·Informatics Research·

Fixed combination of latanoprost and timolol *vs* the individual components for primary open angle glaucoma and ocular hypertension: a systematic review and meta-analysis

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Abstract

• AIM: To assess the effects of the fixed combination of 0.005% latanoprost and 0.5% timolol (FCLT) *vs* their individual components for primary open angle glaucoma (POAG) and ocular hypertension (OHT).

• METHODS: After searched PubMed, EMBASE, the Cochrane Library and SCI, all randomized controlled clinical trials (RCTs) and cross-over studies were included. The control groups were the monotherapy or the concomitant therapy of latanoprost and timolol. The outcomes were visual field defect, optic atrophy, mean intraocular pressure (IOP) and IOP fluctuation. The analysis was carried out in RevMan version 5.1 software.

• RESULTS: The post-intervention mean IOP of FCLT was significantly lower compared to timolol [mean difference (MD) -2.92, 95%CI -3.28 to -2.55, *P*<0.00001] and latanoprost (MD -1.11, 95%CI -1.51 to -0.72, *P*<0.00001). The post-intervention IOP fluctuation was also significantly lower compared to timolol (MD -0.88, 95%CI -1.23 to -0.53, *P*<0.00001) and latanoprost (MD -0.63, 95%CI -1.04 to -0.22, *P*=0.002). The mean IOP was higher in FCLT morning dose group than the one in unfixed combination of 0.005% latanoprost and 0.5% timolol (UFCLT) (MD 1.10, 95%CI 0.81 to 1.39, *P*<0.00001). Otherwise, there was no difference between FCLT evening dose group and UFCLT (MD 0.34, 95%CI -0.01 to 0.69, *P*=0.06). There was no statistical difference for the incidence of visual field defect and optic atrophy between FCLT and the monotherapy of components.

• CONCLUSION: A better IOP lowering effect has been demonstrated for FCLT compared to the monotherapy of components. The IOP lowering effect was worse for FCLT morning dose and almost same for FCLT evening dose compared to the UFCLT. We need more long-term high quality RCTs to demonstrate the outcomes of visual field defect and optic atrophy.

• KEYWORDS: primary open angle glaucoma; ocular hypertension; the fixed combination of latanoprost and timolol; intraocular pressure; meta-analysis
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INTRODUCTION

Medical interventions, laser trabeculoplasty and glaucoma drainage surgery are three main interventions for primary open angle glaucoma (POAG) nowadays. Topical medical therapy is the main intervention for ocular hypertension (OHT) patients who need treatment. All of these interventions focus on lowering the intraocular pressure (IOP). A single topical hypotensive drug is the first line choice, but as many as 40% patients need more than one medication to reach the target IOP [1]. More bottles of drug introduce more exposure to benzalkonium chloride, which has been shown to cause dose-dependent toxic effects on the ocular surface and tear film [2]. More kinds of medication mean more complicated use methods, which lead to worse compliance. The fixed combination solved these problems. The first prostaglandin analog and timolol fixed combination is the fixed combination of 0.005% latanoprost and 0.5% timolol (FCLT). Latanoprost belongs to prostaglandin agonists which increase uveoscleral outflow. This kind of

medication is recommended to use once daily. Timolol maleate is a kind of beta-blockers, which reduce IOP by decreasing the production of fluid. Timolol generally has been administered twice daily. Beta-blockers have little activity during the night-time hours, when aqueous production by the ciliary body is reduced because of natural circadian factors. A single morning dose can achieve most of effect of this medication [3]. FCLT is recommended to use once daily in the morning or in the evening. It is a question whether changed administration method affect the efficacy. For drug approval by the Food and Drug Adminstration, a fixed combination must have better efficacy than each of the component medications used as mono-therapy, and as effective as the component medication given concomitantly in a 2-bottle regimen. The published system reviews did not discuss the effects on visual field (VF) and optic atrophy for FCLT. Their assessments of the evidence quality are uncomprehensive.

To assess the effects of FCLT for POAG and OHT, we summarized the evidence of VF loss, optic atrophy, and IOP compared to the mono-therapy and unfixed combination of 0.005% latanoprost and 0.5% timolol (UFCLT).

SUBJECTS AND METHODS

Criteria for Considering Studies for This Review We included all randomized controlled clinical trials (RCTs) and cross-over studies which comparing FCLT administrated one drop once daily in the morning or in the evening with the mono therapy or UFCLT. The exclusion criterion were the studies that were judged as "high risk of bias" for random sequence generation or without ethical approval of studies and informed consent. We put no treatment duration limitations. The majority participants of the included studies have to be diagnosed as POAG or OHT, with mean IOP above 21 mm Hg as the baseline. There were no age or gender limitations for the patients. The outcomes of this review were: 1) the incidence of VF defect; 2) the incidence of optic atrophy; 3) IOP: the diurnal or 24h mean IOP, the diurnal or 24h fluctuation of IOP.

Search Methods for Identification of Studies We searched the Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library, MEDLINE, EMBASE, and Science Citation Index in November 2013.

The keywords for the medication were timolol, latanoprost, fixed drug combinations. The keywords for the disease were POAG, OHT. The limit for the research was randomized controlled trial. We placed no language or date restrictions in the searches for trials. The retrieval strategy see the supplemental file. We also searched the reference lists of identified trials and used the Science Citation Index to find reports that cited the identified relevant studies.

Data Collection and Analysis Two authors examined each full-text report to determine the eligibility independently and collected data according to a customized form. We calculated Kappa statistic to measure the agreement between two authors making inclusion/exclusion decisions[4].

Assessment of Risk of Bias in Included Studies Trial quality was assessed according to methods set out in chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions [5]. We used seven components to determine methodological quality: adequate sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data addressed, free of selective reporting, and free of other biases. Each component was graded at low risk of bias, unclear risk of bias, or high risk of bias as outlined in the Cochrane Handbook. We excluded trials scored at "high risk of bias" on adequate sequence generation.

Measures of Treatment Effect We summarized data from the studies, which collected similar outcomes and used similar follow-up times, after the Chi-square test for heterogeneity between trial results. For dichotomous data, we expressed results as risk ratio (RR) estimates [95% confidence interval (CI)]. For continuous data, we obtained the mean and standard deviations. We converted standard errors to standard deviations. We summarized results across studies with mean differences (MD, 95%CI).

UNIT OF ANALYSIS ISSUES

Cross-over Trials The cross-over trials are suitable to evaluate a temporary effect of a stable, chronic condition. The IOP change is a temporary effect. POAG and OHT are stable chronic conditions. So cross-over trials are appropriate for this review. We used five components to determine methodological quality for cross-over trials: suitable design; without carryover effect; not only obtained first period data; correct analysis; comparability of results with those from parallel-group trials. All of these were incorporated in the free of other biases. Only when all of these components were assessed at "low risk of bias", the assessment was "low risk of bias". For the studies with a paired t-test, we used the generic inverse-variance method in review manager. For the trials without paired t-test, we make a approximately paired analysis by imputing missing standard deviations. For the trials that we couldn't impute standard deviations, we incorporated them into analysis as if the trials were parallel group trials.

More Than Two Intervention Groups For a multi-arm study, we chose intervention groups that are relevant to the review and meet the criteria of inclusion. We used two components to determine methodological quality: are data

presented for each of the groups to which participants were randomized? Are reports of the study free of suggestion of selective reporting of comparisons of intervention arms for some outcomes?

Cluster-randomized Trials We dealt with trials made an intra individual comparison of both eyes as cluster-randomized trials. The methodological quality assessment are list as following: 1) recruitment bias; 2) baseline imbalance; 3) loss of clusters; 4) incorrect analysis; and 5) comparability with individually randomized trials.

Assessment of Heterogeneity We identified statistical heterogeneity with I^2 and Chi-squared test. A P value lower than 0.05 provided the evidence of heterogeneity of the effects. The first choice we made to do a meta-analysis is fixed-effect model. When there is heterogeneity that cannot readily be explained, we incorporated it into a random-effects model. If heterogeneity was due to the presence of one or two outlying studies with results that conflict with the rest of the studies, we performed analyses both with and without outlying studies as part of a sensitivity analysis. If the meta-analysis did not include such outlying studies or the heterogeneity could not be explained by exclusion of outlying studies, we explored the heterogeneity by conducting subgroup analyses according to the administration time. A RCT compared the mean 24h IOP between the FCLT used once daily in the morning and in the evening [6]. The evening dose provided a lower mean 24h IOP (MD -0.70, 95%CI -1.21 to -0.19, P =0.007). The differences between two groups for each time points are not statistical significant except at 6 a.m. and 10 a.m. So the evening dose of FCLT provided a lower mean 24h IOP and diurnal IOP than morning dose.

Summary of Findings We rated the quality of the outcomes according to the grades of recommendation, assessment, development, and evaluation (GRADE). Randomized trials begin as high-quality evidence. "Quality" as used in GRADE is compromised by imprecision, inconsistency, indirectness of study results, and publication bias. In addition, several factors can increase our confidence in an estimate of effect. When rated the quality of risk of bias, we assigned random sequence generation, allocation concealment, blinding of participants and personnel, incomplete outcome data, and stopped early in other risk of bias as key domains. We judged "risk of bias" within study and across studies according to chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions [5]. We downgraded the quality of the publication bias for the reasons below: visually asymmetrical funnel plot, statistical significant of the test for funnel plot asymmetry, or the evidence came from a number of commercially funded small studies. The small study was the study whose participants were less than 200. According to the Cochrane Handbook for Systematic Reviews of Interventions, the statistical tests for funnel plot asymmetry only be used for the meta-analysis which included more than 10 studies.

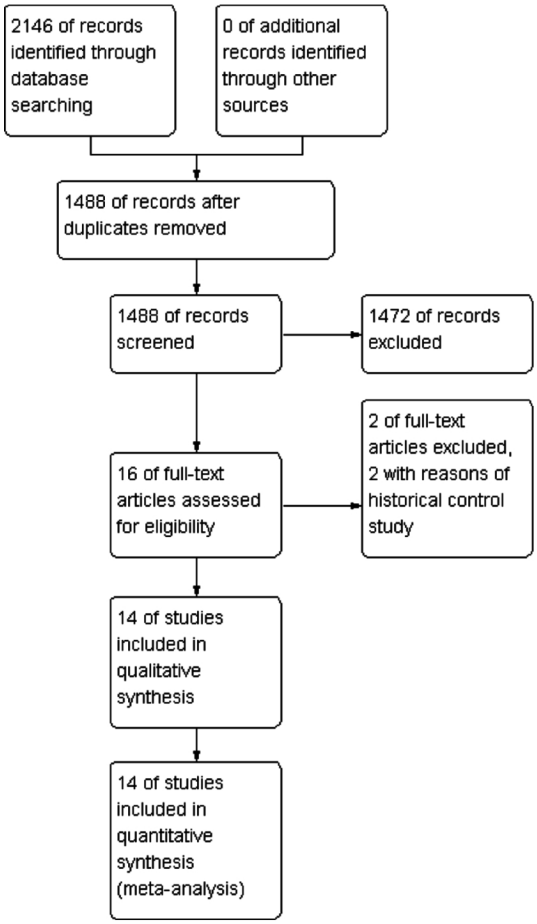

Figure 1 Study flow diagram.

RESULTS

Description of Studies

Results of the search The electronic searches revealed 2146 abstracts of papers. We obtained 1488 records after duplicates removed. Screened the title and the abstract, we could easily exclude 1472 records from further assessment without any doubt. We got the full copies of 16 potentially or definitely relevant papers. After checking reference lists, it didn't reveal a further paper. We screened a total of 16 papers for content and methodological quality according to their full text copies. The value of Kappa was 0.93. Thus, it has left a total of 16 papers describing 14 trials that addressed the FCLT *vs* the monotherapy of the components or the UFCLT for POAG or OHT (Figure 1).

Included Studies We included a total of 16 papers describing 14 trials in this systematic review. Diestelhorst [7] and Diestelhorst and Almegard [8] are two papers of a same trial. Larsson and Diestelhorst [9] and Diestelhorst and Larsson [10] are two papers of another same trial. So we only

Table 1 Characteristics of included studies

Authors	Group design	Length of treatment (wk)	Medication	Total No. patients	Total No. patients remained	Frequency of delivery	Mean age (a)	Sex (M/F)	POAG or OHT (%)
Diestelhorst and Almegard[8]	4 P	4	FCLT	47	37	E	64.5	17/29	98
			T	25	19	E	57.7	11/14	92
			L	21	18	E	61.7	6/15	90
Diestelhorst and Larsson[16]	C	6	FCLT	195	190	M	68 67	40/53 49/48	82 74
			UFCLT			T M+L E+T E			
Diestelhorst and Larsson[10]	2 P	12	FCLT	263	255	E	65	129/126	91
			UFCLT	254	247	T M+L E+T E	65	99/148	90
Higginbotham *et al*[11]	3 P	26	FCLT	138	125	M	61	67/71	94
			T	140	104	M+E	63	80/60	91
			L	140	116	M	63	68/72	92
Higginbotham *et al*[13]	3 P	12	FCLT	129	114	E	64.8	57/72	98
			T	134	113	M+E	63.7	59/75	98
			L	131	111	E	63.5	63/68	98
Konstas *et al*[17]	C	8	FCLT	37	37	E	65.8	14/23	100
			L			E			
Konstas *et al*[18]	C	8	FCLT	34	34	E	62.4	13/21	100
			T			M+E			
Konstas *et al*[19]	C	8	FCLT	30	29	E	63.7	13/16	100
			T			M+E			
Magacho *et al*[21]	2 P	4	FCLT	14[a]	14[a]	M	59.2	NR	71
			L	14[b]	14[b]	M	53.8	NR	60
Olander *et al*[20]	2 P	3	FCLT	176	175	M	64.4	72/103	91
			L	174	173	M	63.2	70/103	93
Palmberg *et al*[14]	3 P	12	FCLT	170	150	E	65.3		96
			L	165	145	E	65.1	152/183	96
			T	165	145	M	64		97
Pfeiffer[12]	3 P	26	FCLT	140	128	M	64	67/73	95
			L	147	119	M	63	77/70	87
			T	149	117	M+E	64	52/97	93
Varma *et al*[15]	3 P	26	FCLT	278	NR	M	62.3	134/144	95
			L	287	NR	M or E	63.2	145/142	90
			T	289	NR	M+E	63.8	132/157	92
Zhao *et al*[22]	2 P	8	FCLT	125	125	E	50	66/59	100
			UFCLT	124	123	T M +L E+T E	47.9	50/74	100

P: Parallel; C: Cross-over; NR: Not report; L: Latanoprost; T: Timolol; FCLT: Fixed combination of latanoprost and timolol; UFCLT: Unfixed combination of latanoprost and timolol; M: Morning; E: Evening; [a]25 eyes from 14 patients; [b]28 eyes from 14 patients.

cited Diestelhorst and Almegard [8] and Diestelhorst and Larsson [10] below. The trials recruited a total of 4135 participants, including 2632 Caucasian, 324 African, 165 Black, and 278 other ethnicity patients. Three trials included 737 patients did not report the ethnicity of participants. There were three kinds of control groups in the included trials: timolol, latanoprost, or UFCLT (Table 1).

Design Six trials had more than two arms and made more than one comparison[8,11-15]. Four trials were cross-over design. The others were two parallel arm studies[16-19].

Sample sizes The sample size ranged from 30 to 854 people. About half of included trials were small sample size studies; eight trials included more than 200 participants (Table 1).

Sample characteristics Five trials only included people with POAG[8,16-19]. Seven trials accepted both POAG and OHT participants [10-13,15,20,21]. But no trial restricted entry to participants with OHT only. In included trials that the percentages of POAG and OHT were less than 100%, the diagnoses of the remaining cases were capsular glaucoma, pigmentary glaucoma, exfoliation glaucoma, and

pseudo-exfoliation glaucoma. One trial made an intra individual comparison of both eyes [21]. The other trials only choose one eye of one participant (Table 1).

Interventions The intervention of treatment groups was FCLT which was used once daily in the morning or in the evening. The interventions of control groups were timolol in eight trials, latanoprost in nine trials, and UFCLT in three trials[8,11-15,18-22] (Table 1).

Outcome measures 1) The reduction of onset or progression of VF loss. Two trials reported the incidence of VF deterioration [11,12]; 2) Reduction of optic nerve head cupping progression (according to objective assessment): Two trials reported the incidence of optic atrophy according to ophthalmoscope [11,12]; 3) Reduction of IOP: all the trials measured the IOP using Goldmann applanation.

Seven studies reported mean IOP of diurnal time points. Five studies[11-16] measured IOP at 8 a.m., 10 a.m., and 4 p.m., two[8, 10] at 8 a.m., 12 a.m., and 4 p.m. Four trials reported mean IOP at 6 a.m., 10 a.m., 2 p.m., 6 p.m.,10 p.m., and 2 a.m. as the mean 24h IOP [17-19,22]. Two trials only recorded and reported IOP of one diurnal time point [20,21]. Four trials recorded daily IOP fluctuation[15,17-19].

Funding source Seven studies were sponsored by Pfizer [10, 13-15, 18, 20, 22]. Two studies were founded by Pharmacia [11,12]. Four studies had not been funded commercially [8,17,19,21]. One study didn't report whether had accepted commercial funding source[16].

RISK OF BIAS IN INCLUDED STUDIES

Allocation (selection bias) Nine studies described the methods of sequence generation, so were judged as "low risk of bias". Among them, four trials used simple randomization, and five were stratified[8,10,11,13,14,16,20-22]. The other five trials only mentioned "randomly assigned" without sufficient information for judgment[12,15,17-19].

Most studies did not describe the methods of allocation concealment in details, so the judgment was "unclear risk of bias" for these trails. Five trials reported the methods, so were judged as "low risk of bias"[11,13,20-22] (Figure 2, Table 2).

Blinding (performance bias and detection bias) Nine trials were double-masked of the participants and study personnel, so were judged as low risk of performance bias [11-14,16-20]. Two trials just reported masked without details, so we were unclear about the risk of performance bias [8,15]. Besides, two trials were only evaluator masked, one trial was open label study [10,21,22]. The outcomes of the three trials are likely to be influenced by lack of blinding. These trials were high risk of performance bias. Six trials were evaluator masked [10,14,17-19,21]. Six trials were not evaluator masked, but outcome measurement is not likely to be influenced by

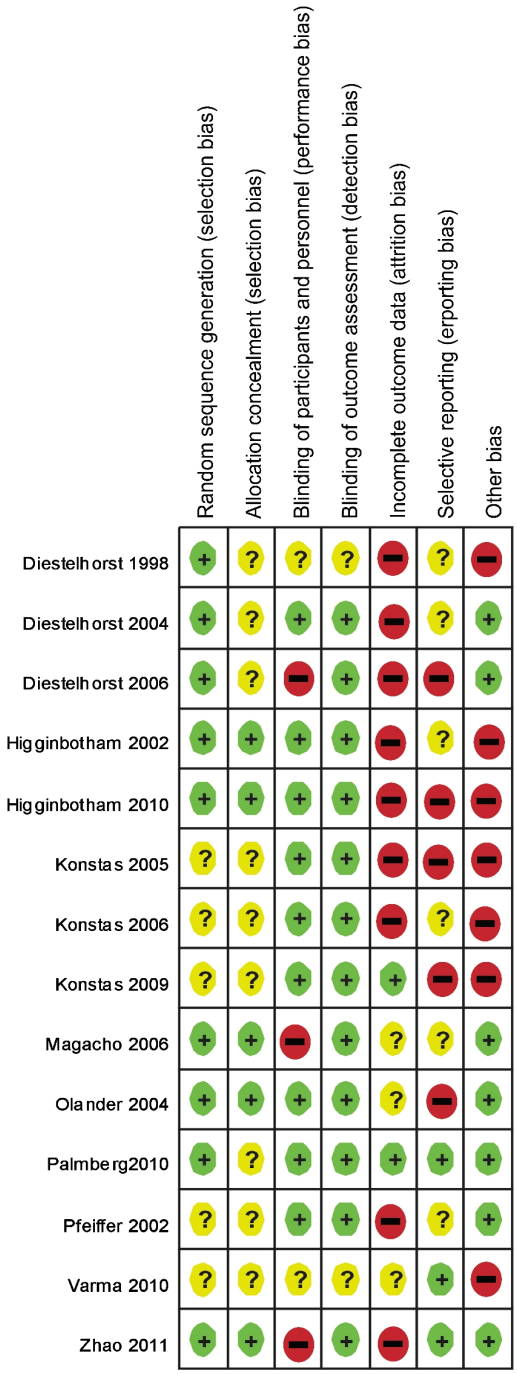

Figure 2 Risk of bias summary.

lacking of blinding [11-13,16,20,22]. These twelve trials were judged as "low risk of detection bias". Two trials[8,15] reported masked without details, so we were unclear about the risk of detection bias (Figure 2, Table 2).

Incomplete Outcome Data (attrition bias) Reasons of withdrew were not related to true outcomes in two trials, so were judged as low risk of attrition bias [14,19]. Three trials did not report the number of participants randomized or the reasons of withdrawal, so we were unclear about the incomplete outcome data [15,20,21]. In the other nine trials, reasons for missing outcome data were likely to be related to true outcome, with both imbalance in numbers and reasons for missing data across intervention groups. These trials were

Table 2 The comment of risk of bias

Trial	Random sequence generation	Allocation concealment	Blinding participants personnel	Blinding outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Diestelhorst and Almegard [8]	SiR	WD	WD	WD	Y	WD	SE
Diestelhorst and Larsson[16]	StR	WD	DM	WEM	Y	WD	WO
Diestelhorst and Larsson[10]	SiR	WD	EM	EM	Y	SR	WO
Higginbotham *et al*[11]	StR	CA	DM	WEM	Y	WD	BI
Higginbotham *et al*[13]	StR	CA	DM	WEM	Y	SR	SD
Konstas *et al*[17]	WD	WD	DM	EM	Y	SR	SE
Konstas *et al*[18]	WD	WD	DM	EM	Y	WD	SE
Konstas *et al*[19]	WD	WD	DM	EM	N	SR	SE
Magacho *et al*[21]	SiR	CA	EM	EM	WD	WD	WO
Olander *et al*[20]	StR	CA	DM	WEM	WD	SR	WO
Palmberg *et al*[14]	SiR	WD	DM	EM	N	PA	WO
Pfeiffer[12]	WD	WD	DM	WEM	Y	WD	WO
Zhao *et al*[22]	StR	SeE	OL	WEM	Y	PA	WO
Varma *et al*[15]	WD	WD	WD	WD	WD	PA	BI

SiR: Simple randomization; StR: Stratified radomization; WD: Without detail; CA: Central allocation; DM: Double masked; EM: Evaluate masked; WEM: Without evaluator masked; SeE: Sealed envelopes; OL: Open label; N: The reasons of withdrew were not related to true outcomes; Y: The reasons of withdrew were related to true outcomes; PA: The protocol is available and without selection report; SR: Selective report; WO: Without other risk of bias; SE: Stop early; BI: Baseline imbalance; SD: Study design.

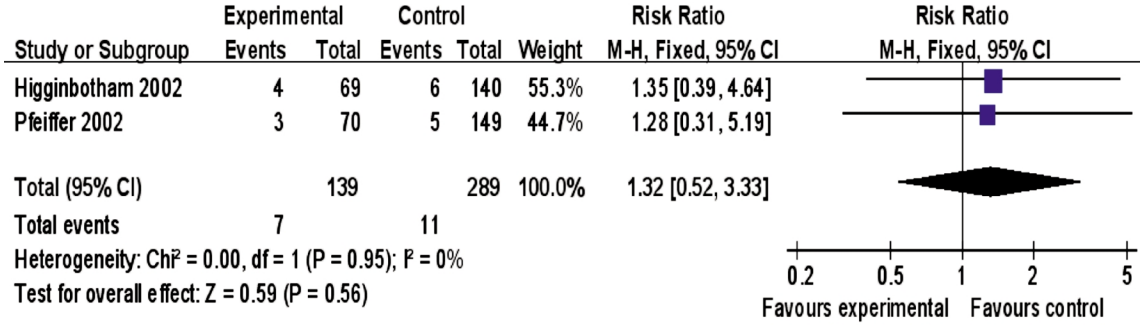

Figure 3 Analysis 1.1 the fixed combination of latanoprost and timolol *vs* timolol: the incidence of visual field defect.

high risk of attrition bias (Figure 2, Table 2).

Selective Reporting (reporting bias) The protocols of three studies were available and all of the study pre-specified (primary and secondary) outcomes that are of interest in the review had been reported in the pre-specified way [14,15,22]. So we judged these trials as "low risk of bias". In two studies, some outcomes of interest in the review were reported incompletely so that they could not be properly entered in meta-analysis [10,19]. Two trials made selectively choice of different time points data [13,20]. One study inadequately reported data. All these trials were judged as "high risk of bias" [17]. The protocols of the other six studies were not available, so we had insufficient information for judgment [8,11,12,16,18,21] (Figure 2, Table 2).

Other potential sources of bias Seven studies appeared to be free of other sources of bias. We judged as "low risk of bias" [10,12,14,16,20-22]. Four studies which didn't mention a pre-specified sample size might have stopped at a point chosen [8,17-19]. Two studies had extreme baseline imbalance [11,15]. For more than two intervention groups: in one study, data were not presented for each of the groups[13]. These trials were judged as having "high risk of bias" (Figure 2, Table 2).

EFFECTS OF INTERVENTIONS

Fixed Combination of Latanoprost and Timolol *vs* timolol We retrieved eight trials comparing FCLT with timolol[8,11-15,18,19]. Three of them were long-term parallel group trials with large sample size [11,12,15]. Another two were short term cross-over studies with small sample size[18,19].

Incidence of Visual Field Defects (Figure 3) Two trials reported data on the incidence of glaucomatous VF defects [11,12]. Meta-analysis failed to achieve clear statistical significant difference for VF protection between two groups (RR 1.32, 95% CI 0.52 to 3.33, P=0.56).

Incidence of Optic Atrophy (Figure 4) Two trials provided data concerning the incidence of optic atrophy[11,12]. There was no significant difference between treatments in the incidence of optic atrophy (RR 1.37, 95% CI 0.23 to 8.02, P=0.73).

Mean Intraocular Pressure (Figure 5) Six trials reported the mean diurnal IOP, two cross-over trials reported data on

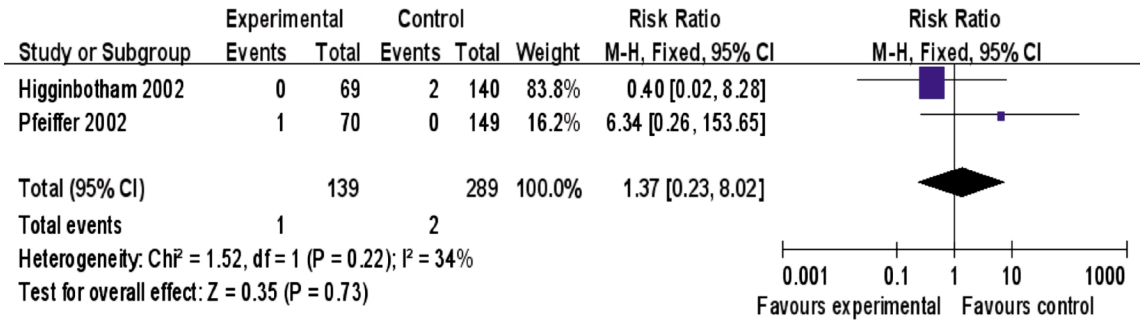

Figure 4 Analysis 1.2 the fixed combination of latanoprost and timolol *vs* timolol: optic atrophy.

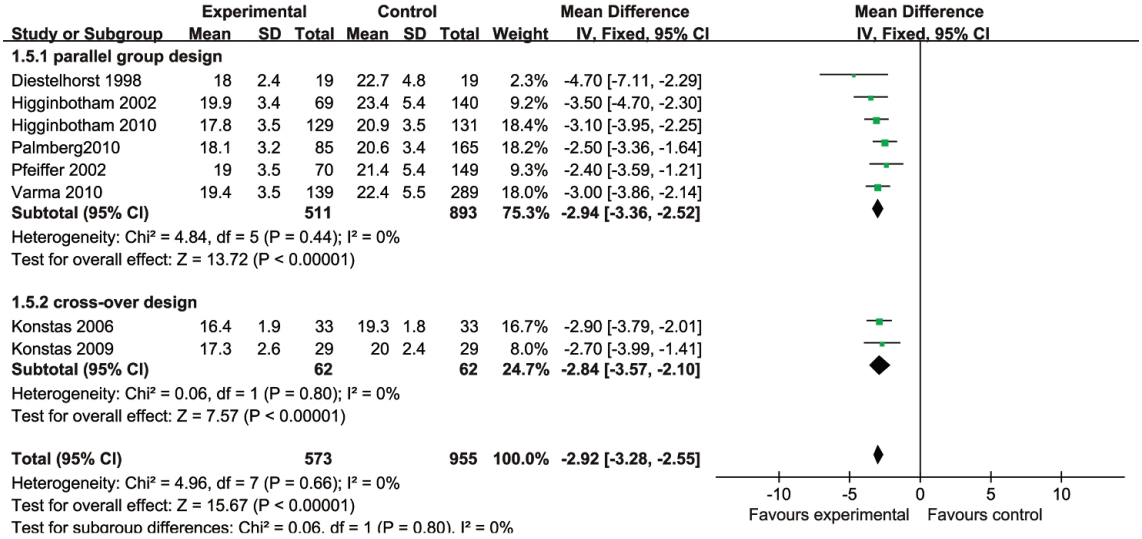

Figure 5 Analysis 1.3 the fixed combination of latanoprost and timolol *vs* timolol: the mean IOP.

the mean of 24-h IOP. According to the data in cross-over trials, the mean 24-h IOP was significantly lower compared FCLT with timolol (MD -2.84, 95%CI -3.57 to -2.12, P< 0.00001)[18,19]. When the data of cross-over trials was inputted as parallel group study and analyzed with invasive-variance method, the result was similarly as above (MD -2.84, 95%CI -3.57 to -2.10, P <0.00001). So we used the imputed data and made a sensitivity analysis. The difference of results for included these two trials (MD -2.92, 95%CI -3.28 to -2.55, P<0.00001) or excluded them (MD -2.94, 95%CI -3.36 to -2.52, P<0.00001) was 0.02 mm Hg, which was not obvious in clinical. Meta-analysis comparing FCLT with timolol provided clear evidence of a positive treatment effect on IOP control (MD -2.92, 95%CI -3.28 to -2.55, P <0.00001).

Fluctuation of Intraocular Pressure (Figure 6) Three trials reported the fluctuation of diurnal IOP [15,18,19]. FCLT provided a lower fluctuation of diurnal IOP compared to timolol (MD -0.88, 95%CI -1.23 to -0.53, P <0.00001).

Fixed Combination of Latanoprost and Timolol *vs* latanoprost We included nine trials in this comparison [8,11-15,17,20,21]. Three trials were long-term studies with large sample size [11,12,15]. The others were short-term studies, the follow-up time ranged from 3 to 12wk.

Incidence of Glaucomatous Visual Field Defects (Figure 7) Two trials reported data on the incidence of glaucomatous VF defects [11,12]. There was no significant difference between treatments in the incidence of the VF defect (RR 1.04, 95% CI 0.43 to 2.52, P =0.94).

Incidence of Optic Atrophy (Figure 8) Two trials provided data concerning the incidence of optic atrophy[11,12]. Meta-analysis failed to achieve clear statistical evidence between FCLT and latanoprost (RR 0.89, 95% CI 0.13 to 5.92, P =0.90)

Mean Intraocular Pressure (Figure 9) Eight trials reported data on the mean diurnal IOP. A cross-over trial reported the mean 24h IOP reduction from baseline rather than the post intervention mean IOP. One trial only reported one time point IOP reduction from the baseline [20]. We imputed the post intervention data for these two trials. Imputed data was imprecision, so we made sensitivity analysis both including and excluding these two trials. According to the data in cross-over trial, the mean 24h IOP was significantly lower compared FCLT with latanoprost (MD -2.50, 95%CI -3.58 to -1.42, P<0.00001)[17]. The mean IOP was lower in FCLT group compared to latanoprost whether including those two trials (MD -1.44, 95%CI -1.89 to -0.99, P<0.00001) or not (MD -1.11, 95%CI -1.51

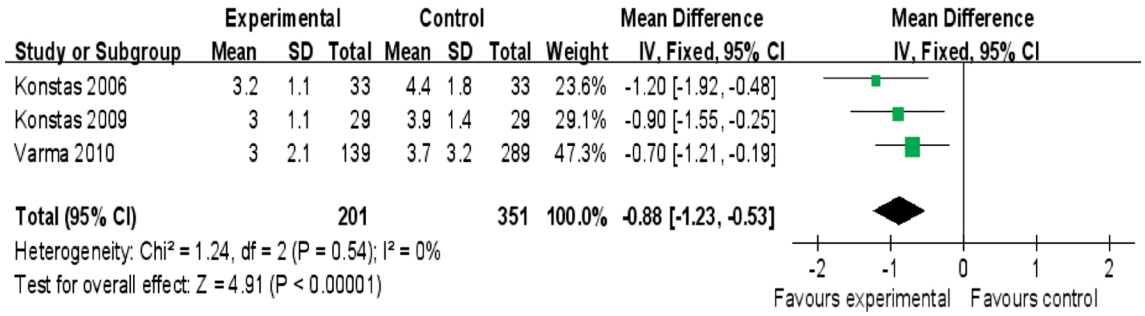

Figure 6 Analysis 1.4 the fixed combination of latanoprost and timolol *vs* timolol: the fluctuation of intraocular pressure.

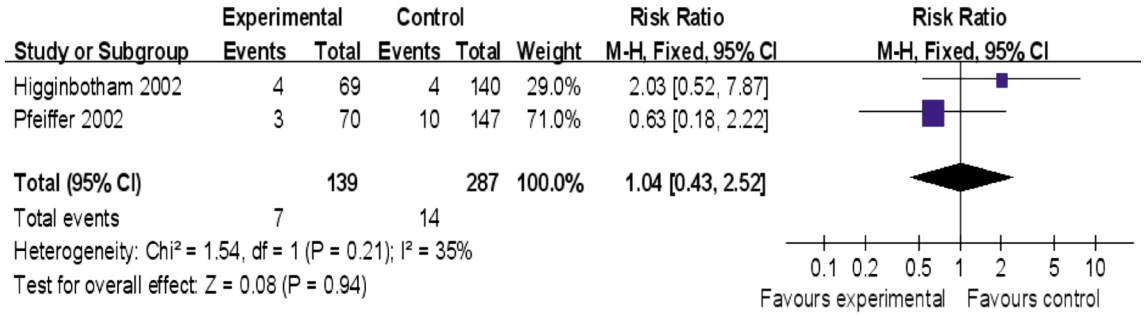

Figure 7 Analysis 2.1 the fixed combination of latanoprost and timolol *vs* latanoprost: the incidence of visual field defect.

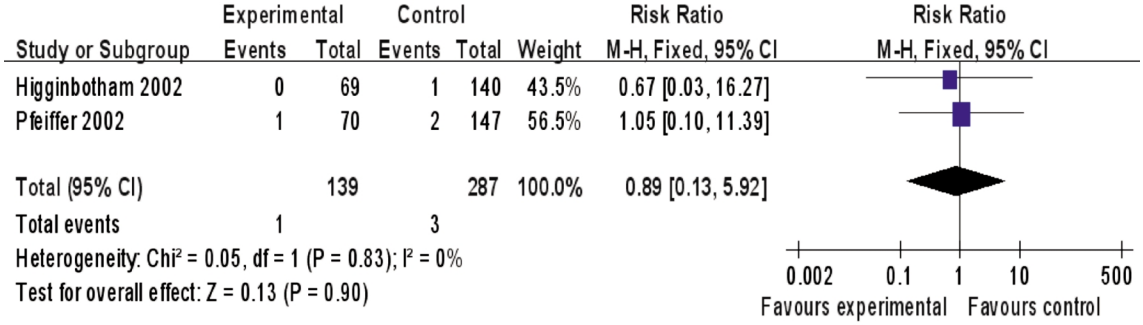

Figure 8 Analysis 2.2 the fixed combination of latanoprost and timolol *vs* latanoprost: optic atrophy.

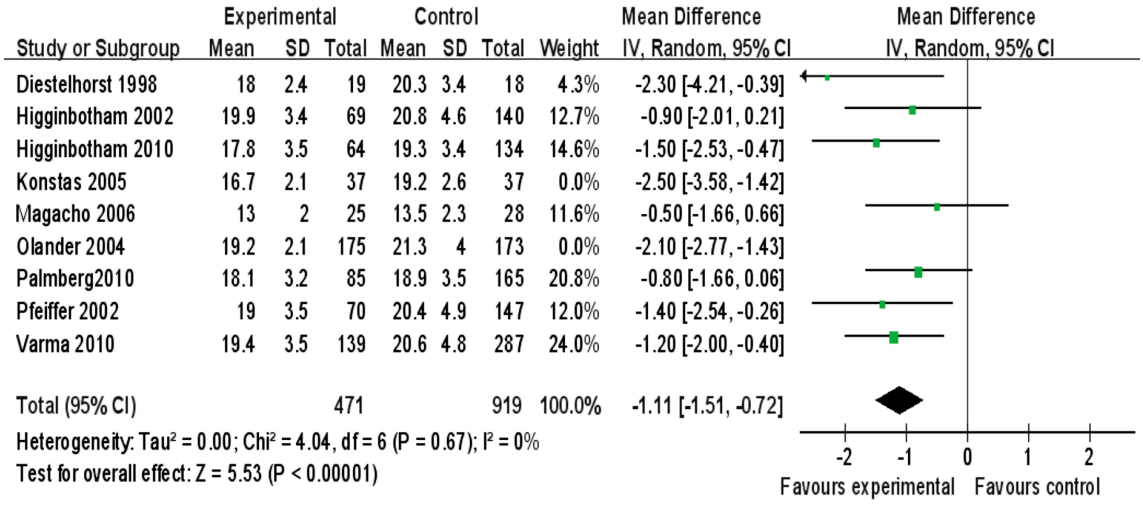

Figure 9 Analysis 2.3 the fixed combination of latanoprost and timolol *vs* latanoprost: the mean IOP.

to -0.72, $P<0.00001$). The sensitivity analysis indicated a 0.33 mm Hg difference, which was unimportant in Clinical. When we included those two trials, this meta-analysis had moderate heterogeneity ($I^2=43\%$). When excluding them, the heterogeneity decreased ($I^2=0\%$). So we showed the result excluding those two trials.

Fluctuation of Intraocular Pressure (Figure 10) Two trials reported the fluctuation of IOP, it was lower in the fixed combination groups compared to latanoprost (MD -0.63, 95%CI -1.04 to -0.22, $P=0.002$)[15,17].

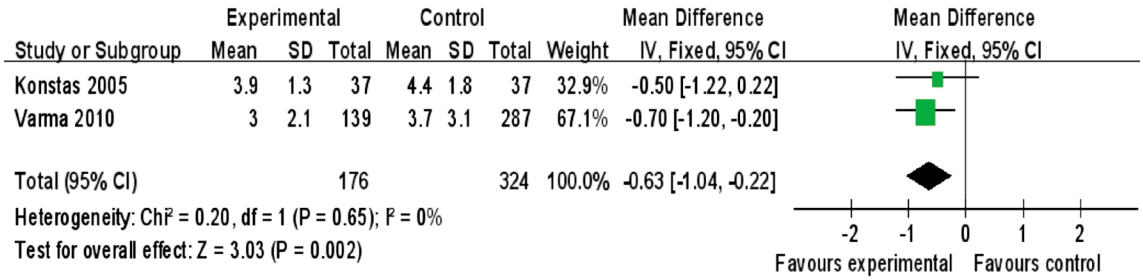

Figure 10 Analysis 2.4 the fixed combination of latanoprost and timolol *vs* latanoprost: the fluctuation of intraocular pressure.

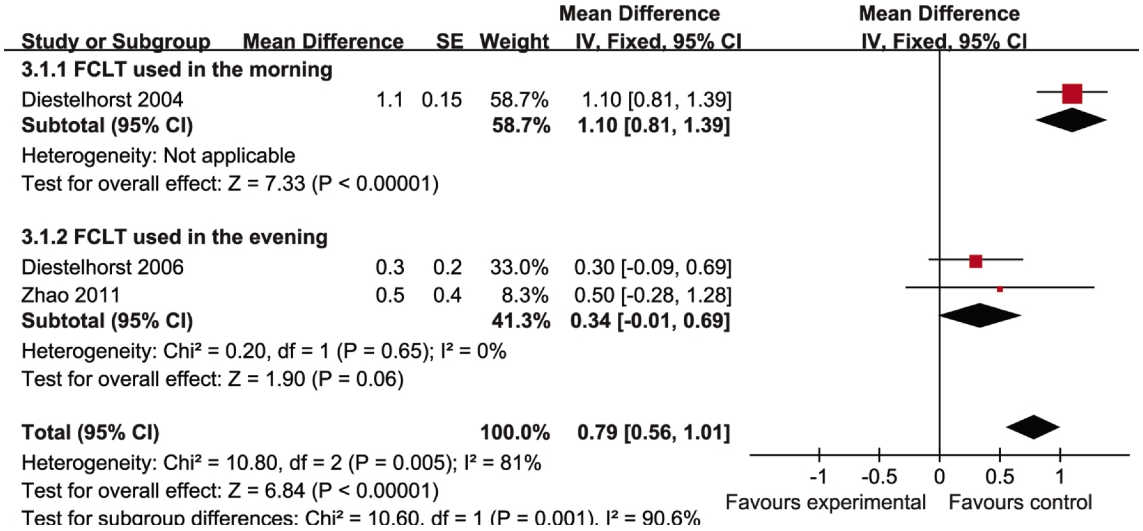

Figure 11 Analysis 3.1 the fixed combination of latanoprost and timolol *vs* UFCLT: the mean IOP.

Fixed combination of Latanoprost and Timolol *vs* Unfixed Fixed Combination of Latanoprost and Timolol There were three studies focused on the comparison of FCLT and UFCLT [10,16,22]. In one cross over study, the FCLT was used once daily in the morning [16]. While in the other two parallel group studies, the FCLT was used in the evening[10,22]. None of the three trials report the incidence of glaucomatous VF defects and optic atrophy.

Mean Diurnal Intraocular Pressure (Figure 11) Because substantial heterogeneity of the mean IOP existed among the three studies (I^2=81%, P=0.005), we made a subgroup analysis according to the administration time. When the FCLT was used in the morning in the cross-over study, the mean IOP was higher in FCLT than in UFCLT (MD 1.10, 95%CI 0.81 to 1.39, P<0.00001). When the FCLT was used in the evening in the parallel studies, meta-analysis failed to achieve Clear statistical evidence in the difference between the two groups (MD 0.34, 95% CI -0.01 to 0.69, P=0.06).

DISCUSSION

Summary of Main Results We failed to achieve clear statistical evidence for the incidence of VF defect and optic atrophy in six months treatment compared FCLT with timolol or latanoprost. However, the mean IOP and the fluctuation of IOP were significantly lower compared to timolol or latanoprost. Statistically significant heterogeneity was observed in the mean diurnal IOP compared FCLT with UFCLT. The mean diurnal IOP was higher in FCLT than in UFCLT when FCLT was used in the morning. Meta-analysis failed to achieve clear statistical evidence of difference when FCLT was used in the evening

Overall Completeness and Applicability of Evidence There was no study focused on the outcomes of VF defects and optic atrophy in the comparison of FCLT and UFCLT. For the studies which compared FCLT with the components, it was insufficient to confirm the difference of the two outcomes between experiment and control groups. All relevant types of participants had been investigated in these studies, but the follow-up time was merely 26wk, which may be not long enough to detect the difference of the interventions for these two outcomes. Besides, to the IOP outcomes, all relevant types of participants and interventions had been investigated. Although the controls were not uniformly well defined across all studies, the results was mainly consistence, the difference did not affect the result. So the outcomes were sufficient to confirm the result.

The interventions in the included studies were applicability for the practice.

Quality of the Evidence

For the incidence of visual field defects Only two trials provided data on the VF defect. The mean follow-up time was 6mo.

Fixed combination of latanoprost and timolol *vs* timolol The 'risk of bias' for this outcome within study and across studies was "high risk of bias". We rated down the risk of bias by one level. The imprecision was rated down by two levels. Optimal information size is not met (11 164 *vs* 428). There were very few events. The 95% CI (0.52 to 3.33) included both appreciable benefit (RR=0.75) and appreciable harm (RR=1.25). All above, the quality of evidence for this outcome is very low.

Fixed combination of latanoprost and timolol *vs* latanoprost The quality of this comparison on risk of bias, publication bias, and imprecision was the same as above. Inconsistence was also a problem in this comparison (I^2=35%). The quality of evidence is very low.

For the the incidence of optic atrophy Only two trials provided data on the incidence of optic atrophy. The mean time of follow-up was 6mo.

Fixed combination of latanoprost and timolol *vs* timolol Both studies were judged as "high risk of bias" for this outcome within study, so we judged "high risk of bias" for this outcome across studies. The imprecision was rated down by two levels. The 95% CI (0.23 to 8.02) included both appreciable benefit (RR=0.75) and appreciable harm (RR=1.25). According to GRADE guidelines: 8.Rating the quality of evidence-indirectness [23], the optic atrophy is not a patient-important outcomes. So we rated down by one level for indirectness. The heterogeneity in this outcome was moderate (I^2=34%) but without statistical significance (P=0.73). The quality of evidence for this outcome is very low.

Fixed combination of latanoprost and timolol *vs* latanoprost The quality of evidences on risk of bias, publication bias, and imprecision, and indirectness for this comparison was the same as above. The result of the included studies was consistence. Thus, the quality of evidence is also very low.

For Mean Intraocular Pressure

Fixed combination of latanoprost and timolol *vs* timolol This outcome is a surrogate outcome, so we need to rate down for indirectness. All six parallel studies measured the mean diurnal IOP. The mean follow-up time was 17.7wk. We judged "high risk of bias" for this outcome within study for three studies, "unclear risk of bias" for another three studies. The proportion of studies at high risk of bias was insufficient to affect the interpretation of results, so we judged as "unclear risk of bias" for this outcome across studies. In conclusion, the quality of evidence for parallel studies is moderate. Both cross-over trials reported the mean 24h IOP. The mean follow-up time was 8wk. We judged "high risk of bias" for this outcome within study and across studies. For continuance outcomes, total population size is less than 400. We rated down one level for imprecision. One trial was commercially funded small study, so the quality of evidence for cross-over studies on publication bias is low. In conclusion, the quality of evidence for cross-over studies is low. The difference whether including the low quality cross-over trials or excluded them was 0.02 mm Hg, which was not obvious in clinical. So we chose the results of high quality parallel trials.

Fixed combination of latanoprost and timolol *vs* latanoprost Nine parallel group studies and one cross-over study provided data on this outcome, the mean time of follow-up was 13.4wk. This outcome is a surrogate outcome, besides, we only used time-point IOP as mean IOP because of the limited data from the two studies, so we need to rate down the outcome for indirectness We judged "high risk of bias" for this outcome within study for six studies[8,10,11,13,17,21]; "unclear risk of bias" for three studies [12,14,20], The proportion of information from studies at high risk of bias was sufficient to affect the interpretation of results. We rated down the quality of risk of bias by one level. The other problem was the inconsistency. The Heterogeneity was I^2 =43%, P=0.08, representing moderate heterogeneity. But the Pvalue showed the heterogeneity without statistical significance. When excluding the two studies with reckoned data, the heterogeneity decreased (I^2=0%). The sensitivity analysis indicated a 0.33 mm Hg difference, which was unimportant in practice. So the inconsistency didn't influence the quality of this outcome. The quality of evidence for this outcome is moderate.

Fixed combination of latanoprost and timolol *vs* unfixed combination of latanoprost and timolol We made subgroup analysis according to administration time of FCLT. In the morning dose group, one parallel study with a follow-up time of 6wk provided the mean diurnal IOP. We judged "high risk of bias" for this outcome within study and across studies, so the quality of evidence for this subgroup is moderate. In the evening dose group, one parallel group study and a cross-over study reported this outcome. The mean time of follow-up was 10wk. Two included trials were large sample size, without heterogeneity between them. We judged as "high risk of bias" for this outcome within and across studies. In subgroup of FCLT of evening dosage, the quality is moderate.

For Fluctuation of Intraocular Pressure

Fixed combination of latanoprost and timolol *vs* timolol We identified one parallel group study and two cross-over studies in this outcome. The mean follow-up time was 14wk. We just need to rate down by one level for risk of bias,

because two trials were judged as "high risk of bias", one trial was "unclear risk of bias" within study. All above, the quality of evidence is moderate.

Fixed combination of latanoprost and timolol *vs* latanoprost One parallel group study and one cross-over study provided the result. The mean time of follow-up was 17wk. We just need to rate down by one level for risk of bias, because one trial was judged as "high risk of bias", one trial was "unclear risk of bias" within study. Thus, the total quality of evidence is moderate.

Agreements and disagreements with other studies or reviews We had not found other reviews focused on the evidence of VF defect and optic atrophy for FCLT. Most studies chose the IOP as the main outcome. A Cochrane review investigate medical interventions for POAG and OHT [24]. This review got the conclusion that medical IOP lowering treatment has a VF protective effect. Positive but weak evidence for a beneficial effect of the class of beta-blockers has been shown; direct comparisons of prostaglandins to placebo are not available. This review didn't research the FCLT. According to our review, FCLT is more effective in lowering IOP in patients with POAG or OHT, but not in optic atrophy or VF defect. First, the follow-up time was 26wk, which may not long enough to detect the difference of the interventions for these two outcomes. Second, they were reported as incidence of ocular adverse events, a dichotomous data, which may not precision compared to the quantifiable results (such as mean deviation, pattern standard deviation of Humphrey VF parameters and nerve fiber layer thickness). Third, the quality of these outcomes were rated down for imprecision, the sample size was not large enough to detect the difference.

We found three systematically reviews about the FCLT *vs* timolol, latanoprost, or UFCLT [25-27]. One review discuss IOP at 9 a.m., noon, 4 p.m. and diurnal curve for FCLT *vs* timolol or latanoprost[25]. This review searched all RCTs up to the end of August 2010. IOP reduction with FCLT was significant on the mean diurnal curve compared to timolol (MD=-2.73 mm Hg) and latanoprost (MD=-1.31 mm Hg), which was similar as our result. Another one researched the absolute and relative IOP reduction of FCLT from baseline, without a control group [26]. The other one discussed the prostaglandin analogs and timolol-fixed *vs* unfixed combinations or mono therapy for POAG [27]. The conclusion was that FCs (fixed combinations) are more efficacious than their individual components, but less efficacious than their respective UCs. This review didn't make separate analysis about three kinds of FCs, the heterogeneity between studies was I^2=52%, P=0.08.

AUTHORS´ CONCLUSIONS

Implications for Practice FCLT provided a better mean IOP lowering effect compared to timolol (-2.94 mm Hg) and latanoprost (-1.11 mm Hg). A better IOP fluctuation control has been clearly demonstrated on FCLT than timolol (-0.88 mm Hg) and latnaoprost (-0.63 mm Hg). We could get a comparable effect compared FCLT evening dose with UFCLT (0.34 mm Hg) and a worse IOP lowering effect compared FCLT morning dose with UFCLT (1.1 mm Hg). There was no evidence of FCLT *vs* UFCLT and no powerful evidence of FCLT *vs* timolol or latanoprost for VF and optic atrophy. Whether use FCLT for patients uncontrolled with mono-therapy should remain individualized, considering the compliance, the level of IOP, age and other risk factors.

Implications for Research This review shows that the question of the effects on mean IOP and IOP fluctuation of FCLT *vs* timolol or latanoprost in POAG and OHT patients should now be regarded as answered. Further research may be justified to investigate the IOP reduction effect compared to UFCLT. Further high quality RCTs with long term follow-up on VF defect, optic atrophy are needed.

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REFERENCES

1 Lundberg LU, Thygesen J, Damgaard-Jensen L, Serup L, Kessing S V. Glaucoma patients treated by practicing ophthalmologists in Denmark. Estimated number of patients and the extent of visual field defects. *Ugeskr Laeger* 2000;162(21):3028-3033

2 Baudouin C. Side effects of antiglaucomatous drugs on the ocular surface. *Curr Opin Ophthalmol* 1996;7(2):80-86

3 Zimmerman TJ, Kaufman HE. Timolol, dose response and duration of action. *Arch Ophthalmol* 1977;95(4):605-607

4 Higgins JPT, Deeks JJ. Selecting Studies and Collecting Data. In Higgins JPT, Green S, ed. *Cochrane Handbook for Systematic Reviews of Interventions.* Version 5.1.0 [updated March 2011] www. cochrane-handbook.org. The Cochrane Collaborationl; 2011:7.4-7.5

5 Higgins JP, Altman DG, Sterne JA. Assessing Risk of Bias in Included Studies. In Higgins JPT, Green S, ed. *Cochrane Handbook for Systematic Reviews of Interventions.* Version 5.1.0 [updated March 2011] www. cochrane-handbook.org. The Cochrane Collaborationl; 2011:8.13-8.19

6 Takmaz T, Aşik S, Kürkçüoglu P, Gurdal C, Can I. Comparison of intraocular pressure lowering effect of once daily morning *vs* evening dosing of latanoprost/timolol maleate combination. *Eur J Ophthalmol* 2008;18(1): 60-65

7 Diestelhorst M. Comparison of fixed-ratio combinations of latanoprost and timolol: a randomised, double-masked, multicentre study in glaucoma patients with timolol and latanoprost as controls. Annual Meeting of the Association for Research in Vision and Ophthalmology, Parts 1-2 May 11-16, 1997 Fort Lauderdale, Florida, USA. *Invest Ophthalmol Vis Sci* 1997;38(4 PART 1-2):S280

8 Diestelhorst M, Almegard B. Comparison of two fixed combinations of latanoprost and timolol in open-angle glaucoma. *Graefes Arch Clin Exp Ophthalmol* 1998;236(8):577-581

9 Larsson LI, Diestelhorst M. A 12-week, randomized, double-masked multicenter study of the fixed combination latanoprost and timolol in the evening *vs* the individual components. Annual Meeting of the Association for Research in Vision and Ophthalmology, May 01-05, 2005 Ft Lauderdale, FL, USA. *Invest Ophthalmol Vis Sci* 2005;46(Suppl. S):2459

10 Diestelhorst M, Larsson LI; European-Canadian Latanoprost Fixed Combination Study Group. A 12-week, randomized, double-masked, multicenter study of the fixed combination of latanoprost and timolol in the evening versus the individual components. *Ophthalmology* 2006;113 (1): 70-76

11 Higginbotham EJ, Feldman R, Stiles M, Dubiner H; Fixed Combination Investigative Group. Latanoprost and timolol combination therapy *vs* monotherapy: one-year randomized trial. *Arch Ophthalmol* 2002;120 (7): 915-922

12 Pfeiffer N; European Latanoprost Fixed Combination Study Group. A comparison of the fixed combination of latanoprost and timolol with its individual components. *Graefes Arch Clin Exp Ophthalmol* 2002;240(11): 893-899

13 Higginbotham EJ, Olander KW, Kim EE, Grunden JW, Kwok KK, Tressler CS; United States Fixed-Combination Study Group. Fixed combination of latanoprost and timolol *vs* individual components for primary open-angle glaucoma or ocular hypertension a randomized, double-masked study. *Arch Ophthalmol* 2010;128(2):165-172

14 Palmberg P, Kim EE, Kwok KK, Tressler CS; Canada and United States Fixed Combination Latanoprost/Timolol Study Group. A 12-week, randomized, double-masked study of fixed combination latanoprost/timolol versus latanoprost or timolol monotherapy. *Eur J Ophthalmol* 2010;20(4): 708-718

15 Varma R, Hwang LJ, Grunden JW, Bean GW. Using diurnal intraocular pressure fluctuation to assess the efficacy of fixed-combination latanoprost/timolol versus latanoprost or timolol monotherapy. *Br J Ophthalmol* 2010;94(1):80-84

16 Diestelhorst M, Larsson LI; European Latanoprost Fixed Combination Study Group. A 12 week study comparing the fixed combination of latanoprost and timolol with the concomitant use of the individual components in patients with open angle glaucoma and ocular hypertension. *Br J Ophthalmol* 2004;88(2):199-203

17 Konstas AG, Boboridis K, Tzetzi D, Kallinderis K, Jenkins JN, Stewart WC. Twenty-four-hour control with latanoprost-timolol-fixed combination therapy *vs* latanoprost therapy. *Arch Ophthalmol* 2005;123(7):898-902

18 Konstas AG, Lake S, Economou AI, Kaltsos K, Jenkins JN, Stewart WC. 24-Hour control with a latanoprost-timolol fixed combination *vs* timolol alone. *Arch Ophthalmol* 2006;124(11):1553-1557

19 Konstas AG, Pikilidou MI, Tsironi S, Mikropoulos D, Kozobolis VP, Sarafidis PA, Lasaridis AN, Nelson LA, Stewart WC. 24-Hour intraocular pressure and blood pressure levels with latanoprost/timolol fixed combination versus timolol. *Curr Eye Res* 2009;34(5):369-377

20 Olander K, Zimmerman TJ, Downes N, Schoenfelder J; Xalacom/ Latanoprost Study Group. Switching from latanoprost to fixed-combination latanoprot-timolol: a 21-day, randomized, double-masked, active-control study in patients with glaucoma and ocular hypertension. *Clin Ther* 2004; 26(10):1619-1629

21 Magacho L, Reis R, Shetty RK, Santos CC, Avila MP. Efficacy of latanoprost or fixed-combination latanoprost-timolol in patients switched from a combination of timolol and a nonprostaglandin medication. *Ophthalmology* 2006;113(3):442-445

22 Zhao JL, Ge J, Li XX, Li YM, Sheng YH, Sun NX, Sun XH, Yao K, Zhong Z; Xalacom Study Group in China. Comparative efficacy and safety of the fixed vs unfixed combination of latanoprost and timolol in chinese patients with open-angle glaucoma or ocular hypertension. *BMC Ophthalmol* 2011;11:23

23 Rasch A, Perleth M, Langer G, Meerpohl JJ, Gartlehner G, Kaminski-Hartenthaler A, Schunemann HJ. GRADE Guidelines: 8. Rating the quality of evidence - indirectness. *Z Evid Fortbild Qual Gesundhwes* 2012;106 (10):745-753

24 Vass C, Hirn C, Sycha T, Findl O, Bauer P, Schmetterer L. Medical interventions for primary open angle glaucoma and ocular hypertension. *Cochrane Database Syst Rev* 2007;(4):CD003167

25 Aptel F, Cucherat M, Denis P. Efficacy and tolerability of prostaglandin-timolol fixed combinations: a meta-analysis of randomized clinical trials. *Eur J Ophthalmol* 2012;22(1):5-18

26 Cheng JW, Cheng SW, Gao LD, Lu GC, Wei RL. Intraocular pressure-lowering effects of commonly used fixed-combination drugs with timolol: a systematic review and meta-analysis. *PLoS One* 2012;7 (9): e45079

27 Quaranta L, Biagioli E, Riva I, Rulli E, Poli D, Katsanos A, Floriani I. Prostaglandin analogs and timolol-fixed versus unfixed combinations or monotherapy for open-angle glaucoma: a systematic review and meta-analysis. *J Ocul Pharmacol Ther* 2013;29(4):382-389