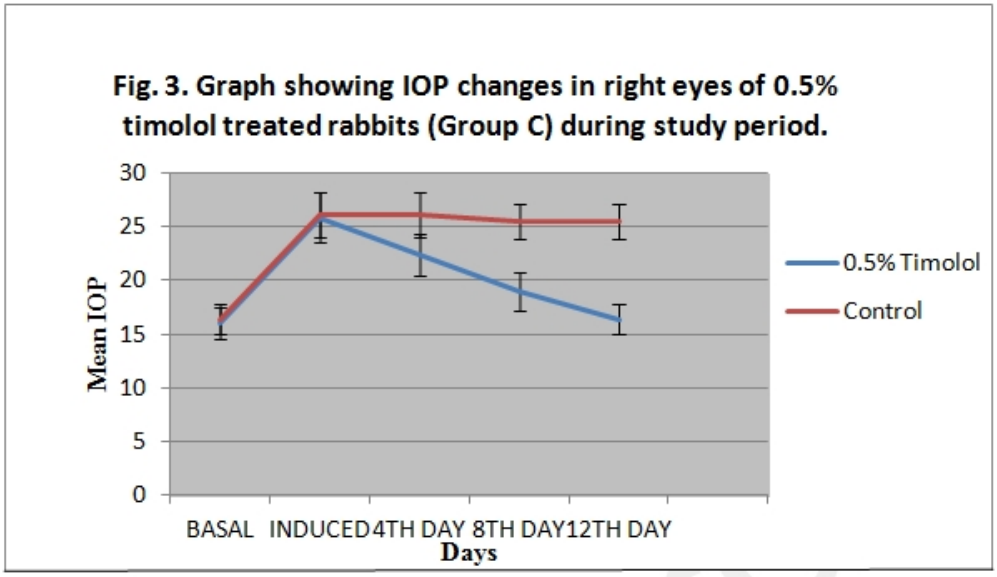


Fig. 2. Graph showing IOP changes in right eyes of 0.1% verapamil treated rabbits (Group B) during study period.





There was no statistical difference (*P*>.05) in mean baseline IOPs for the left and right eyes of all the rabbits suggesting baseline IOPs were in the same range (Tale 1). At the end of 40 days, there was 56.9% and 55.5% increase in mean IOPs of left and for right eyes of all rabbits respectively following steroid treatment when compared to baseline IOP. 34% reduction in IOP was noted in 0.5% diltiazem treated eyes (S, *P*<.001) and 37% reduction in 0.1% verapamil treated eyes (*P*<.001) during the study period. Whereas, 0.5% timolol instillation resulted in 36% reduction in IOP of group C rabbits(S, *P*=.001).No significant (NS, *P*>.05) reduction in the IOP during the treatment period was observed in left eyes of group A, B and C (control-distilled water treated) rabbits, suggesting control drug has no ocular hypotensive effect. There was no statistical significant difference in the inter groups (*P*>.05), suggesting these drugs are equally effective in reducing IOP as the percentage reduction of IOP by these drugs are in same range (34%-37%).

4. DISCUSSION

Calcium (Ca^{2+}) plays a pivotal role in the physiology and biochemistry of every cell. It plays an important role in signal transduction pathways, where it acts as a second messenger, in neurotransmitter release from neurons, and contraction of muscle cells. Calcium influx has several effects on aqueous humour dynamics including hydrostatic component due to effect on arterial blood pressure, ciliary body perfusion, and on the active secretion of Na^{+},Ca^{2+} ions by ciliary epithelium.[19] Several studies have shown, increase IOP after topical administration of calcium ionophores in animal models [20].

Calcium channels (L-type and T-type) found to have role in cellular growth, proliferation of vascular smooth muscle and fibroblasts. Thus, CCBs may have beneficial effect in glaucoma by inhibiting the synthesis of extracellular collagen matrix [21]. Contraction of trabecular meshwork cells depend on Ca^{2+} influx by L-type channels. By blocking L-type channels, CCBs cause relaxation of trabecular meshwork cells, thus increasing outflow of aqueous humour. According to the glutamate theory of cell death, calcium influx is the terminal step in axon death. The ability to block calcium influx by CCBs can theoretically

produce a neuroprotective benefit [22]. Moreover, gap junctions which exist between non-pigmented and pigmented ciliary epithelial cells, possibly regulated by calcium. Verapamil interferes with these gap junctions, altering cellular permeability of the ciliary epithelium and thus inhibiting aqueous humour formation [17].

The perfusion studies in dissected human eyes showed dose dependant increase in outflow facility after verapamil administration [13,23]. Many previous studies have reported ocular hypotensive effect of CCBs after oral, intravenous and topical application [6-13]. In different studies by Netland, Abelson and Mooshian have reported decrease in the IOP after single topical dose of verapamil and diltiazem in rabbits as well in human [24-26]. Recent study shown that chronic administration of nivaldipine increased choroidal blood flow and slowed the progression of visual field changes in open angle glaucoma patients [27]. In another randomized, double blind, placebo controlled study demonstrated ocular hypotensive effect and increased ocular haemodynamic parameters after oral nimodipine administration in normal tension glaucoma patients [28].

On the other hand, Beatty et al. found an increase in IOP of rabbits after intravenous and topical application of verapamil, nifedipine and diltiazem, probably due to CCB induced ocular vasodilation [14]. Study by Abelson et al. has shown that CCBs have a biphasic effect on IOP, with an ocular hypotensive action at low and an ocular hypertensive action at high concentrations of CCBs [29]. However, study by Melena et al. did not support this hypothesis as decrease in IOP was noted even at very high concentrations of diltiazem [18].

Present study which was undertaken to evaluate the role of CCBs in animal model of glaucoma has shown significant reduction in the IOP after topical instillation of CCBs without producing any adverse effects. Moreover, present study has shown no contralateral ocular hypotensive effect in contrast to previous studies which have shown contralateral ocular hypotensive effect in animal models of glaucoma [7,25,29].

Results of the present study are in accordance with previous reports suggesting that CCBs are effective in the management of glaucoma [25,29]. But it is difficult to extrapolate these findings to humans. However, steroid induced glaucoma model is closely resembles to the chronic-angle glaucoma in human and also it is found that endogenous glucocorticoids play a role in the pathogenesis of glaucoma in human [30,31]. Thus, CCBs can have beneficial effect in preventing glaucoma in subjects receiving topical steroids prescribed for other indications. Nevertheless, further studies are needed to clarify the ocular effects, efficacy, and safety profile of CCBs both in preclinical and clinical subjects.

4. CONCLUSION

The findings of the present study suggest that topical 0.5% diltiazem and 0.1% verapamil significantly reduces the IOP in prednisolone induced glaucoma model of rabbits. Percentage reduction IOP by topical CCBs is comparable to timolol. Thus, topical CCBs can be alternative to conventional anti-glaucoma drugs. However, further research has to be carried out both in experimental and clinical subjects to reveal its efficacy and safety profile.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Institutional Animal Ethical Committee (No. CPCSEA / 362/01/ab) approval was obtained on 16-11-2009; Ref NO:JJMMC/32/2009-10 from J.J.M Medical College, Davangere, Karnataka, India. All animals were handled and taken care according to guidelines of "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, Revised 1985) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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