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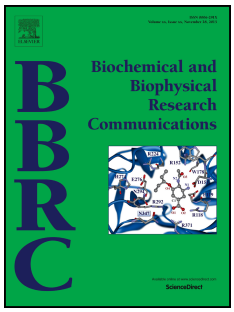

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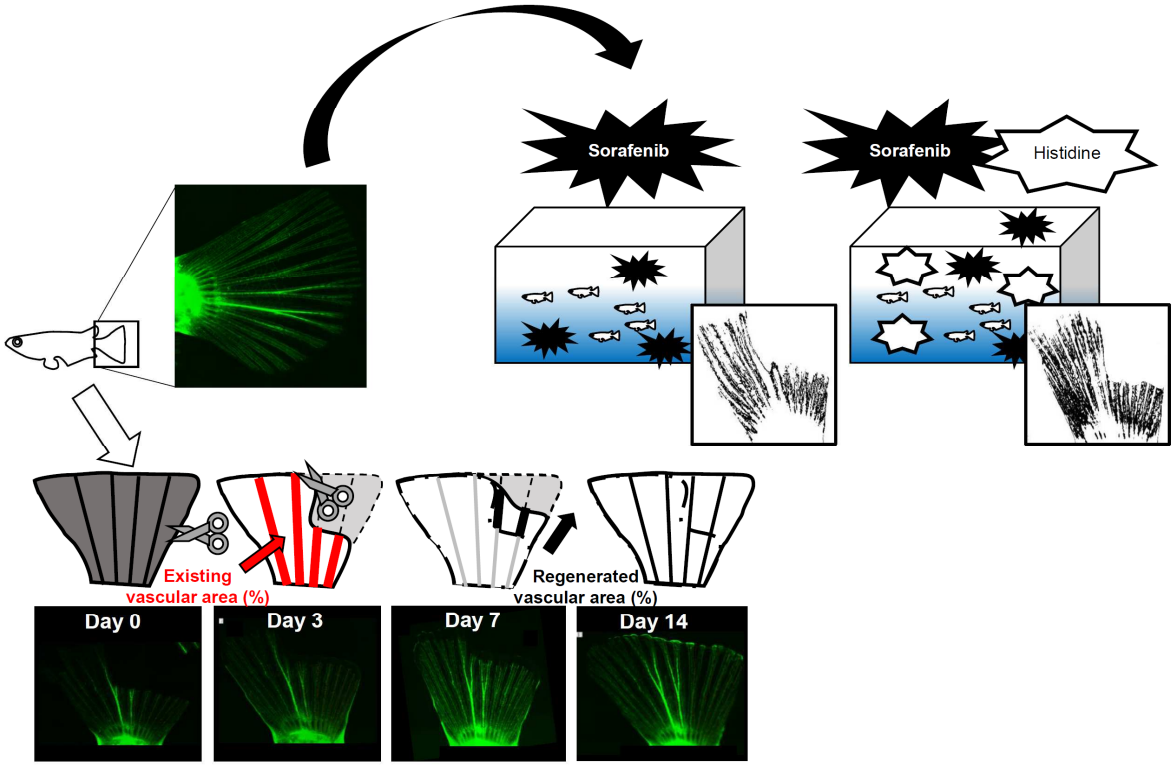
Graphical Abstract



Effect of Histidine on Sorafenib-induced Vascular Damage: Analysis using Novel Medaka Fish Model

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Abstract

Background

Sorafenib (SFN) is an anti-angiogenic chemotherapeutic that prolongs survival of patients with hepatocellular carcinoma (HCC); its side effects, including vascular damages such as hand-foot syndrome (HFS), are a major cause of therapy discontinuation. We previously reported that maintenance of peripheral blood flow by intake of dried bonito broth (DBB) significantly prevented HFS and prolonged the administration period. The amino acids contained in DBB probably contribute to its effects, but the mechanism has not been clarified. We hypothesized that histidine, the largest component among the amino acids contained in DBB, has effects on SFN-induced vascular damage, and evaluated this possibility using a novel medaka fish model.

Methods

The *fli*::GFP transgenic medaka fish model has a fluorescently visible systemic vasculature. We fed the fish with SFN with and without histidine to compare blood flow and vascular structure among the differently fed models. The vascular cross-sectional area of each fish was measured to determine vascular diameter changes.

Results

Our results demonstrated that SFN-fed medaka developed a narrower vascular diameter. In addition, this narrowing was counteracted by addition of histidine to the medaka diet. We

observed no positive effect of histidine on regeneration of cut vessels or on cell growth of endothelial cells and HCC cell lines.

Conclusion

We proved the efficacy of the medaka model to assess vascular changes after administration of specific chemicals. And our results suggest that SFN causes vascular damage by narrowing peripheral vessel diameter, and that histidine effectively counteracts these changes to maintain blood flow.

Keywords: sorafenib; vascular damage; hand-foot syndrome; histidine; medaka

Introduction

Sorafenib (SFN) is expected to prolong survival of advanced hepatocellular carcinoma (HCC) patients [1-3] and, therefore, management of its side effects is essential. The hand-foot syndrome (HFS) [4-6] occurs in 30%–50% of patients [6-8] and seriously diminishes the patient's quality of life. We have shown that SFN-induces HFS by decreasing peripheral blood flow, and that dried-bonito broth (DBB) consumption helps prevent HFS [9]. DBB has been shown to increase blood flow and improve peripheral blood flow in humans [4, 10] and cerebral blood flow in mice [11] and rats [12]. This increase in peripheral blood flow results in an anti-oxidative effect and mood improvement in humans [10, 13]. In addition, histidine, the largest component among the amino acids in bonito extract [11], showed an antihypertensive effect in spontaneously hypertensive rats (SHR) due to attenuation of sympathetic output via the central H3 receptor when administered orally [14]. Thus, the mechanism of blood flow improvement by DBB seems to depend on the effects of histidine.

In this study, we visualized the effects of histidine on the fluorescent systemic vascular structure of the medaka model fish treated with SFN.

Materials and Methods

Animal model

All animal experiments were conducted in full compliance with the regulations of the Institutional Animal Care and Use Committee at Niigata University (Niigata, Japan), which approved the study protocol. We used *fli*::GFP transgenic medaka fish generated by injecting the *fli* gene promoter::green fluorescent protein cassette [15] into the Kyoto-Cab strain. Medaka fish approximately 6–7 months old were used in the experiments. The fish were fed Hikari Crest food (Kyorin, Hyogo, Japan) and were kept in 2 L of tap water in plastic tanks illuminated with fluorescent light from 8 a.m. to 8 p.m. The water temperature was maintained at 25°C ± 1°C.

Cells

Human umbilical vein endothelial cells, HUVECs or HUVEC-Umbilical Vein Endo Cells (EGM-2, cryo amp, C2517A; Lonza Walkersville, Basel, Switzerland) were cultured in EGM™-2BulletKit™ (Lonza Walkersville, Basel, Switzerland). Human liver cancer tumor cell lines (HLE, HepG2) were cultured in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Analysis of vasculature in medaka model

The effect of SFN on the vascular cross-sectional area was examined *in vivo* by using transgenic medaka (*fli*::GFP medaka) as an animal model with a fluorescently visible vascular system structure. In this medaka model, a GFP-expressing cassette was inserted under the control of the *fli* promoter, which drives gene expression in all blood vessels in fish [15]. To assess the vascular cross-sectional area in medaka fed with SFN and histidine, the lower half of the medaka's tail fin was cut with scissors. Vessels in the remaining fin were considered existing vessels, and vessels in the regenerating fin were considered regenerating vessels. Changes in the existing and regenerating vessel areas were monitored at appropriate time points using a fluorescent stereomicroscope (BZ-9000; Keyence Corporation, Osaka, Japan). The captured images were quantitatively analyzed using the ImageJ software (version 1.6.0_20, National Institutes of Health), as previously reported [16].

Preparation of SFN and histidine

SFN (Sorafenib Free Base, S-8599; LCL, Woburn, MA, USA) was dissolved in dimethyl sulfoxide (to 200 mg/ml and then into water) at concentrations of 0, 75, 150, 300, 600, and 2400 μg/L in the tank. Histidine (L-Histidine Monohydrochloride Monohydrate, Animal-Free, 13017-42; Nacalai Tesque, Kyoto, Japan) was dissolved in water at concentrations of 0, 12, and 60 mg/L. The following concentrations of SFN were used: human SFN dose (10 mg/kg);

and an SFN concentration in the tank of 0–2500 μg/L (equivalent to 0–2.5 mg/kg).

MTT cell growth assay

HUVEC and human liver cancer tumor cell lines (HLE, HepG2) were plated in 96-well tissue culture plates (Microplate 96 Well with Lid; Iwaki Asahi Glass, Tokyo, Japan) at a concentration of 1,000 cells per well in 100 μl volumes, and incubated at 37°C in a humidified incubator containing 5% CO_2. The cells were either treated with SFN and histidine or with mock treatment. MTT reagents were added to the cells at the indicated times after the treatment, followed by counting them using a Premix WST-1 Cell Proliferation Assay System (Takara, Kyoto, Japan) and a microplate reader.

Statistical analysis

The data obtained were analyzed either by using the Student's *t*-test, or a one- or two-way factor repeated-measure analysis of variance (ANOVA) followed by a Bonferroni's multiple comparison test.

Results

Effect of SFN on vascular cross-sectional area in medaka

We examined the effect of SFN on the vascular cross-sectional area *in vivo* in a transgenic medaka model. The advantage of this animal model is that the systemic vascular structure can be fluorescently seen in the live animal to assess vascular cross-sectional area and blood flow. After injecting fish with SFN, we excised the lower half of the fish tail fin with scissors (**Fig. 1** and **Materials and Methods**), and monitored changes in existing and regenerating vessel areas at appropriate time points. In order to assess the effect of the different treatments, we looked at the cross-sectional area in the remaining part of the fin and compared it to the regenerating fin a number of days after the excision, assessing vascular cross-sectional area, dilation, and proliferation changes. In addition, the effect of histidine on regeneration of the vasculature after SFN-caused vascular damage was assessed by the degree of recovery of the vascular cross-sectional area on the regenerated vascular area **(Fig. 1)**. For each concentration of SFN administered in this model, the blood vessel area change rate at each concentration after 14 days was determined. At the SFN concentrations of 0, 75, 150, 300, 600, and 2400 μg/L, the changes in the vascular cross-sectional areas were 1.8% ± 4.3%, −8.1% ± 8.3% ($p < 0.05$), −9.7% ± 9.3% ($p < 0.05$), −15.8% ± 5.8% ($p < 0.05$), −13.5% ± 25.7%, and −20.0% ± 15.5%, respectively **(Fig. 2)**. Our results showed that the vascular cross-sectional area in the

medaka model fish decreased in an SFN-dose-dependent manner, and this thus proves the utility of the model to assess drug-mediated vascular damage.

Effect of histidine on vascular cross-sectional area

To examine the effect of DBB on the vascular cross-sectional area of medaka fed with SFN, histidine (the largest component among the amino acids contained in the broth) was dissolved into the tank water. DBB itself was not used because it causes lower visibility by polluting the water in the tank (probably due to bacterial growth), leading to a reduction in the O_2 concentration in the water.

The effects of various concentrations of histidine in the tank water on the recovery of the vascular cross-sectional area affected by SFN were examined **(Fig. 2)**. In the absence of SFN administration, the existing vascular cross-sectional areas increased by 12.3% ± 7.8% ($p < 0.05$) and 17.8% ± 5.0% ($p < 0.05$) from baseline after administration of histidine at concentrations of 12 mg/L and 60 mg/L, respectively. The same cross-sectional areas recovered from −8.1% of the original area after treatment with 75 μg/L SFN to 5.6% ± 1.0% ($p < 0.01$) and 10.9% ± 12.1% ($p < 0.05$) at the same histidine concentrations of 12 mg/L and 60 mg/L, respectively. The effects of histidine at concentrations of 12 mg/L and 60 mg/L were also demonstrated in fish treated with other SFN concentrations. At 150 μg/L SFN

recovery was seen from −9.7% to 2.7% ± 7.2% and 4.3% ± 10.9%, respectively ($p < 0.05$). At 300 μg/L, the recovery was from −15.8% to −6.3% ± 4.4% and −1.2% ± 1.2%, respectively ($p < 0.05$)]. And, at 600 μg/L the recovery was observed from −13.5% to −8.1% ± 2.9% and −4.5% ± 19.3%, respectively. At an SFN concentration of 2,400 μg/L, no recovery effect of histidine was observed, and some medaka fish died because of the toxicity of SFN itself.

No further increase in recovery was seen with higher concentrations of histidine in this experiment. These results demonstrated that histidine causes vascular dilatation and recovery of the decreased vascular cross-sectional area in SFN-treated fish.

Effect of histidine on vascular regeneration

The recovery of vascular cross-sectional area in the regenerative vessels was examined in the medaka model fish. Our results showed a 61.0% ± 9.2% recovery in the regenerative vascular cross-sectional area after 14 days, which was not significantly different from the recovery effect after histidine administration at 12 mg/L or 60 mg/L (N.S.) **(Fig. 3a)**. With increasing SFN concentrations to 75 **(Fig. 3b),** 150 **(Fig. 3c)**, and 300 μg/L **(Fig. 3d),** the regeneration decreased to 11.5% ± 8.5% and 3.8% ± 1.4%, and 3.2% ± 0.9%, respectively, in a dose-dependent manner **(Figs. 3b-d)**. No recovery was seen at SFN concentrations >600 μg/L. The fish which were administered histidine showed relatively higher vasculature

regenerations (not statistically significant) at 15.3% ± 5.5% and 7.6% ± 0.5% with SFN concentrations of 75 **(Fig. 3b)** and 150 μg/L **(Fig. 3c)**. Fish treated with 300 μg/L SFN showed no significant recovery **(Fig. 3d)**.

These results indicate that SFN causes decreases in regeneration of vascular vessels in a concentration-dependent manner and that the effects of histidine on vascular regeneration are not significant.

Effect of histidine on cellular proliferation

To determine if histidine had an effect on cellular proliferation for angiogenesis or for progression of cancer, endothelial and HCC cell lines were cultured with histidine at differing concentrations, and their growth rates were assessed **(Figs. 4a–i)**. Huvec, an endothelial cell line, and HLE and HepG2, which are HCC cell lines, showed no increase in cell growth in response to histidine **(Figs. 4a, d, g)**. Next, the effect of histidine on cell growth of SFN-treated cells was assessed. At concentrations of 5 and 10 μM of SFN in the culture medium, cell growth was significantly suppressed by SFN in a concentration-dependent manner in Huvec **(Figs. 4b, c)**, HLE **(Figs. 4e, f)**, and HepG2 (**Figs. 4h, i**). However, no recovery/increase of cell growth was observed with histidine administration in the medium. These results indicate that histidine had no effect on the cell proliferation in the endothelial

cells or HCC cell lines, and no effect on tumor progression; therefore, the blood flow increase was probably caused by vasodilatation and not by angiogenesis.

Discussion

SFN inhibits multiple tumor growth-related kinases, including VEGF, VEGFR, and PDGFR, and contributes to prolongation of survival in patients with advanced HCC [1-3]. Among various side effects related to SFN administration, hypertension (HT) and HFS are known to occur frequently and are associated with damage to the peripheral vessels, which leads to an increase in vascular resistance and a decrease in blood flow [5]. We showed before that the decrease in peripheral blood flow detected by Doppler ultrasound is associated with HFS in SFN-treated patients; and that intake of DBB recovers the blood flow and prevents occurrence therein of HFS [9]. The mechanism underlying the recovery of blood flow is thought to be vasodilatation by DBB or its specific components. DBB increases peripheral blood flow in humans [4, 10] and cerebral blood flow in mice [11] and rats [12]. In addition, anti-oxidative effects and improvement of mood have been shown [10, 13]. The antihypertensive and vasodilation effects of bonito [17, 18] and DBB have been reported in stroke-prone SHRs, in which the consumption of bonito extract suppressed the decrease in cerebral blood flow and reduced the risk of stroke [12]. In addition, histidine, the largest component among the amino acids contained in bonito extract [11], also has shown antihypertensive effects in SHRs by attenuating sympathetic output via the central H3 receptor in SHR [14]. These results suggest that the histidine in DBB may cause a vasodilatation effect that is exerted via central nerve signaling. We studied the effect of

histidine on vascular diameters in medaka model fish treated with SFN as a first step to explore the possibility of giving histidine to SFN-treated patients to prevent the SFN-induced vascular damage that was seen in HFS.

Our study demonstrated that SFN decreased the cross-sectional vascular diameter in the live *fli*::GFP medaka model fish fed with SFN **(Fig. 1),** and that histidine, reversed this effect and resulted in recovered normal vascular structure in a dose-dependent manner **(Fig. 2)**. This medaka fish model allows for a large number of animals to be examined more easily than in other animal models. The vascular structure and blood flow in each live fish can be assessed in a time-dependent manner, and similar serum concentrations of SFN and histidine can be expected in all fish because the animals are kept together in water tanks with precise concentrations of the test substances. The levels of SFN and histidine in each medaka fish body were consistent with those in the other fish kept in the same tank. Our results also suggest that histidine is effective in dilating existing vessels in the fins of medaka fish **(Fig. 2)**; however, we observed no effects on the growth rate of regenerative vasculature **(Fig. 3)**. Our results indicate that the anti-tumor effect of SFN, exerted by inhibiting development of tumor feeding arteries, is maintained and not affected by histidine. The *in vitro* cell growth assay also showed that histidine had no effect on tumor cells or endothelial cellular proliferation **(Fig. 4)**.

The possibility that histidine accumulating in mast cells synthesizing histamine could directly stimulate the H1 receptor in the blood vessels causing dilation is unlikely, given the report that shows that orally administered L-histidine gets converted into histamine (an effector in the central nervous system) and shows hypotensive effects via H3 receptors [14]. In fact, oral administration of L-histidine showed no effect on the peripheral histamine concentration, whereas the concentration of histamine in cerebrospinal fluid increased [14].

DBB does not contain peptides [17, 18], and the vascular dilation by DBB can be considered to be caused by histidine, its largest component. However, there is a possibility that carnosine (beta-alanyl-L-histidine), consisting of the amino acids beta-alanine and histidine, a proven reactive oxygen species scavenger that helps dilate vessels, might also have helped vasodilatation, because histidine is its precursor [19].

Our previous results showing less SFN-related HFS events in patients consuming DBB [9], as well as the findings in this current study support a mechanism where the histidine in the broth helps counteract the SFN-related vasoconstriction that leads to HFS, without inducing tumor cell proliferation. A future clinical trial is necessary to determine if histidine can effectively prevent HFS and to rule out any possible negative tumor-growth effects. However, our results suggest that SFN-related HFS depends on narrowing of the pre-existing vascular structure and that histidine may help prevent HFS by causing vascular dilatation without inducing angiogenesis.

Our study demonstrated that SFN caused narrowing in the medaka vasculature, decreasing blood flow; and that histidine effectively inhibits these changes, improving the vascular diameter of the vessels. Since histidine did not affect tumor progression or the novel regenerating vessels, its administration could help prevent SFN-related vascular damage including HFS, enabling a longer period of SFN administration and improving the prognosis of patients.

In conclusion, the medaka fish model is useful for analyzing vascular changes under the administration of SFN and histidine; and histidine may prove a valuable co-adjuvant in the management of SFN-related vascular damage to help prolong survival in HCC patients.

Conflicts of interests

The authors declare that they have no competing interests.

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References

1. J.M. Llovet, S. Ricci, V. Mazzaferro, et al, Sorafenib in advanced hepatocellular carcinoma, N. Engl. J. Med. 359 (2008) 378–390.
2. A.L. Cheng, Y.K. Kang, Z. Chen, et al, Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial, Lancet Oncol. 10 (2009) 25–34.
3. J. Furuse, H. Ishii, K. Nakachi, et al, Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma, Cancer Sci. 99 (2008) 159–165.
4. L.T. Macedo, J.P. Lima, L.V. Dos Santos, et al, Prevention strategies for chemotherapy-induced hand-foot syndrome: a systematic review and meta-analysis of prospective randomised trials, Support Care Cancer. 22 (2014) 1585–1593.
5. R.S. Ishak, S.A. Aad, A. Kyei, et al, Cutaneous manifestations of anti-angiogenic therapy in oncology: Review with focus on VEGF inhibitors, Crit. Rev. Oncol. Hematol. 90 (2014) 152–164.

6. M.E. Lacouture, L.M. Reilly, P. Gerami, et al, Hand foot skin reaction in cancer patients treated with the multikinase inhibitors sorafenib and sunitinib, Ann. Oncol. 19 (2008) 1955–1961.

7. M. Iijima, K. Fukino, M. Adachi, et al, Sorafenib-associated hand-foot syndrome in Japanese patients, J. Dermatol. 38 (2011) 261–266.

8. J.H. Lee, Y.H. Chung, J.A. Kim, et al, Genetic predisposition of hand-foot skin reaction after sorafenib therapy in patients with hepatocellular carcinoma, Cancer. 119 (2013) 136–142.

9. K. Kamimura, Y. Shinagawa, K. Ogawa, et al, Effective prevention of hand-foot syndrome by the consumption of dried bonito broth, Gan. To. Kagaku. Ryoho. 43 (2016) 463–465.

10. Y. Nozawa, T. Ishizaki, M. Kuroda, et al, Effect of dried-bonito broth intake on peripheral blood flow, mood, and oxidative stress marker in humans, Physiol. Behav. 93 (2008) 267–273.

11. M. Honda, Y. Nozawa, T. Ishizaki, et al, Ingestion of bonito extract ameliorates peripheral blood flow in mice loaded from over crowding stress, Biomed. Res. 30 (2009) 129–135.

12. M. Honda, H. Yamada, Y. Nozawa, et al, Consumption of bonito extract suppresses the decrease in cerebral blood flow in stroke-prone spontaneously hypertensive rats, Biomed. Res. 31 (2010) 251–258.

13. Y. Nozawa, M. Kuroda, N. Takanori, Consumption of dried-bonito broth acutely increases peripheral blood flow in humans, J. Health Sci. 53 (2007) 339–343.

14. H. Toba, A. Nakamori, Y. Tanaka, et al, Oral L-histidine exerts antihypertensive effects via central histamine H3 receptors and decreases nitric oxide content in the rostral ventrolateral medulla in spontaneously hypertensive rats, Clin. Exp. Pharmacol. Physiol. 37 (2010) 62–68.

15. N.D. Lawson, B.M. Weinstein, In vivo imaging of embryonic vascular development using transgenic zebrafish, Dev. Biol. 248 (2002) 307–318.

16. T. Vrekoussis, V. Chaniotis, I. Navrozoglou, et al, Image analysis of breast cancer immunohistochemistry-stained sections using ImageJ: an RGB-based model, Anticancer Res. 29 (2009) 4995–4998.

17. R. Norris, R.J. FitzGerald, Antihypertensive peptides from food proteins, in: B. Hernandez-Ledezma, B. Hseih (Eds.), Bioactive food peptides in health and disease, In Tech, Croatia, 2013, pp. 45–72.

18. H. Fujita, M. Yoshikawa, LKPNM: a prodrug-type ACE-inhibitory peptide derived from fish protein, Immunopharmacology. 44 (1999) 123–127.

19. K. Nagai, M. Tanida, A. Niijima, et al, Role of L-carnosine in the control of blood glucose, blood pressure, thermogenesis, and lipolysis by autonomic nerves in rats: involvement of the circadian clock and histamine, Amino Acids. 43 (2012) 97–109.

Figure legends

Figure 1 Development of a medaka model to examine the effect of SFN on the vascular cross-sectional area *in vivo*

(a) The lower half of the medaka's tail fin was cut with scissors. Vessels in the remaining fin were considered pre-existing vessels (shown in red bold lines), and vessels in the regenerating fin were considered regenerating vessels (shown in black bold lines). **(b-e)** Natural course of regeneration of a cut fin and vessels

Figure 2 Effect of SFN and histidine on vascular cross-sectional area in medaka fish

Changes in the vascular cross-sectional area of existing vessels caused by SFN administration were compared between groups of fish treated with histidine at different concentrations.

Three FLI-GFP fish were examined using the ImageJ software to quantitate the vascular cross-sectional area of existing vessels before and after the regeneration time. The changes in area were presented as percentages (%) for each fish. Values in the figure represent the mean ± SD (n = 3 for each group).

* $p < 0.05$, ** $p < 0.01$. One-way ANOVA followed by Bonferroni's multiple comparison test

Figure 3 Effect of histidine on vascular regeneration

Three FLI-GFP medaka fish were examined using the ImageJ software to perform a

quantitative analysis of the vascular cross-sectional area of regenerative vessels at 3, 7, and 14 days after the fin tail excision procedure. SFN concentrations of 0 **(a)**, 75 **(b)**, 150 **(c)**, and 300 μg/L **(d)** were tested. Differences are presented as the change in area (%) in each case. The values represent the mean ± SD of for each group (n = 3 for each group). N.S., no statistical significance. Two-way ANOVA followed by Bonferroni's multiple comparison test

Figure 4 Effect of histidine on cellular proliferation

Cell growth of HUVEC **(a-c)**, HLE **(d-f)**, and HepG2 **(g-i)** cell lines with and without SFN and histidine at appropriate doses. The values represent the mean ± SD (n = 3 for each group). N.S., no statistical significance. Two-way ANOVA followed by Bonferroni's multiple comparison test

Figure 1

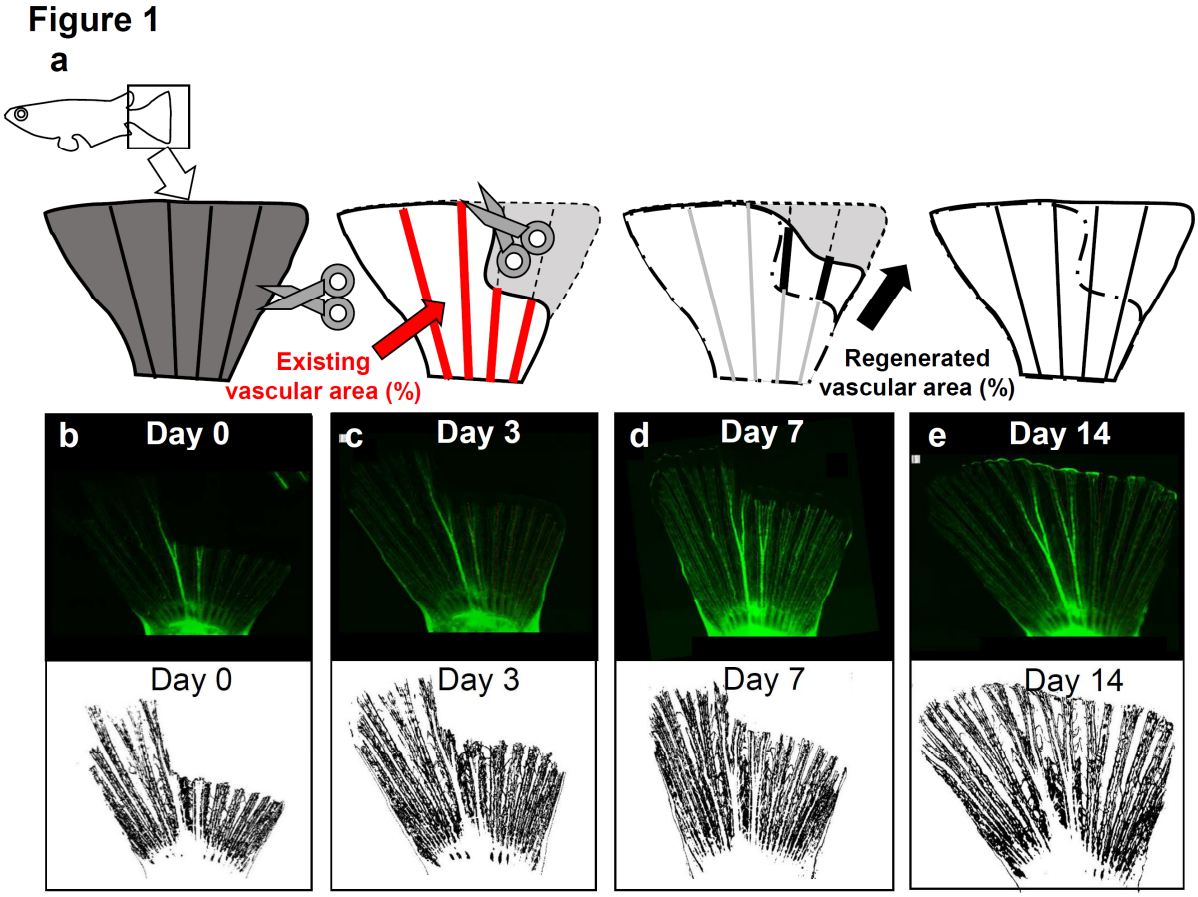
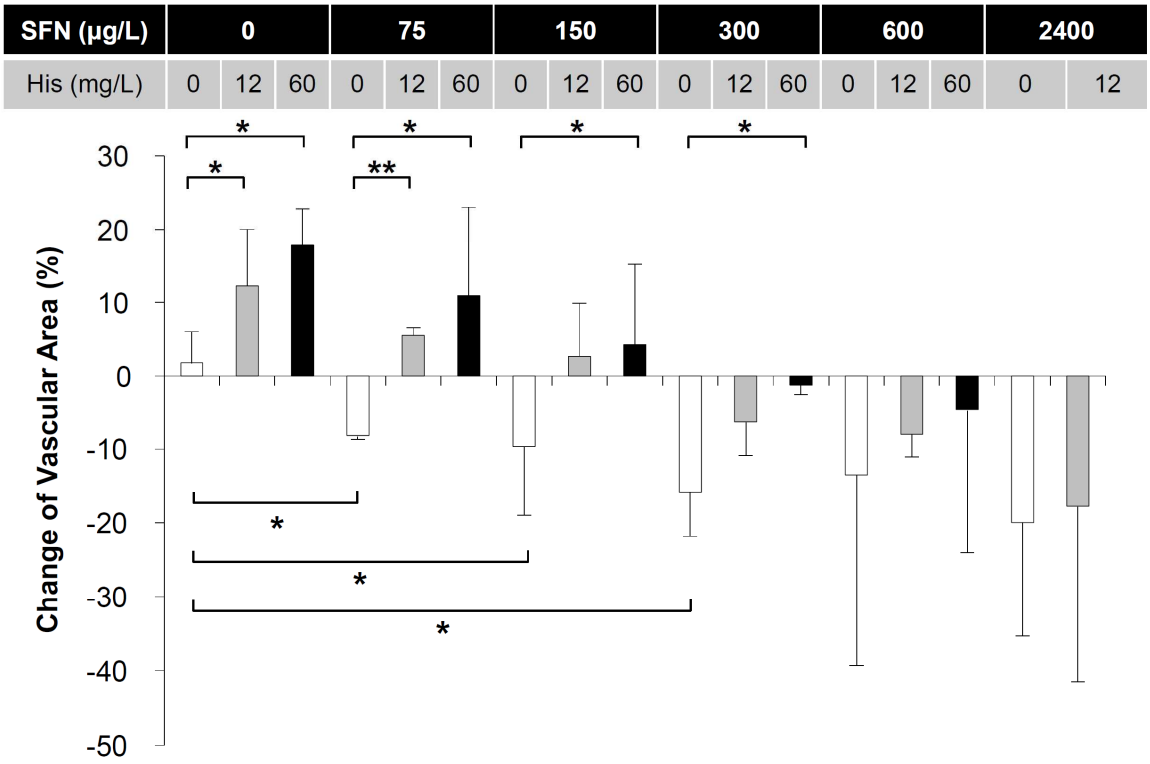


Figure 2



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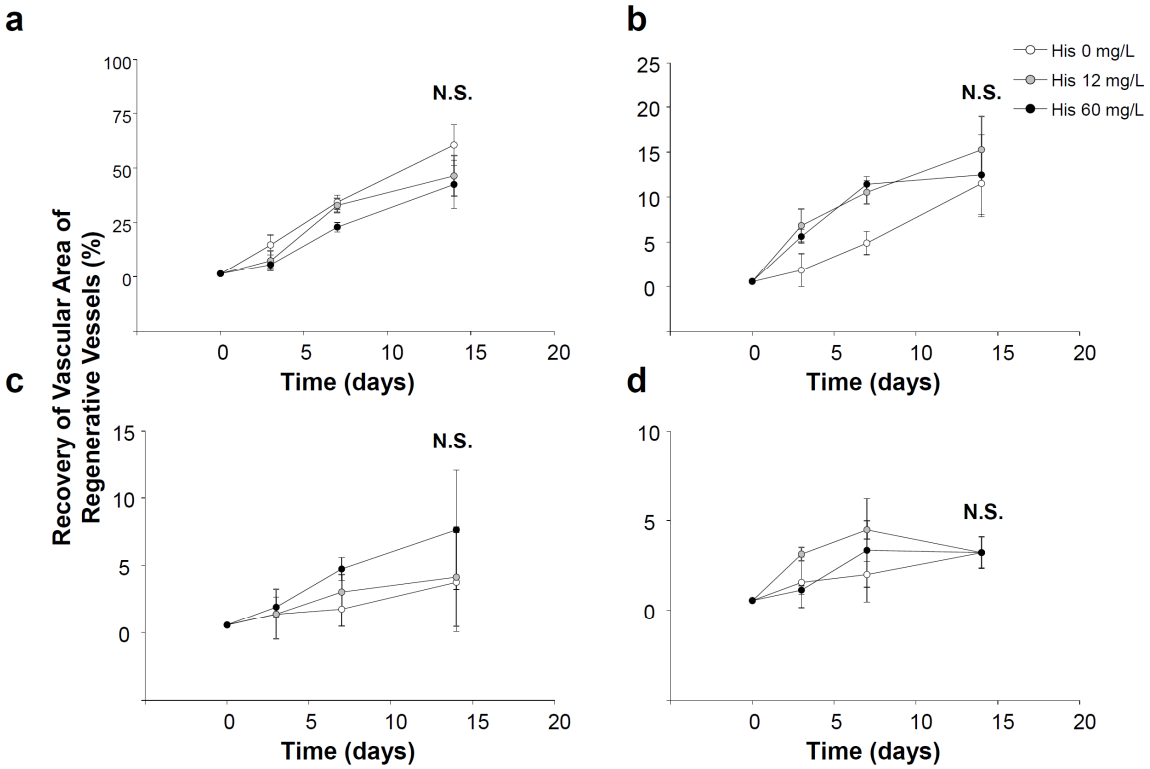
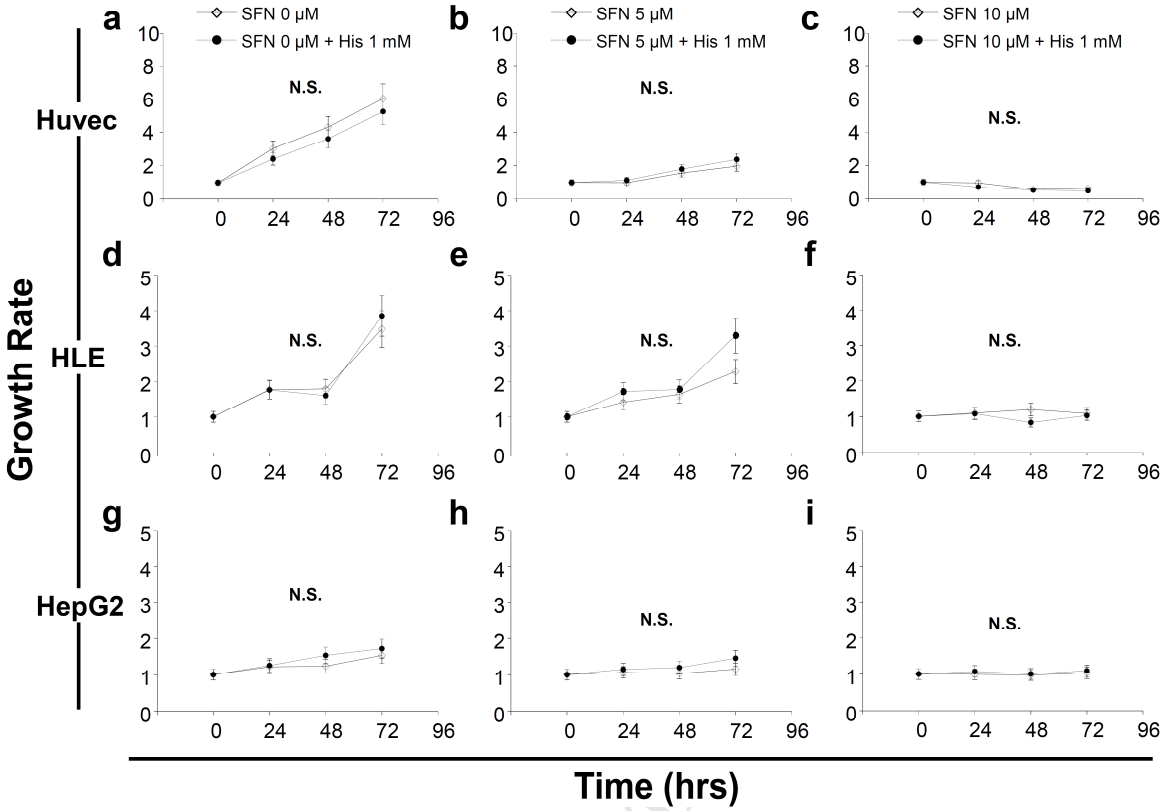


Figure 4



Highlights

1. Sorafenib decreased blood flow in the vasculature by narrowing the vascular diameter.
2. Histidine maintained the blood flow by dilating the vascular diameter of the existing vessel.
3. Histidine was useful for managing the sorafenib related vascular damage.
4. No positive effect of histidine was seen on the tumor progression or on the novel regenerating vessels
5. A *fli*::GFP transgenic medaka fish generated by injecting the *fli* gene promoter::green fluorescent protein cassette into the Kyoto-Cab strain is useful for analyzing the vascular changes under the administration of these chemicals.