Original Article / Özgün Araştırma

Investigating the Effect of Rosuvastatin, Paracetamol and Co-administration of Rosuvastatin and Paracetamol on Ocular Tissue

Statin, Parasetamol ve Statin-parasetamol Beraber Kullanımının Göz Dokularına Etkisinin Araştırılması

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Abstract

Objective: Statins and Paracetamol are two drugs that have a high prescription rate all over the world. Possible side effects can easily be augmented because they use the same cytochrome oxidase enzymes in liver. This study aimed to investigate the effect single or combined administration of these drugs on ocular tissues.

Materials and Methods: Twenty-eight 12- to 15-month-old rats were divided in four groups: Control, Rosuvastatin (10 mg/kg/day for 7 times a week), Paracetamol (50 mg/kg/day for 5 times a week) and Rosuvastatin (10 mg/kg/day for 7 times a week) + Paracetamol (50 mg/kg/day for 5 times a week) for 8 weeks. At the end of study, intraocular pressure (IOP) was measured and ocular tissues were obtained for histopathological evaluation under anaesthesia with Ketamine and Xylasine (50 mg/kg and 5 mg/kg, respectively).

Results: Rosuvastatin showed an IOP dropping effect and paracetamol did not prevent it. Histopathological evaluation mainly revealed retinal nerve fibre layer degeneration. Additionally, different pathological alterations such as corneal oedema and polypoid proliferation were observed in all the treated groups, although they were rare.

Conclusion: The IOP dropping effect of rosuvastatin shows that it is safe in glaucoma patients, but this beneficial effect was not observed with Paracetamol. Retinal nerve fibre layer degeneration with both drugs might be one of the reasons for visual disturbances in real life conditions.

Öz

Amaç: Statinler ve Parasetamol, tüm dünyada en çok reçete edilme oranına sahip ilaçlardan ikisidir. Olası yan etkileri, karaciğerde aynı sitokrom enzimini kullanmasına bağlı olarak kolaylıkla artabilir. Bu çalışmayla her bir ilacın ayrı ayrı ya da birlikte kullanımı sonucunda göz dokusuna olan etkisi incelendi.

Gereç ve Yöntemler: On iki - on beş aylık 28 sıçan 4 gruba ayrıldı: Kontrol, Rosuvastatin (10 mg/kg/gün, haftada 7 kez), Parasetamol (50 mg/kg/gün, haftada 5 kez) ve Rosuvastatin (10 mg/kg/gün, haftada 7 kez) + Parasetamol (50 mg/kg/gün, haftada 5 kez) 8 hafta boyunca uygulandı. Çalışmanın sonunda, Ketamine ve Ksilasin (50 mg/kg and 5 mg/kg) anestezisi altında, göz içi basıncı (GİB) ölçülüp, göz dokuları histopatolojik inceleme için alındı.
Bulgular: Statin GİB düşürücü etkiye sahiptir ve bu etki Parasetamol ile engellenmemektedir. Histopatolojik inceleme başlıca retinal sinir lifi tabakası hasarını ortaya çıkardı. Buna ek olarak, korneal ödem ve polipoid proliferasyon gibi farklı patolojik değişimler tüm tedavi gruplarında görüldü, ancak nadirdi.
Sonuç: Statin GİB düşürücü etkisi bu ilacın glokom hastaları için güvenli olduğunu gösterir, ancak bu faydalı etki Parasetamol'de belirlenmedi. Her iki ilaç ile belirlenen retinal sinirde hasar daha çok araştırma gerektirir; vizüel şikayetlerin bir nedeni olabilir.

Introduction

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, are widely prescribed for their cholesterol-lowering properties. In addition to lipid-lowering properties, it has been stated that statins have pleiotropic effects including anti-inflammatory, anti-apoptotic and antiproliferative effects (1,2). It has been suggested for several ophthalmic conditions including age-related macular degeneration, glaucoma, diabetic retinopathy and uveitis (2), although some ocular side effects has been reported such as blurred vision, visual impairment, visual field defect, reduced visual acuity, myopia, hypermetropia, presbyopia,and astigmatism which might be associated with muscle or liver problems (3).

Paracetamol (Acetaminophen, APAP) is one of the most common analgesics and antipyretics applied in health care and oral or intravenous routes can be administered at various stages of the pain treatment: pre-emptive, post-operative, and chronic pain (4). It is consumed as an over-the-counter medicine in many countries. Its responsible metabolism is cytochrome oxidase enzyme systems similar to statins and its highly toxic metabolite N-acetyl-p-benzoquinonimine (NAPQI) experimentally induced cataract formation has been reported (5).

Statin treatment is quite high in clinics and these patients have exposures many drugs together, such as APAP. Therefore, in this study we evaluated possible drug interactions between Rosuvastatin (RSV) and APAP on ocular tissues clinically and histopathologically.

Materials and Methods

A total of 28 male rats (12-15 months old) were obtained from Experimental Animal Center of University and all applicable international, national and institutional guidelines for the care and use of animals were followed.

The reason, statins are mostly used after middle age; we used 12-15 months old rats (their life span is approximately 26-28 months) for our study. The study mainly planned to assess possible hepatic and renal adverse drug interaction of RSV and APAP and supported by the grant of The Scientific and Technological Research Council of our country (TUBITAK 114S567). To decrease the animal number used in medical researches, the remained eye tissues have been evaluated after taking relevant ethical approval with this study. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (HADYEK 64583101/2014/076).

The rats were randomized into 4 groups (seven in each group):

Control group; healthy, no drug was applied.

RSV group; was given 10 mg/kg RSV/daily with drinking water 7 times a week for 8 weeks.

APAP group; received 50 mg/kg paracetamol through intraperitonal injection, 5 days a week for eight weeks.

RSV+APAP group; was applied 50 mg/kg paracetamol through intraperitonal injection, 5 days a week for eight weeks and 10 mg/kg RSV with drinking water at the same time.

The rats were balanced every monday and the doses of RSV and APAP were adjusted for every cage. While giving the drugs, we did not want to use toxic doses, tried to mimic daily posology. At the end of experiment, under the anesthesia with Ketamine and Xylasine (50 mg/kg and 5 mg/kg, respectively), intraocular eye pressure (IOP) were measured with a Schiotz indentation tonometer three times for both eyes by the same masked researcher, and both eye tissues were harvested and kept in 10% formalin solution.

After routine tissue following, the obtained samples were sliced in 5 micrometer thickness and stained by hematoxylin-eosin. Histopathological examination was performed by using a light microscope (Zeiss Primo Star, Ankara, Turkey). Cornea, conjunctiva, retina and vascular structures were detected.

Statistical Analysis

The normality of IOP variable was analyzed with Kolmogorov-Smirnov test. The statistical evaluation was assessed with Kruskall-Wallis. Descriptive statistics were presented as median (interquartile range) and p values below 0.05 were considered significant.

Results

IOP of control rats were 11 (3.25) mm/Hg; RSV 7 (5) mm/Hg; APAP 14 (4) mm/Hg and co-administration of RSV+APAP 6.5 (4) mm/Hg. While APAP did not affect IOP (p=1.000), RSV dropped the IOP significantly (p<0.001) and this RSV effect did not prevented by co-administration of APAP (p<0.001). The comparison of IOP measurements between groups were summarized on Table 1.

Histopathological analyses of all groups did not show uniform pathological phenomenon. Some examples of findings have photographed in Figure 1. Control groups photographs of cornea and retina have been shown in Figure 1A (X40 magnification) and Figure 1B (X10 magnification). Corpora amylacea formation was present in all groups, one of examples in Figure 1C is belong to APAP group (X40 magnification). Degeneration of retinal nerve fiber layer has seen in RSV, APAP and RSV+APAP group (Figure 1D, E and F, respectively, X10 magnification). Neovascularization in Retinal nerve fiber layer (D X10 magnification) and cornea (G X10 magnification) with RSV; vascular endothelial polypoid proliferation in RSV+APAP (H X10 magnification), corneal edema in RSV (J X40 magnification) were the other rare findings. Corneal edema and thickness, conjunctival epithelial degeneration, retinal nerve fiber layer and vascular structure alteration numbers are as shown in Table 2.

Discussion

This study has been mainly planned to investigate APAP exposure during statin treatment as a "rational drug therapy" approach. Liver toxicity of statins is well known, but there was not any satisfactory report about adverse effects of co-administration of APAP which is another liver toxic agent. Meanwhile, we extended our study to ophthalmic effects. Highly toxic metabolite of APAP, NAPQI, consumes not only liver

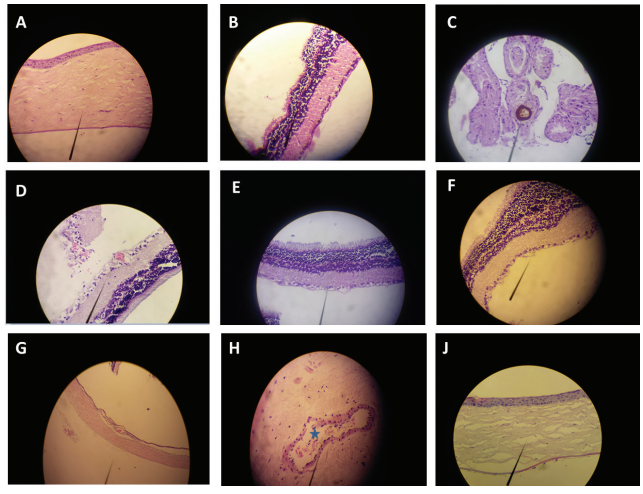

Figure 1. Some examples of histopathological findings in all groups. Control groups' cornea and retina in A (X40 magnification) and B (X10 magnification) respectively; Corpora amylacea formation in APAP group (C X40 magnification); Retinal nerve fiber layer degeneration in RSV, APAP and RSV+APAP group (D, E and F, respectively, X10 magnification); Neovascularization in RSV (G X10 magnification), vascular endothelial polypoid proliferation (blue star) in RSV+APAP (H X10 magnification), corneal edema in RSV (J X40 magnification)

RSV: Rosuvastatin; APAP: Paracetamol (Acetaminophen)

Table 1. Intraocular pressure evaluation of ocular tissues in all experimental groups

Group vs Group	IOP			p
Control vs Rosuvastatin	11 (3.25)	vs	7 (5)	<0.001
Control vs APAP	11 (3.25)	vs	14 (4)	1.000
Control vs Rosuvastatin + APAP	11 (3.25)	vs	6.5 (4)	<0.001
Rosuvastatin vs APAP	7 (5)	vs	14 (4)	<0.001
Rosuvastatin vs Rosuvastatin + APAP	7 (5)	vs	6.5 (4)	1.000
APAP vs Rosuvastatin + APAP	14 (4)	vs	6.5 (4)	<0.001

APAP: Paracetamol (Acetaminophen), IOP: Intraocular pressure

Table 2. Histopathological evaluation of ocular tissues in all experimental groups. Numbers show the pathological sample numbers

	Control	Rosuvastatin	APAP	Rosuvastatin + APAP
Corneal thickness	0	1	1	2
Corneal edema	3	7	4	5
Conjunctival epithelial degeneration	0	3	0	0
Retinal nerve fibril layer degeneration	0	6	5	2
Endothelial polypoid proliferation	0	0	0	1
Corpora amylacea in lacrimal gland	2	2	2	2

APAP: Paracetamol (Acetaminophen)

glutathione, but also tissue levels as well. It has been shown that there is not any significant difference of pharmacokinetic parameters between concentrations of Paracetamol and Paracetamol-glucuronide in the plasma and aqueous humour (4). On the other hand, one of the earlier studies demonstrated morphological changes in the retina of rats following direct intra vitreal injection of lovastatin (0.25 micromol in 7.5 microlitre of 10 mM, Tris-HCl, pH 7.4). This study described degeneration of photoreceptor inner and outer segments, rosette formation, and the appearance of debris-filled macrophages (6); it led us to expand our research on ocular tissue and performed histopathological analyses as well. Therefore, the first question was to understand individual side effect of APAP and statin, and second question was the outcome of these agents co-administration on ocular tissues in chronic usage.

Here, the prominent clinical finding of this study is RSV treatment has 32.84% IOP decreasing effect on healthy rats; this beneficial effect was not obtained with APAP and APAP did not prevent statins' IOP dropping effect. Contrary, a clinical study demonstrated that 1 g orally given APAP for 2 weeks significantly reduced IOP of 9 open angle glaucoma patients (7); but, Jampel HD study supports our findings. He planned a small randomized clinical trial with 10 glaucoma patients, he gave 650 mg APAP four times a day for 7 days, did not get any significant IOP lowering effect of APAP (8). On the other hand, statins IOP dropping effect is previously reported clinical glaucoma study (1). Our study have shown that this effect can be seen on healthy subjects too, without depending on lipid lowering or any suggested impact on vascular system; at some point, statin treatment might have prophylactic effect if the patient takes any statin therapy for any reason.

Searching of the literatures, histopathological side effects of these two medications are very rarely studied and reported. In our study, the most important histopathological finding is degeneration of retinal nerve fiber layer in all treatment groups. For statin, this finding is contradictory of previous experimental and observational studies that it has been suggested neuro-protective on ocular diseases (1). A report for Lovastatin and Simvastatin with 150 patients was provided good evidence that there was not any toxicity finding on lens and other ocular tissues (9). Contrary, a detailed retrospective analyses of statin users indicate that 1.6% (301/18.395) RSV patients developed ocular side effects, including blurred vision, visual impairment and reduced visual acuity between 1988 and 2013, but this study could not performed an adjustment of age (3). The prevalence of visual disturbances of statin seems highly low, but at some point this might be explained with shrinking of retinal nerve fiber layer that we have found in our study. On the other hand, for APAP, Nassini et al. (10) proved that NAPQI metabolite is responsible of releasing sensory neuropeptides that mediates neurogenic inflammation in conjunctiva at therapeutic doses. This paper might support our observations that chronic APAP treatment results in degeneration of retinal nerve fiber layer of six animals out of seven. So, damaging of retinal nerve fiber layer with both drugs should take more attention; it can bring some clinical important side effects.

Corpora amylacea formation has seen in all groups as a common findings and this thought to be because of the animals' age. Although, edema has seen even

in control group, the medications are increased its forming too. Previously, histopathological analysis of rabbit eyes treated with topical application of APAP shown an edematous cornea (11), we have given systemic administration and detected four rats have corneal edema and one rat with corneal thickness in out of seven. Conjunctival epithelial degeneration was only seen with RSV in three animals out of seven. Endothelial polypoid proliferation has determined only in one rat from the combine treatment group. Considering that we have determined neovascularization in retinal nerve fiber layer in one rat and in cornea in another rat with RSV, this endothelial polypoid proliferation might be related with this structural changing. However, we would like to emphasize that these pathological findings are really rare, therefore we are not sure about their presence are due to the medications. Beside the beneficial reports of statins, retrospective 95 case reports analyzed, although, some patients also received medications known to increase bleeding times, it is suspected ocular hemorrhage is ''possibly'' due to statin therapy (12). We could not determine any bleeding focus on the ocular tissue layers. APAP metabolite NAPQI, has induced cataract formation experimentally (5), we could not detect any lens tissue changing as well with the chronic administrations of medications.

Conclusion

Statin IOP dropping effect shows this medication is safe in glaucoma patient, but we cannot see this beneficial effect with Paracetamol. Retinal nerve fiber layer degeneration with both drugs needs more attention; it might be one of the reasons of visual disturbances.

Ethics

Ethics Committee Approval: Aydın Adnan Menderes University for Animal Experiments (HADYEK 64583101/2014/076).

Informed Consent: This is an animal experiment study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.G., B.D., Design: A.G., B.D., Data Collection or Processing: A.İ.A.Ü., F.K., B.D., Analysis or Interpretation: A.İ.A.Ü., F.K., Y.Ö., B.D., Literature Search: A.İ.A.Ü., F.K., B.D., Writing: A.İ.A.Ü., B.D.

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