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The food dye Tartrazine disrupts vascular formation both in zebrafish larvae and in human primary endothelial cells

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Tartrazine (E102) is a controversial coloring agent whose potential impacts on human health are not fully understood. Our study reveals the vascular disrupting effects of tartrazine (TTZ) on developing zebrafish embryos in vivo and on human umbilical vein endothelial cells in vitro. The dye was shown to cause dose-dependent hemorrhages in zebrafish embryos. Analyzing transgenic zebrafish harboring fluorescent endothelial cells revealed that TTZ treatment disrupted cell organization into vessels in both the sub-intestinal vein and the brain area. Assays on human umbilical vein endothelial cells demonstrated that TTZ inhibited endothelial proliferation, tube formation, and migration in a dose-dependent manner. Taken together, our results indicate for the first time that TTZ can affect endothelial cell properties, possibly by disrupting Rho family GTPase pathways which control the cytoskeleton. Our finding provides a credible explanation for many reported human health impacts and offers prospective applications for biomedicine.

Keywords Tartrazine, Blood vessels, Zebrafish, HUVEC, BMP pathways, Rho GTPases

Tartrazine (E102, TTZ), a lemon-yellow monoazo dye, is one of the most widely used coloring agents in food products, medicines, and cosmetics. Despite being extensively studied in recent decades, TTZ remains among the most controversial food additives, with various debates over its potential safety and toxicity[1–3]. The 2009 EFSA Panel on food additives set its acceptable daily intake (ADI) to 7.5 mg/kg bw based on the "no observed adverse effect level" (NOAEL) at 750 mg/kg bw/day determined in a rat chronic toxicity assay[1], while the Joint FAO/WHO expert committee in 2017 approved a higher ADI at 10 mg/kg bw[3].

However, recent studies have challenged the safety of TTZ even at those ADI levels or lower. For instance, 30-day TTZ feeding to rats at 7.5 mg/kg bw induced liver and kidney histopathology, in addition to leucocyte DNA damage[4]. Another study described fetal mortality and teratogenicity following 0.45 and 4.5 mg/kg bw TTZ administration to pregnant rats on the 6th −15th days of gestation[5]. Similarly, neurotoxic[6]and haemato-immunotoxic[7]impacts have been reported as the results of TTZ treatment at ADI and below. The main suspect to trigger all these effects is oxidative stress resulting from TTZ metabolites produced by the gut microbiota[8,9].

In humans, the most controversial health aspect of TTZ is its association with attention-deficit hyperactivity disorder (ADHD). Dated back to the 70s[10], that allegation once again drew public attention in 2007 thanks to the RCT study of McCann et al.[11], listing TTZ among the infamous "Southampton six" – food additives whose mixture could possibly induce hyperactivity in children. Besides, immune (hypersensitivity) and non-immune (intolerance) reactions to the dye have been long reported, albeit rare[3,10,12,13]. One notable TTZ-induced symptom in several cases is the occurrence of purpuric spots (subcutaneous hemorrhage)[13–15].

While most of the studies until now were based on cell culture and rodent models, zebrafish embryo is increasingly used for toxicological investigations[16]. Generally considered as a non-regulated embryonic in vivo model (until 120 h old)[17], this embryo can be used in complement to conventional in vitro and animal models: its transparent body allows visualization of all standard vertebrate systems and organs; its rapid development allows assessment of multiple toxicological targets and processes within a few days; more importantly, research

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using zebrafish embryos can provide predictive value not only for human health risk but also for ecotoxicity assessment[16,18,19].

Here, by combining tests on zebrafish embryos and human cells, we show that TTZ displays a disruptive effect on vascular formation, which may help shed light on the possible processes involved in some of TTZ's adverse effects.

Methods

Materials

The zebrafish wild type (WT) AB strain, and transgenic strains *Tg(fli1:EGFP) (y1Tg)*[20] and *Tg(kdrl:mCherry)*(y171Tg)[21]were maintained within the Zebrafish Facility in GIGA-Research, University of Liège. Fish were reared in a Techniplast (Buguggiate, Italy) recirculating system under a 14:10-h light/dark photocycle. The isolation and maintenance of human umbilical vein endothelial cells (HUVECs) were previously described[22,23]. The cells were cultured in EGM2 (Lonza, Verviers, Belgium) supplemented with 5% FCS (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Tartrazine (#86310, Sigma Aldrich, Hoeilaart, Belgium) 100 g/L stock solution was prepared and diluted as appropriate in E3 (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, and 0.16 mM $MgSO_4$) for zebrafish experiments and in PBS for cell experiments.

Zebrafish embryonic assays

General toxicity evaluation The zebrafish embryonic toxicity (ZET) assay was adapted from the OECD Test No.236 (Fish Embryo Toxicity Test)[19]. After breeding, eggs were collected into E3 medium. At 3–4 h post-fertilization (hpf), fertilized and healthy embryos were selected and distributed into 6-well plates at 25 embryos/well containing 5 mL of E3 medium supplemented or not with TTZ. Embryos were raised until four days post-fertilization (4 dpf), solutions were renewed and dead embryos were removed daily. Lethality and malformation rates were recorded. Phenotypes were compared with those described previously by Kimmel et al.[24]

Blood vessel assessment: Transgenic embryos were obtained by crossing heterozygous *Tg(fli1:EGFP)* or *Tg(kdrl:mCherry)* with WT and exposed to 5 and 10 g/L TTZ from 3-4hpf. Visual analysis was performed at 3 dpf using a Leica (Wetzlar, Germany) M165FC fluorescent dissecting microscope. Both transgenic lines are vasculogenic reporters whose endothelial cells (ECs) are labeled with respectively green or red fluorescence. Despite displaying a brighter fluorescence signal, the *Tg(fli1:EGFP)* was used solely for the sub-intestinal vein (SIV) assessment due to the intense fluorescence in the pharyngeal arch cranial neural crest cells. Therefore, the pan-vasculature observation was performed on the *Tg(kdrl:mCherry)* line. All experiments were performed in triplicate, $n = 50$.

Human umbilical vein endothelial cell (HUVEC) assays

Human umbilical vein endothelial cells were obtained from Lonza (Verviers, Belgium) and used between passages 3 and 8. All experiments were performed in triplicate, and each included five wells per TTZ concentration (including control), each time cell viability was ensured by staining with trypan blue before seeding.

Cell proliferation Cells were seeded into a 96-well plate for 48 h at 8000 cells/well. Selected concentrations of TTZ (0–1000 ug/mL) were then added, and proliferation was analyzed after 24 h using the Cell Proliferation ELISA BrdU Colorimetric kit (Roche, Mannheim, Germany) following the manufacturer's instructions. Absorbance at 355 nm was measured on a VICTOR X3 Multilabel Reader (PerkinElmer, Waltham, USA).

Tubulogenesis HUVECs were seeded onto a Matrigel-coated 96-well plate at 3000 cells/well into 100 µl of medium supplemented with selected concentrations of TTZ (0–1000 µg/ml), then incubated at 37 °C for 6 h. Tubulogenic pictures were taken under a CKX41 microscope (Olympus, Tokyo, Japan) and analyzed using ImageJ.

Migration HUVECs were plated into 48-well plates and cultured to confluency in EGM2. A scratch wound was then created in the monolayers using a pipette tip. The scratched wells were washed with PBS and incubated in EBM2 0.5% DBS for 6 h. The wound size (i.e., the distance between two sides of the wound) was measured at time points 0 h and 6 h with a CKX41 microscope (Olympus, Tokyo, Japan). The difference between wound sizes at 6 h and 0 h (i.e., wound closure) was calculated and represented as relative to the respective control per replicate.

TargetNet prediction

The online platform TargetNet (http://targetnet.scbdd.com, accessed January 2022) was used to predict potential protein targets of TTZ using its molecular fingerprint. TTZ's SMILES string was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov). TargetNet parameters were set at default values (AUC $\geq$ 0.7; Fingerprint type ECFP4). Potential target hits were defined as having a probability score > 0.

Data and statistical analysis

All data analysis and graphs were performed using R 4.3.2. All scripts and raw data are available upon request from the authors.

Concerning zebrafish embryo development assessment, toxicity indices (i.e. LC50 and EC50) of TTZ were estimated by fitting dose response models with two-parameter log-logistic functions using package "drc"[25]. The teratogenic index (TI) was then calculated by dividing the LC50 by EC50. Mixed effect models (using package "lme4"[26]) were fitted to analyze the observed survival and malformation rates at different treatment conditions due to the multilevel nature of the experiments (including both biological and technical replications). The average survival and malformation rates and their 95% prediction intervals (error bars) were obtained using the function *predictInterval()*in packages "merTools"[27] and then plotted using Prism. A similar approach was also employed for ectopic sprouting data.

The same analytical pipeline and R packages ("drc", "lme4", and "merTools") were used for the results from HUVEC cell experiments. The IC50 of TTZ on HUVEC proliferation was determined using the same method as the ZET assay. For tube formation data, since both tube length and branching results are bounded by zero and not normally distributed, they were first log-transformed before analyzing and model fitting.

Results

In vivo effect of TTZ on the developing zebrafish embryos

Hemorrhage as a remarkable malformation

Following a preliminary range-finding experiment, wild type zebrafish larvae were exposed to TTZ concentrations ranging from 0 to 50 g/L in order to identify toxicity indices (including LC50, EC50, and TI - teratogenic index) as well as the types of malformations caused by TTZ exposure. Compared to controls, the test revealed TTZ to be non-aquatoxic with an $LC50_{96hpf}$ of 9.31 g/L and an $EC50_{96hpf}$ of 5.49 g/L (Fig. 1A) (more description below). Thus, with a TI (teratogenic index, defined as the ratio between LC50 and EC50) of 1.7, the compound can be considered slightly teratogenic for zebrafish embryonic development (Fig. 1A).

Compared to the control (Fig. 1B), one remarkable malformation caused by TTZ exposure was the occurrence of multiple hemorrhagic sites across the embryonic body, starting at 2 dpf. The severity of hemorrhage was dose-dependent: at lower concentrations, the sites mainly appeared around the duct of Cuvier (i.e., the common cardinal vein) region (Fig. 1C) and then expanded to other body parts at higher concentrations, including brain

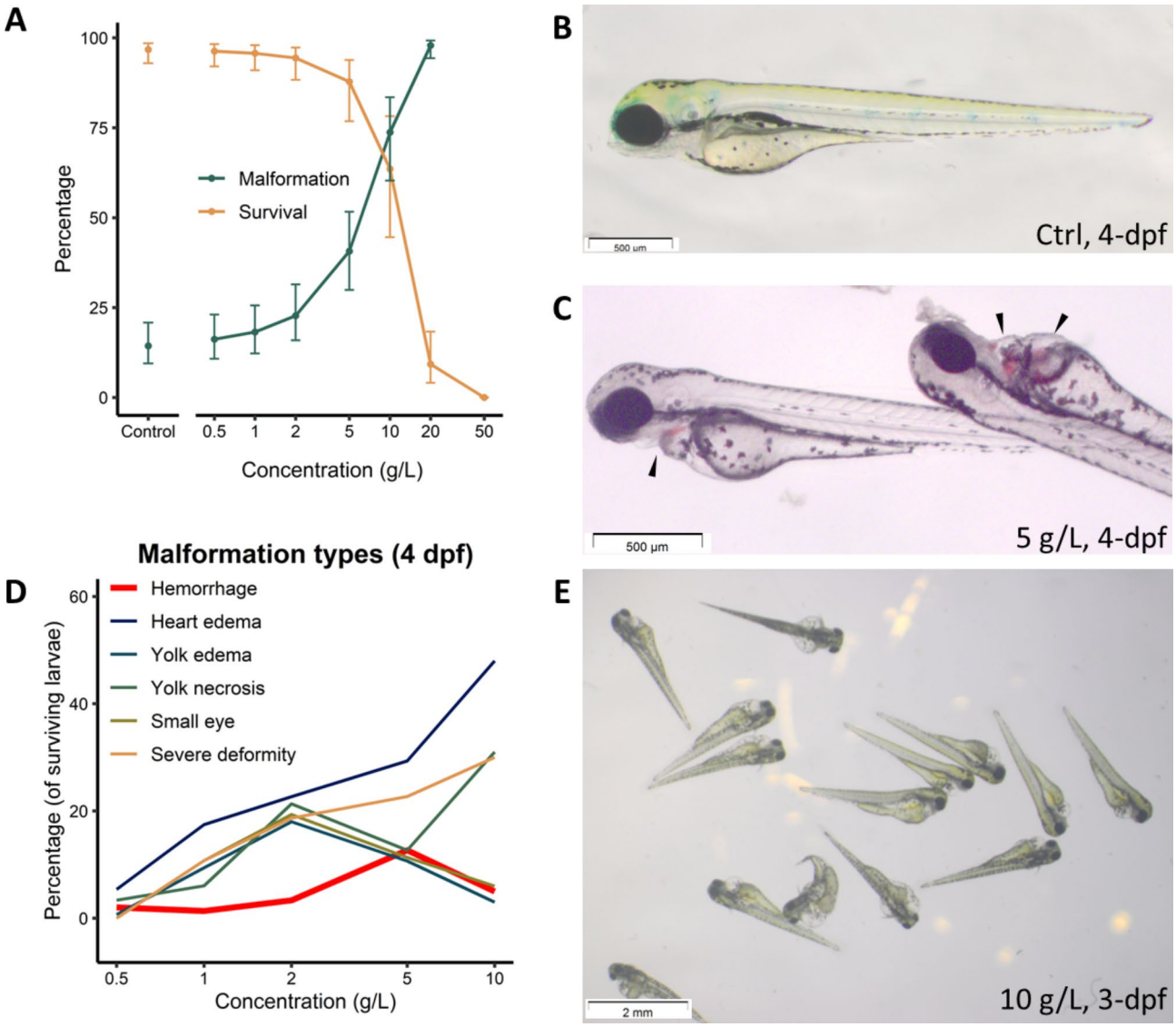

Fig. 1. ZET results for TTZ. *(A) Dose-response curves showing survival and occurrence of any malformation induced by 96 h TTZ exposure, X-axis is logarithmically scaled. (B) A control embryo at 4 dpf showing normal morphology. (C) Hemorrhagic sites (arrowheads) induced by TTZ exposure. (D) Percentages of surviving embryos with different phenotypes at 4 dpf (Standard Deviations were excluded for visual clarity). (E) Severe deformities at high TTZ concentration.*

hemorrhage. Other notable defects included pericardial edema, yolk edema, yolk necrosis, small eye, and whole-body deformities (Fig. 1D-E). However, the percentage of TTZ-induced bleeding larvae did not follow the typical dose-response relationship (Fig. 1D, thick red line). The decrease in the percentage of larvae with specific defects (hemorrhage, small eye, yolk edema and necrosis, Fig. 1D) at high concentrations suggests that these treatments lead to multiple, more severe defects[16]. Also, the biphasic curves of these specific defects, and the transitions of dominant phenotypes, suggest multiple modes of action involved[16]. The hemorrhage phenotype, which reached 14% at 5 g/L then declined at 10 g/L, was genuinely remarkable, thus follow-up investigations were conducted.

TTZ treatment of transgenic reporter fish lines revealed impairment of in vivo angiogenesis

Upon discovering the TTZ-induced hemorrhagic phenotype in wild type zebrafish embryos, further experiments were conducted using transgenic reporter zebrafish lines to verify whether TTZ could affect blood vessel formation. The exposure doses were selected at 5 and 10 g/L as these concentrations produced the most hemorrhagic phenotypes without inducing too much mortality or other deformities (Fig. 1A, E).

Firstly, whole vascular observation was conducted on the *Tg(kdrl:mCherry)* embryos at 3 dpf. The results revealed that TTZ exposure could disrupt endothelial cell (EC) organization into the vascular system to various extents in the larvae. The effect was more evident at 10 g/L TTZ, where many larvae had impaired branching and segmentation in both the cephalic vasculature and the caudal vein plexus (CVP) (Fig. 2A). Notably, the dorsal lateral anastomosis vessels (DLAV) and intersegmental vessels (ISV) are only marginally affected.

Next, to quantify the inhibitory effect of TTZ on the zebrafish blood vessel formation, further experiments were conducted with emphasis on SIV formation[18,28] using the *Tg(fli1:GFP)*. At 3 dpf, a normally developed SIV forms a smooth, basket-shaped structure on each side of the embryonic yolk (Fig. 2B). In our experiments, TTZ exposure at 5 and 10 g/L respectively induced a smaller basket size and abnormal SIV ectopic sprouting in 50% and 14% of 3-dpf embryos (Fig. 2C) – following the same trend of hemorrhage occurrences at 4-dpf larvae (Fig. 1E) but at higher percentage.

TTZ inhibits HUVEC tubulogenesis, migration, and proliferation

After confirming the vascular disrupting potential of TTZ on in vivo zebrafish embryos, the subsequent step is to verify if it was due to the direct effect of the food dye on ECs or possibly another indirect mechanism. Furthermore, to verify whether these effects would be observed in human cells, several in vitro experiments were carried out using primary Human Umbilical Vascular Endothelial Cells (HUVECs).

BrdU incorporation assays revealed that TTZ could inhibit HUVEC proliferation with an IC_{50} of 30 mg/L (56 μM) (Fig. 3A). Tubulogenesis assays showed that the compound also inhibited HUVEC tube formation (Fig. 3B-D). Practically, tubes and branches stopped forming at 267 mg/L (500 μM) (Fig. 3C-D). Additionally, wound healing assays (Fig. 3E-F) revealed that HUVEC cell migration was substantially inhibited by TTZ. All tested effects of TTZ on HUVEC activities (proliferation, tubulogenesis, and migration) were dose-dependent, while statistical analyses indicated that the observed inhibition was significant even at the lowest tested dose (10.7 mg/L or 20 μM, $p < 0.005$).

Discussion

TTZ affects blood vessel formation, possibly through interaction with the BMP signaling pathway

Throughout decades of research on the food dye tartrazine with numerous investigations, this is the first study reporting that TTZ has vascular disrupting properties. We have shown that the dye can impair angiogenesis by disrupting blood vessel formation in developing zebrafish embryos and by inhibiting human primary ECs from proliferating, migrating, and forming tubular networks using an array of simple experiments. Combining tests using zebrafish embryos and mammalian ECs has previously been used as a cost-effective tool in studying angiogenic factors[29–32], here it helped reveal that the vascular disrupting effect of TTZ is universally conserved across multiple species, possibly pan-vertebrate.

In zebrafish, the formation of both the DLAV and the ISV are mainly regulated by the vascular endothelial growth factor (VEGF) signaling pathway (Vegf-A and Vegf-C)[33]. On the other hand, SIV development is dependent on both the bone morphogenic protein (BMP) and the VEGF pathway[33–36], while brain vascularization[37]and EC migration during CVP formation require mainly BMP signaling[34,38]. Thus, the effects of TTZ observed on zebrafish embryos predominantly on SIV, CVP, and cranial vessels, while DLAV and ISV are unaffected (Table 1), strongly suggest that this compound may affect vascularization by interacting with the BMP pathway, rather than the more popular VEGF-A signaling.

Rho GTPases as the main suspect

The action of BMP signaling in zebrafish blood vessel formation is closely linked with activities of the Rho GTPase family, which regulates cell migration by controlling the formation of cytoskeleton processes[32,39–41]. Wakayama et al. (2015) reported BMP action mediated by Cdc42, a Rho GTPase family member whose inhibition leads to a fused CVP network without clear spaces between capillaries[34]. Cdc42 has been shown to control EC directional sensing through projection of filopodia[39], whose disruption could lead to inhibition of the wound healing process[31]. It also regulates microtubule alignment and EC elongation[29,42]; hence its loss-of-function would facilitate EC lumen collapse, which, together with the impaired migration, could further contribute to tubulogenic failure. Another family member is RAC1, which plays multiple roles in endothelial cell activities and vascular development[43], contributing to EC survival[41], migration[39], and zebrafish brain vascular pruning[44]. Interestingly, human RAC1 protein was predicted to interact with TTZ by the TargetNet platform (Supplementary Table S1). The third family member RHOA also controls EC directional migration[45,46]and

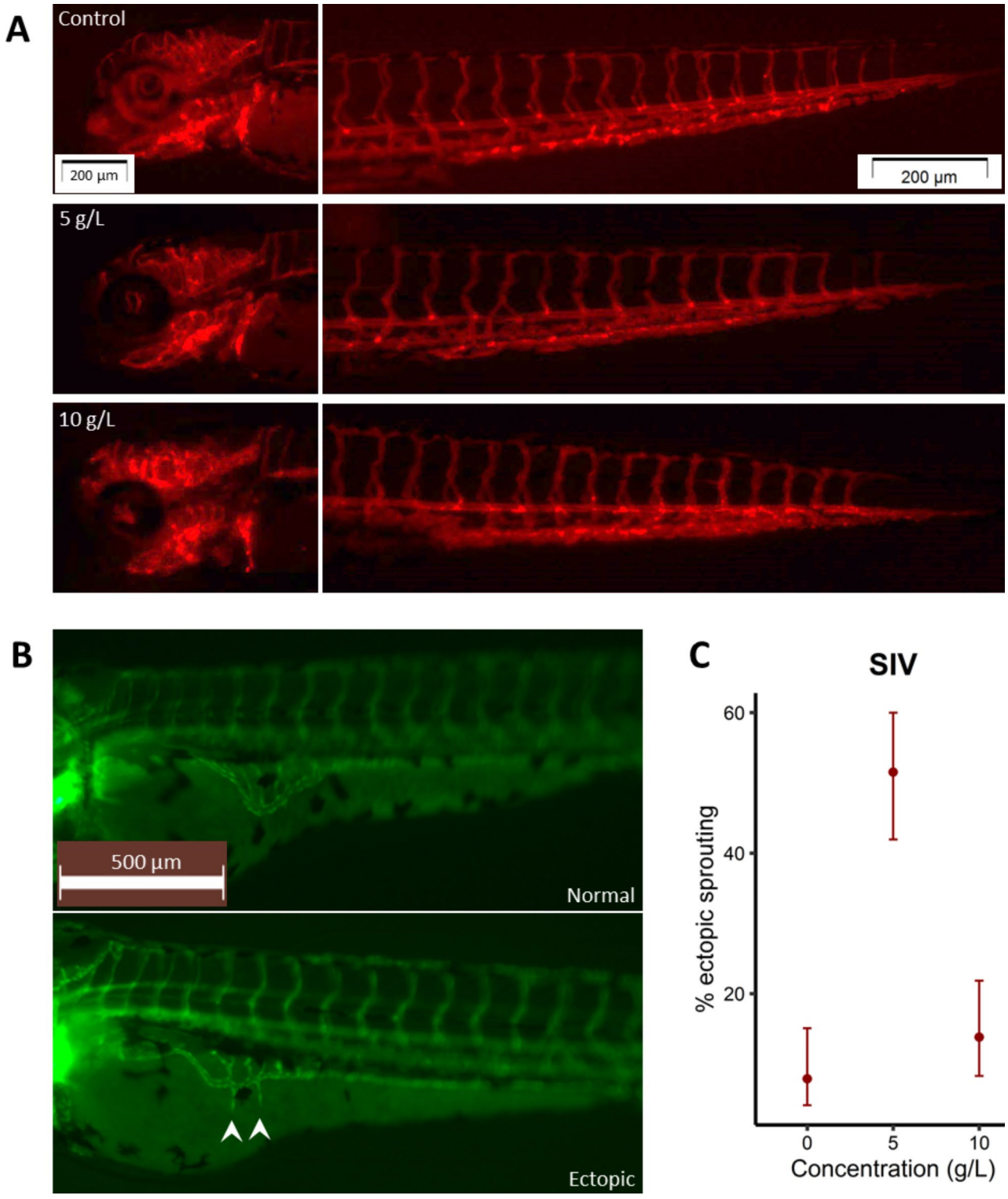

Fig. 2. Vascularization analysis on transgenic fluorescent zebrafish lines. *(A) Cephalic vessel and caudal venous plexus vascularization are dose-dependently affected by TTZ; Vessels appear to be increasingly merged/clumped together instead of forming distinguishable fine structures; Notably , the three-longitudinal-vessel pattern in the CVP gradually merged (5 g/L) into a two-vessel pattern (10 g/L). (B) Normal (basket-shaped) and ectopically sprouted (arrowheads) SIV formation. (C) Percentages of ectopic sprouting at different TTZ concentrations.*

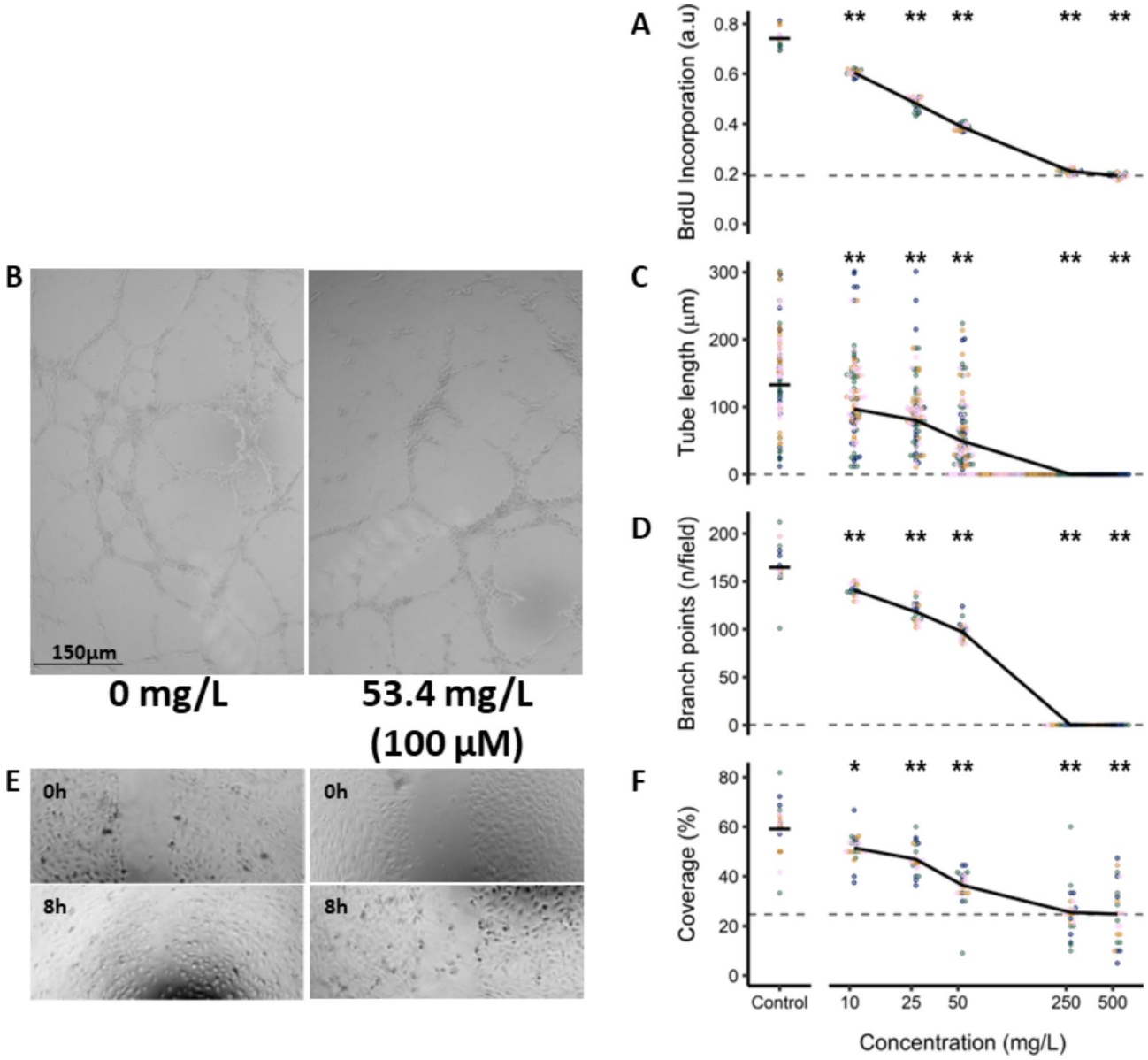

Fig. 3. TTZ inhibits HUVEC angiogenic activities in a dose-dependent manner. *(A) TTZ inhibits HUVEC proliferation in the BrdU assay. (B-D) TTZ treatment leads to reduced tube extension and branching during tubulogenesis. (E-F) Wound healing assay showing anti-migratory effect of TTZ. Concentrations are log-scaled , and biological replicates are represented using different colors. Asterisks denote statistical significance: *$p < 0.01$; **$p < 0.001$.*

Zebrafish embryos						HUVECs		
Hemorrhage	**SIV**	**CVP**	**Cephalic vessels**	**DLAV**	**ISV**	**Proliferation**	**Migration**	**Tube formation**
Yes	Ectopic sprouting	Undeveloped network		N/A	N/A	Inhibited	Partially impaired	Inhibited

Table 1. Summary of TTZ's angiogenic effects on zebrafish embryos and HUVECs.

tube collapse[47]. A recent paper also suggested key roles of Rac1 and RhoA in the zebrafish BMP angiogenic pathways[32].

While no direct relationship between Rho GTPases and zebrafish SIV ectopic formation was found in the literature, indirect evidence exists, such as via elements of the lipid metabolism pathways[48]– for example, treatment with the cholesterol-reducing drug atorvastatin led to both SIV ectopic sprouting in zebrafish[49]and an increase of GTP loading of all Rho GTPases in HUVECs[50]. Another indirect evidence is the family of cerebral cavernous malformations genes (CCMs), whose pathways regulate Rho GTPases in ECs[51,52]. Loss-of-function

of CCM orthologs in zebrafish causes ectopic SIV sprouting and fused CVP[53,54], and induce diverse effects on human EC proliferation, migration, and tube formation depending on both EC type (HUVEC or brain EC)[55]and the CCM gene involved (CCM1, 2, or 3)[52,55,56]. Noteworthily, variants of the CCM genes are also responsible for many cases of brain hemorrhage[51,52,54,55]. On the other hand, TTZ treatment in vitro (in human hepatoma cells)[57]and in vivo (in CD1 mice)[58]led to changes in expression of several genes participating in RhoA GTPase signaling pathways: JUN, MAPK8 (JNK1)[59,60], MAP2K6 (MKK6)[60,61], and PIK3C2A[62,63]. Re-analyzing the transcriptomic changes following TTZ treatment in human hepatoma cells[57] further revealed regulation of genes related to Rho GTPase-activated formins, Rho GTPase effectors, and signaling by Rho GTPases.

In addition to RAC1, TargetNet also suggests other Rho GTPase signaling-related proteins capable of interacting with TTZ (Supplementary Table S1). They include HDAC4, which could antagonize KLF2-induced RHOA activation and promote random cell motility[64], or PTGS2, which is downstream of RHOA/RAC1/CDC42 in endothelia[65]and fibroblasts[66]. These observations further support the possible interactions between TTZ and Rho family GTPase activities.

Other possible interactions

Aside from the possibility of interacting with Rho GTPases, TTZ may act via multiple pathways, as the compound was reported to have both estrogenic[67]and oxidative[68]potentials – both of which are related to the angiogenesis pathway[69,70]and GTPase activities[69,71]. Indeed, our ZET results corroborate the involvement of multiple toxicological mechanisms beside vascularization (Fig. 1E). Still, further investigations are required to comprehensively understand TTZ's vascular disruptive effect and other reported bioactivities.

In this study, one striking observation is the difference in effective concentrations of TTZ in the two systems: in zebrafish larvae, substantial effects on vascular development are seen at 5–10 g/L, while HUVECs display decreased activities at 10.7 mg/L (20 μM) and completely malfunction at 267 mg/L (0.5 mM). It is important to note here that TTZ is a hydrophilic compound, dissolved in water, thus the chorionic or other cell membranes may protect the larvae from its influence, while HUVECs are directly exposed to it. In addition, the detoxifying organs (i.e., liver and kidney) may be involved, as well as the affinity of serum albumin to TTZ[1,3].

While the exact mechanism of TTZ's vascular disrupting feature is yet to be determined, our discovery may already offer several applied implications. Tartrazine is a common, yet controversial food additive that is alleged to cause multiple adverse effects, including genotoxicity, oxidative stress, and behavioral alteration[1,2,4,6–10,12,13,15,67,68,72]. However, no report has mentioned its effect on the vascular system or endothelium, even though hemorrhagic sites can be observed in previous studies[73,74]. This newly found anti-angiogenic feature may shed light on the presence of purpura experienced by some individuals[14,15], which has long been attributed to an immune response[12,14,15]. Our discovery may offer a straightforward explanation for these cases: ECs exposed to TTZ will malfunction, leading to vascular defect and internal hemorrhage. The reason why TTZ-induced purpura rarely happens could be explained by either a high individual sensitivity to its effects, or the fact that more often TTZ does not reach an effective concentration within the blood vessels: less than 5% of TTZ is absorbed after oral uptake, then predominantly excreted via urine[1], whereas a large proportion of the chemical may have been metabolized by the gut microflora[2]. Regarding the emerging recognition of gut microbiota in food allergy cases[75], it is reasonable to hypothesize a link between the microbiota and TTZ hypersensitivity. Another health aspect of TTZ, perhaps the most controversial, is its alleged link with ADHD in children[3,11]. If the link is valid, one possible explanation is the indirect impact of TTZ consumption on brain function via blood vessel function[76].

The discovered vascular effect of TTZ may also contribute to the safety regulation of the colorant, especially in food and medical applications. Methods have been published using TTZ as a photoabsorber for light-based hydrogel bioprinting to be used as scaffolds for endothelial cell seeding and tissue engineering[77–80]. Even though there is a washing step between scaffold printing and cell seeding, the risk of TTZ residues affecting HUVECs behaviors should not be neglected. More importantly, TTZ as a common drug excipient and food additive may interfere with proangiogenic therapy such as in coronary and peripheral artery disease or ischemic stroke[81]. On the other hand, this TTZ's function could lead to potential applications in treating conditions such as cancer or macular degeneration[28,81,82]. Given the extensive information on TTZ toxicity and safety, its status as a food additive, and its abundant and cheap production, the chemical may serve as an affordable alternative for existing anti-angiogenic drugs.

Data availability

All scripts and raw data are available upon request from the authors - namely M.M., D.D.T., and N.B.N.

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References

1. EFSA. Scientific opinion on the re-evaluation tartrazine (E 102). *EFSA J.* **7**, 1331. https://doi.org/10.2903/j.efsa.2009.1331 (2009).
2. EFSA. Statement on Allura Red AC and other sulphonated mono azo dyes authorised as food and feed additives. *EFSA J.* **11**, 3234. https://doi.org/10.2903/j.efsa.2013.3234 (2013).
3. JECFA. *Safety Evaluation of Certain food Additives: Prepared by the Eighty-second Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*v, 493 (p. (World Health Organization, 2017).
4. Khayyat, L., Essawy, A., Sorour, J. & Soffar, A. Tartrazine induces structural and functional aberrations and genotoxic effects in vivo. *PeerJ* **5**, e3041. https://doi.org/10.7717/peerj.3041 (2017).
5. Hashem, M. M., Abd-Elhakim, Y. M., Abo-El-Sooud, K. & Eleiwa, M. M. E. Embryotoxic and Teratogenic effects of Tartrazine in rats. *Toxicol. Res.* **35**, 75–81. https://doi.org/10.5487/tr.2019.35.1.075 (2019).

6. Bhatt, D., Vyas, K., Singh, S., John, P. J. & Soni, I. Tartrazine induced neurobiochemical alterations in rat brain sub-regions. *Food Chem. Toxicol.* **113**, 322–327. https://doi.org/10.1016/j.fct.2018.02.011 (2018).
7. Abd-Elhakim, Y. M. et al. Comparative haemato-immunotoxic impacts of long-term exposure to tartrazine and chlorophyll in rats. *Int. Immunopharmacol.* **63**, 145–154. https://doi.org/10.1016/j.intimp.2018.08.002 (2018).
8. Gao, Y. et al. Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved. *J. Food Sci.* **76**, T125–129. https://doi.org/10.1111/j.1750-3841.2011.02267.x (2011).
9. Himri, I. et al. A 90-day oral toxicity study of Tartrazine, a synthetic food dye, in Wistar rats. *Int. J. Pharm. Pharm. Sci.* **3**, 159–169 (2011).
10. Simon, R. A. Adverse reactions to food additives. *Curr. Allergy Asthma Rep.* **3**, 62–66. https://doi.org/10.1007/s11882-003-0014-9 (2003).
11. McCann, D. et al. Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. *Lancet* **370**, 1560–1567. https://doi.org/10.1016/S0140-6736(07)61306-3 (2007). https://doi.org/http://dx.
12. Miller, K. Sensitivity to tartrazine. *Br. Med. J. (Clin Res. Ed)*. **285**, 1597–1598 (1982).
13. Gultekin, F. & Doguc, D. K. Allergic and immunologic reactions to food additives. *Clin. Rev. Allergy Immunol.* **45**, 6–29. https://doi.org/10.1007/s12016-012-8300-8 (2013).
14. Criep, L. H. Allergic vascular purpura. *J. Allergy Clin. Immunol.* **48**, 7–12. https://doi.org/10.1016/0091-6749(71)90049-2 (1971). https://doi.org/http://dx.
15. Parodi, G., Parodi, A. & Rebora, A. Purpuric Vasculitis due to Tartrazine. *Dermatology* **171**, 62–63 (1985).
16. Babayigit, A. et al. Assessing the toxicity of Pb- and Sn-based perovskite solar cells in model organism Danio rerio. *Sci. Rep.* **6**, 18721. https://doi.org/10.1038/srep18721 (2016).
17. European Council. (2010).
18. Serbedzija, G. N., Flynn, E. & Willett, C. E. Zebrafish angiogenesis: a new model for drug screening. *Angiogenesis* **3**, 353–359. https://doi.org/10.1023/a:1026598300052 (1999).
19. OECD. *Test No. 236: Fish Embryo Acute Toxicity (FET) Test* (OECD Publishing, 2013).
20. Lawson, N. D. & Weinstein, B. M. In vivo imaging of embryonic vascular development using transgenic zebrafish. *Dev. Biol.* **248**, 307–318. https://doi.org/10.1006/dbio.2002.0711 (2002).
21. Fujita, M. et al. Assembly and patterning of the vascular network of the vertebrate hindbrain. *Development* **138**, 1705–1715. https://doi.org/10.1242/dev.058776 (2011).
22. Jaffe, E. A., Nachman, R. L., Becker, C. G. & Minick, C. R. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J. Clin. Invest.* **52**, 2745–2756. https://doi.org/10.1172/JCI107470 (1973).
23. Fontaine, M. et al. Extracellular vesicles mediate communication between endothelial and vascular smooth muscle cells. *Int. J. Mol. Sci.* **23** https://doi.org/10.3390/ijms23010331 (2021).
24. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. & Schilling, T. F. Stages of embryonic development of the zebrafish. *Dev. Dyn.* **203**, 253–310. https://doi.org/10.1002/aja.1002030302 (1995).
25. Ritz, C., Baty, F., Streibig, J. C. & Gerhard, D. Dose-response analysis using R. *PLoS One*. **10**, e0146021. https://doi.org/10.1371/journal.pone.0146021 (2015).
26. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear mixed-effects models Usinglme4. *J. Stat. Softw.* **67** https://doi.org/10.18637/jss.v067.i01 (2015).
27. Knowles, J. & Frederick, C. *merTools: Tools for Analyzing Mixed Effect Regression Models*, < (2020). https://CRAN.R-project.org/package=merTools
28. Chimote, G. et al. Comparison of effects of anti-angiogenic agents in the zebrafish efficacy-toxicity model for translational anti-angiogenic drug discovery. *Drug Des. Devel Ther.* **8**, 1107–1123. https://doi.org/10.2147/DDDT.S55621 (2014).
29. Hetheridge, C. et al. The formin FMNL3 is a cytoskeletal regulator of angiogenesis. *J. Cell. Sci.* **125**, 1420–1428. https://doi.org/10.1242/jcs.091066 (2012).
30. McCollum, C. W. et al. Identification of vascular disruptor compounds by analysis in zebrafish embryos and mouse embryonic endothelial cells. *Reprod. Toxicol.* **70**, 60–69. https://doi.org/10.1016/j.reprotox.2016.11.005 (2017).
31. Lavina, B. et al. Defective endothelial cell migration in the absence of Cdc42 leads to capillary-venous malformations. *Development* **145** https://doi.org/10.1242/dev.161182 (2018).
32. Veloso, A. et al. The cytoskeleton adaptor protein Sorbs1 controls the development of lymphatic and venous vessels in zebrafish. *BMC Biol.* **22**, 51. https://doi.org/10.1186/s12915-024-01850-z (2024).
33. Nakajima, H., Chiba, A., Fukumoto, M., Morooka, N. & Mochizuki, N. Zebrafish Vascular Development: General and tissue-specific regulation. *J. Lipid Atheroscler.* **10**, 145–159. https://doi.org/10.12997/jla.2021.10.2.145 (2021).
34. Wakayama, Y., Fukuhara, S., Ando, K., Matsuda, M. & Mochizuki, N. Cdc42 mediates Bmp-induced sprouting angiogenesis through Fmnl3-driven assembly of endothelial filopodia in zebrafish. *Dev. Cell.* **32**, 109–122. https://doi.org/10.1016/j.devcel.2014.11.024 (2015).
35. Goi, M. & Childs, S. J. Patterning mechanisms of the sub-intestinal venous plexus in zebrafish. *Dev. Biol.* **409**, 114–128. https://doi.org/10.1016/j.ydbio.2015.10.017 (2016).
36. Koenig, A. L. et al. Vegfa signaling promotes zebrafish intestinal vasculature development through endothelial cell migration from the posterior cardinal vein. *Dev. Biol.* **411**, 115–127. https://doi.org/10.1016/j.ydbio.2016.01.002 (2016).
37. Rochon, E. R., Menon, P. G. & Roman, B. L. Alk1 controls arterial endothelial cell migration in lumenized vessels. *Development* **143**, 2593–2602. https://doi.org/10.1242/dev.135392 (2016).
38. Phng, L. K., Stanchi, F. & Gerhardt, H. Filopodia are dispensable for endothelial tip cell guidance. *Development* **140**, 4031–4040. https://doi.org/10.1242/dev.097352 (2013).
39. Lamalice, L., Le Boeuf, F. & Huot, J. Endothelial cell migration during angiogenesis. *Circ. Res.* **100**, 782–794. https://doi.org/10.1161/01.RES.0000259593.07661.1e (2007).
40. van der Meel, R. et al. The VEGF/Rho GTPase signalling pathway: a promising target for anti-angiogenic/anti-invasion therapy. *Drug Discov Today.* **16**, 219–228. https://doi.org/10.1016/j.drudis.2011.01.005 (2011).
41. Boueid, M. J., Mikdache, A., Lesport, E., Degerny, C. & Tawk, M. Rho GTPases signaling in zebrafish development and disease. *Cells* **9** https://doi.org/10.3390/cells9122634 (2020).
42. Phng, L. K. et al. Formin-mediated actin polymerization at endothelial junctions is required for vessel lumen formation and stabilization. *Dev. Cell.* **32**, 123–132. https://doi.org/10.1016/j.devcel.2014.11.017 (2015).
43. Tan, W. et al. An essential role for Rac1 in endothelial cell function and vascular development. *FASEB J.* **22**, 1829–1838. https://doi.org/10.1096/fj.07-096438 (2008).
44. Chen, Q. et al. Haemodynamics-driven developmental pruning of brain vasculature in zebrafish. *PLoS Biol.* **10**, e1001374. https://doi.org/10.1371/journal.pbio.1001374 (2012).
45. Vega, F. M., Fruhwirth, G., Ng, T. & Ridley, A. J. RhoA and RhoC have distinct roles in migration and invasion by acting through different targets. *J. Cell. Biol.* **193**, 655–665. https://doi.org/10.1083/jcb.201011038 (2011).
46. Wu, C. et al. Rab13-dependent trafficking of RhoA is required for directional migration and angiogenesis. *J. Biol. Chem.* **286**, 23511–23520. https://doi.org/10.1074/jbc.M111.245209 (2011).
47. Bayless, K. J. & Davis, G. E. Microtubule depolymerization rapidly collapses capillary tube networks in vitro and angiogenic vessels in vivo through the small GTPase rho. *J. Biol. Chem.* **279**, 11686–11695. https://doi.org/10.1074/jbc.M308373200 (2004).

48. Kalaivani, V. & Jaleel, A. Apolipoprotein(a), an enigmatic anti-angiogenic glycoprotein in human plasma: a curse or cure? *Pharmacol. Res.* **158**, 104858. https://doi.org/10.1016/j.phrs.2020.104858 (2020).
49. Avraham-Davidi, I. et al. ApoB-containing lipoproteins regulate angiogenesis by modulating expression of VEGF receptor 1. *Nat. Med.* **18**, 967–973. https://doi.org/10.1038/nm.2759 (2012).
50. Xiao, H., Qin, X., Ping, D. & Zuo, K. Inhibition of rho and Rac geranylgeranylation by atorvastatin is critical for preservation of endothelial junction integrity. *PLoS One*. **8**, e59233. https://doi.org/10.1371/journal.pone.0059233 (2013).
51. Whitehead, K. J. et al. The cerebral cavernous malformation signaling pathway promotes vascular integrity via rho GTPases. *Nat. Med.* **15**, 177–184. https://doi.org/10.1038/nm.1911 (2009).
52. Richardson, B. T., Dibble, C. F., Borikova, A. L. & Johnson, G. L. Cerebral cavernous malformation is a vascular disease associated with activated RhoA signaling. *Biol. Chem.* **394**, 35–42. https://doi.org/10.1515/hsz-2012-0243 (2013).
53. Hogan, B. M., Bussmann, J., Wolburg, H. & Schulte-Merker, S. ccm1 cell autonomously regulates endothelial cellular morphogenesis and vascular tubulogenesis in zebrafish. *Hum. Mol. Genet.* **17**, 2424–2432. https://doi.org/10.1093/hmg/ddn142 (2008).
54. Voss, K. et al. Functional analyses of human and zebrafish 18-amino acid in-frame deletion pave the way for domain mapping of the cerebral cavernous malformation 3 protein. *Hum. Mutat.* **30**, 1003–1011. https://doi.org/10.1002/humu.20996 (2009).
55. Zhu, Y. et al. Differential angiogenesis function of CCM2 and CCM3 in cerebral cavernous malformations. *Neurosurg. Focus*. **29**, E1. https://doi.org/10.3171/2010.5.FOCUS1090 (2010).
56. Zhu, Y. et al. In vitro characterization of the angiogenic phenotype and genotype of the endothelia derived from sporadic cerebral cavernous malformations. *Neurosurgery* **69**, 722–731. https://doi.org/10.1227/NEU.0b013e318219569f (2011). discussion 731 – 722.
57. Gijbels, E., Devisscher, L. & Vinken, M. Testing in vitro tools for the prediction of cholestatic liver injury induced by non-pharmaceutical chemicals. *Food Chem. Toxicol.* **152**, 112165. https://doi.org/10.1016/j.fct.2021.112165 (2021).
58. Raposa, B. et al. Food additives: Sodium benzoate, potassium sorbate, azorubine, and tartrazine modify the expression of NFkappaB, GADD45alpha, and MAPK8 genes. *Physiol. Int.* **103**, 334–343. https://doi.org/10.1556/2060.103.2016.3.6 (2016).
59. Teramoto, H. et al. Signaling from the small GTP-binding proteins Rac1 and Cdc42 to the c-Jun N-terminal kinase/stress-activated protein kinase pathway. A role for mixed lineage kinase 3/protein-tyrosine kinase 1, a novel member of the mixed lineage kinase family. *J. Biol. Chem.* **271**, 27225–27228. https://doi.org/10.1074/jbc.271.44.27225 (1996).
60. Canovas, B. & Nebreda, A. R. Diversity and versatility of p38 kinase signalling in health and disease. *Nat. Rev. Mol. Cell. Biol.* **22**, 346–366. https://doi.org/10.1038/s41580-020-00322-w (2021).
61. Anton, R., Bauer, S. M., Keck, P. R., Laufer, S. & Rothbauer, U. A p38 substrate-specific MK2-EGFP translocation assay for identification and validation of new p38 inhibitors in living cells: a comprising alternative for acquisition of cellular p38 inhibition data. *PLoS One*. **9**, e95641. https://doi.org/10.1371/journal.pone.0095641 (2014).
62. Yoshioka, K. et al. Endothelial PI3K-C2alpha, a class II PI3K, has an essential role in angiogenesis and vascular barrier function. *Nat. Med.* **18**, 1560–1569. https://doi.org/10.1038/nm.2928 (2012).
63. Sunitha, P. et al. Temporal VEGFA responsive genes in HUVECs: gene signatures and potential ligands/receptors fine-tuning angiogenesis. *J. Cell. Commun. Signal.* **13**, 561–571. https://doi.org/10.1007/s12079-019-00541-7 (2019).
64. Cernotta, N., Clocchiatti, A., Florean, C. & Brancolini, C. Ubiquitin-dependent degradation of HDAC4, a new regulator of random cell motility. *Mol. Biol. Cell.* **22**, 278–289. https://doi.org/10.1091/mbc.E10-07-0616 (2011).
65. Schmeck, B. et al. Rho protein inhibition blocks cyclooxygenase-2 expression by proinflammatory mediators in endothelial cells. *Inflammation* **27**, 89–95. https://doi.org/10.1023/a:1023278600596 (2003).
66. Chang, Y. W. et al. Differential regulation of cyclooxygenase 2 expression by small GTPases ras, Rac1, and RhoA. *J. Cell. Biochem.* **96**, 314–329. https://doi.org/10.1002/jcb.20568 (2005).
67. Axon, A. et al. Tartrazine and sunset yellow are xenoestrogens in a new screening assay to identify modulators of human oestrogen receptor transcriptional activity. *Toxicology* **298**, 40–51. https://doi.org/10.1016/j.tox.2012.04.014 (2012).
68. Albasher, G. et al. Perinatal exposure to Tartrazine Triggers Oxidative Stress and neurobehavioral alterations in mice offspring. *Antioxid. (Basel)*. 9. https://doi.org/10.3390/antiox9010053 (2020).
69. Moldovan, L., Mythreye, K., Goldschmidt-Clermont, P. J. & Satterwhite, L. L. Reactive oxygen species in vascular endothelial cell motility. Roles of NAD(P)H oxidase and Rac1. *Cardiovasc. Res.* **71**, 236–246. https://doi.org/10.1016/j.cardiores.2006.05.003 (2006).
70. Fatima, L. A. et al. Estrogen receptor 1 (ESR1) regulates VEGFA in adipose tissue. *Sci. Rep.* **7**, 16716. https://doi.org/10.1038/s41598-017-16686-7 (2017).
71. Briz, V. & Baudry, M. Estrogen Regulates Protein Synthesis and actin polymerization in hippocampal neurons through different molecular mechanisms. *Front. Endocrinol. (Lausanne)*. **5**, 22. https://doi.org/10.3389/fendo.2014.00022 (2014).
72. Amin, K., Hameid, I. I., Abd Elsttar, A. & H. A. & Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. *Food Chem. Toxicol.* **48**, 2994–2999 (2010).
73. Jiang, L. L. et al. Toxicity Assessment of 4 Azo Dyes in zebrafish embryos. *Int. J. Toxicol.* **39**, 115–123. https://doi.org/10.1177/1091581819898396 (2020).
74. Gupta, R. et al. Toxic effects of Food colorants Erythrosine and Tartrazine on zebrafish embryo development. *Curr. Res. Nutr. Food Sci. J.* **7**, 876–885. https://doi.org/10.12944/crnfsj.7.3.26 (2019).
75. Shu, S. A. et al. Microbiota and Food Allergy. *Clin. Rev. Allergy Immunol.* **57**, 83–97. https://doi.org/10.1007/s12016-018-8723-y (2019).
76. Cannon Homaei, S. et al. ADHD symptoms in neurometabolic diseases: underlying mechanisms and clinical implications. *Neurosci. Biobehav Rev.* **132**, 838–856. https://doi.org/10.1016/j.neubiorev.2021.11.012 (2022).
77. Benjamin, A. D. et al. Light-based 3D printing of hydrogels with high-resolution channels. *Biomedical Phys. Eng. Express*. **5** https://doi.org/10.1088/2057-1976/aad667 (2019).
78. Grigoryan, B. et al. Multivascular networks and functional intravascular topologies within biocompatible hydrogels. *Science* **364**, 458–464. https://doi.org/10.1126/science.aav9750 (2019).
79. Kinstlinger, I. S. et al. Perfusion and endothelialization of engineered tissues with patterned vascular networks. *Nat. Protoc.* **16**, 3089–3113. https://doi.org/10.1038/s41596-021-00533-1 (2021).
80. Yu, K. et al. Printability during projection-based 3D bioprinting. *Bioact Mater.* **11**, 254–267. https://doi.org/10.1016/j.bioactmat.2021.09.021 (2022).
81. Rust, R., Gantner, C. & Schwab, M. E. Pro- and antiangiogenic therapies: current status and clinical implications. *FASEB J.* **33**, 34–48. https://doi.org/10.1096/fj.201800640RR (2019).
82. Fallah, A. et al. Therapeutic targeting of angiogenesis molecular pathways in angiogenesis-dependent diseases. *Biomed. Pharmacother.* **110**, 775–785. https://doi.org/10.1016/j.biopha.2018.12.022 (2019).

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Author contributions

D.D.T. and M.M. conceived the study; D.D.T. carried out the zebrafish experiments; N.B.N. performed data analysis and visualization; C.P., A.C., S.H., and I.S. carried out the HUVEC experiments; D.D.T., M.M., N.B.N., S.H., and I.S. contributed to writing the paper.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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