CCL2, TNFα, IL-6, and IL-8.[3] The implication of microglial activation in the pathogenesis of DR has led to the concept of microglial modulation as a therapeutic strategy. Recent clinical trials have investigated the inhibition of retinal microglia as a method to inhibit DR. As a result, several drugs including minocycline, doxycycline, and dextromethorphan, which are capable of inhibiting microglia activation, have been recently evaluated for the treatment of DME.[3,4]

In a previous study, our group investigated the effect of crocin on BV-2 retina microglial cells.[5] According to our findings, crocin significantly reduced gene expression of the proinflammatory markers IL-6, CCL2, and iNOS in lipopolysaccharide-challenged BV-2 microglial cells and potently blocked nitric oxide production in these microglia cells. Our findings support the anti-inflammatory and immunomodulatory effects of crocin on retinal microglial cells and indicated that its direct effects on microglia homeostasis could be mediated by its anti-inflammatory effects in animal models of neuronal degeneration.

As in line with this evidence, Sepahi's study results could also be attributed to the possible anti-inflammatory effect of the crocin on retinal microglia cells. However, all available studies aiming to determine the effects of crocin on microglial cells have been performed in the absence of a diabetic insult. In addition, as hyperreflective foci detected on spectral-domain optical coherence tomography (SDOCT) have been used as a surrogate inflammatory marker of DR and also may correspond to activated microglia,[6] the authors may also evaluate the change of hyperreflective foci on SDOCT in their study group.

Finally, while appreciating the initiatives aiming to evaluate the effects of herbal supplements as an adjunct to traditional treatment, further studies evaluating the exact role of these products on the pathophysiologic process of DME are needed.

MÜCELLA ARıKAN YORGUN
Ankara, Turkey

FUNDING/SUPPORT: NO FUNDING OR GRANT SUPPORT. Financial Disclosures: The author has no financial disclosures. The author attests to meeting the current ICMJE criteria for authorship.

REFERENCES

1. Sepahi S, Mohajeri SA, Hosseini SM, et al. Effects of crocin on diabetic maculopathy: a placebo-controlled randomized clinical trial. *Am J Ophthalmol* 2018;190:89–98.
2. Karlstetter M, Scholz R, Rutar M, Wong WT, Provis JM, Langmann T. Retinal microglia: just bystander or target for therapy? *Prog Retin Eye Res* 2015;45:30–57.
3. Altmann C, Schmidt MHH. The role of microglia in diabetic retinopathy: inflammation, microvasculature defects and neurodegeneration. *Int J Mol Sci* 2018;19.
4. Valent DJ, Wong WT, Chew EY, Cukras CA. Oral dextromethorphan for the treatment of diabetic macular edema: results from a phase I/II clinical study. *Transl Vis Sci Technol* 2018;7:24.
5. Yorgun MA, Rashid K, Aslanidis A, Bresgen C, Dannhausen K, Langmann T. Crocin, a plant-derived carotenoid, modulates microglial reactivity. *Biochem Biophys Rep* 2017;12:245–250.
6. Kang JW, Chung H, Chan Kim H. Correlation of optical coherence tomographic hyperreflective foci with visual outcomes in different patterns of diabetic macular edema. *Retina* 2016;36:1630–1639.

REPLY

WE THANK DR MÜCELLA ARıKAN YORGUN FOR HER ATTENtion and comments. As she mentioned in the letter, the pathogenesis of diabetic retinopathy (DR) has not been completely elucidated. The vascular endothelial growth factor (VEGF) causes leakage of neighboring capillaries; and it also stimulates the capillaries to grow ("new blood vessels"). The leakage causes the retina to swell up a little and become waterlogged, a bit like a sponge. This swelling then damages the retinal cells themselves. This is the main mechanism in "maculopathy" and "macular edema." Anti-VEGF treatment is only partially effective against diabetic macular edema.[1] Avastin injections are used to treat diabetic retinopathy. Avastin is an anti-VEGF drug. By blocking the effect of VEGF, Avastin stops the new vessels growing and reduces retinal leakage for a while.[2]

Reactive oxygen species (ROS) generated from mitochondria, NADPH oxidase, and other oxidases are known to play an essential role in the pathogenesis of DR. ROS modify redox-sensitive kinases and transcription factors such as NF-κB, signal transducers and activators of transcription proteins, and activator protein 1 and therefore induce inflammatory gene expression in DR.[3]

Increasing evidence indicates that inflammation is a key player in DR. Increases in vitreous inflammatory cytokines such as IL-6, VEGF, MCP-1, and IP-10 have been found to be positively associated with the progression of DR and the severity of macular edema. The concentrations of inflammatory cytokines such as IL-1β, IL-6, IL-8, MCP-1, IP-10, and VEGF are positively associated with macular edema, whereas levels of anti-inflammatory cytokines such as IL-10 and IL-12 are negatively associated with macular edema.[4]

In the pathogenesis of diabetic retinopathy, mitochondria are damaged and inflammatory mediators are elevated before the histopathology associated with the disease can be observed. Matrix metalloproteinases (MMPs) regulate a variety of cellular functions including apoptosis and angiogenesis. Diabetic environment stimulates the

secretion of several MMPs that are considered to participate in complications, including retinopathy, nephropathy, and cardiomyopathy. Patients with diabetic retinopathy and also animal models have shown increased MMP-9 and MMP-2 in their retina and vitreous.[5]

Also, in a study by Yang and associates,[6] the role of microglial cells in DR was investigated, suggesting that DR may cause the overactivation of microglial cells and induce oxidative stress and the release of proinflammatory factors. Microglial cells BV-2 and N9 were cultured, and high glucose (HG) and free fatty acid (FFA) were used to simulate diabetes. They found that crocin prevented the oxidative stress and proinflammatory response induced by HG-FFA co-treatment.[6]

Following our previous clinical trial study, which is published in *AJO*, our group are investigating the effect of crocin on VEGF, VEGFR, MMP-2, and MMP-9 expression in HG culture.

Therefore, understanding the biochemical changes and the molecular events under diabetic conditions are essential to develop novel therapeutic tools to combat DR disease.

SAMANEH SEPAHI
SEYED AHMAD MOHAJERI
SEYEDEH MARYAM HOSSEINI
ELHAM KHODAVERDI
NASSER SHOEIBI
MARAL NAMDARI
SAYYED ABOLGHASEM SAJADI TABASSI
Mashhad, Iran

CONFLICT OF INTEREST DISCLOSURES: SEE THE ORIGINAL article for any disclosures of the authors.

REFERENCES

1. Ford JA, Lois N, Royle P, et al. Current treatments in diabetic macular oedema: systematic review and meta-analysis. *BMJ Open* 2013;3(3):e002269.
2. Arevalo JF, Sanchez JG, Lasave AF, et al. Intravitreal bevacizumab (Avastin) for diabetic retinopathy: The 2010 GLADAOF Lecture. *J Ophthalmol* 2011;2011:584238.
3. Al-Shabrawey M, Zhang W, McDonald D. Diabetic retinopathy: mechanism, diagnosis, prevention, and treatment. *Biomed Res Int* 2015;2015:854593.
4. Dong N, Xu B, Wang B, Chu L, Tang X. Aqueous cytokines as predictors of macular edema in patients with diabetes following uncomplicated phacoemulsification cataract surgery. *Biomed Res Int* 2015;2015:126984.
5. Kowluru RA, Zhong Q, Santos JM. Matrix metalloproteinases in diabetic retinopathy: potential role of MMP-9. *Expert Opin Investig Drugs* 2012;21(6):797–805.
6. Yang X, Huo F, Liu B, et al. Crocin inhibits oxidative stress and pro-inflammatory response of microglial cells associated with diabetic retinopathy through the activation of PI3K/Akt signaling pathway. *J Mol Neurosci* 2017;61(4):581–589.