[9] Nishida K, Kobayashi M, Ogihara H, Takeo T, Kitano M, Takeuchi K. Clinical usefulness of smell identification test card: Open Essence. Nippon Jibiinkoka Gakkai Kaiho (Tokyo) 2010;113:751–7 [in Japanese].
[10] Fujio H, Doi K, Hasegawa S, Kobayakawa T, Nibu K. Evaluation of card-type odor identification test for Japanese patients with olfactory disturbance. Ann Otol Rhinol Laryngol 2012;121:413–8.
[11] Okutani F, Hirose K, Kobayashi T, Kaba H, Hyodo M. Evaluation of "Open Essence" odor-identification test card by application to healthy volunteers. Auris Nasus Larynx 2013;40:76–80.
[12] Kobayashi M, Saito S, Kobayakawa T, Deguchi Y, Costanzo R. Cross-cultural comparison of data using the odor stick identification test for Japanese (OSIT-J). Chem Senses 2006;31:335–42.

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Reduced Nasal Transport of Insulin-Like Growth Factor-1 to the Mouse Cerebrum With Olfactory Bulb Resection

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Abstract

Although the olfactory nerve is involved in nasal transport of insulin-like growth factor-1 (IGF-1) to the brain, to our knowledge there have been no direct assessments of the effects of olfactory nerve damage on this transport. To determine whether olfactory bulb resection resulted in reduced transport of nasally administered human recombinant IGF-1 (hIGF-1) to the cerebrum, we measured the uptake of nasally administered iodine-125 hIGF-1 (^{125}I-hIGF-1) in the cerebrum as a percentage of that in the blood in male ICR mice subjected to left olfactory bulb resection (model mice) and in sham-operated male ICR mice (control mice). Phosphorylated extracellular signal–regulated kinase (ERK) 1/2 (Thr202/Tyr204)/(Thr185/Tyr187) as a percentage of total ERK 1/2 in the left cerebrum was also assessed by using enzyme-linked immunosorbent assay after nasal administration of hIGF-1. Uptake of nasally administered ^{125}I-hIGF-1 in the cerebrum as a percentage of that in the blood was significantly lower in the model group than in the control group 30 min after nasal administration of hIGF-1. Unilateral olfactory bulb resection prevented nasally administered hIGF-1 from increasing the phosphorylation of ERK 1/2 in the mouse cerebrum *in vivo*. These findings suggest that olfactory bulb damage reduces nasal transport of hIGF-1 to the brain *in vivo*.

Key words: extracellular signal–regulated kinase 1/2, insulin-like growth factor-1, olfactory bulb resection, olfactory transport

Introduction

Magnetic resonance imaging (MRI) has demonstrated that patients with posttraumatic olfactory dysfunction have injury to the olfactory bulb and tract (88% of patients), subfrontal region (60%), or temporal lobe (32%), but the sites of these injuries are not well correlated with olfactory test scores (Yousem et al. 1996) and it is difficult to visualize damage to the olfactory nerve fibers with MRI (Fujii et al. 2002). Furthermore, MRI often cannot be used on individuals who have metal in their bodies. It would be useful to have objective measurements for assessing damage to the olfactory nerve fibers and predicting the course of traumatic olfactory impairment. Other neuroimaging techniques therefore need to be developed to diagnose patients with traumatic olfactory impairment.

To evaluate olfactory nerve connectivity in patients with impaired olfaction, we previously established a method of assessing the migration of nasally administered thallium-201 (^{201}Tl) to the olfactory bulb (Shiga et al. 2011, 2013). However, it takes 24 h to assess ^{201}Tl migration to the olfactory bulb in subjects. For clinical application, there is therefore a need for an olfactory nerve tracer that can rapidly enter the central nervous system.

Nasal administration has been shown *in vivo* to give rapid delivery of insulin-like growth factor-1 (IGF-1) to the brain beyond the blood–brain barrier (Thorne et al. 2004). However, to our knowledge, the effects of olfactory bulb damage on transport of human recombinant IGF-1 (hIGF-1) from the nasal epithelium to the brain have not yet been

directly assessed. IGF-1 receptor is expressed in the olfactory bulb, anterior olfactory nucleus, frontal cortex, and other parts of the central nervous system (Bohannon et al. 1988). hIGF-1 treatment increases the phosphorylation of extracellular signal–regulated kinase (ERK) 1/2 *in vivo* (Thordarson et al. 2004).

Our aim here was to determine whether unilateral olfactory bulb resection would result in reduced delivery of nasally administered iodine-125 hIGF-1 (^{125}I-hIGF-1) to the cerebrum and prevent nasally administered hIGF-1 from increasing ERK 1/2 phosphorylation in the cerebrum in mice subjected to unilateral olfactory bulb resection. Our results show that assessment of nasally administered hIGF-1 transport to the cerebrum is useful for detecting olfactory bulb damage *in vivo*.

Materials and methods

Materials

Male ICR mice aged 8 weeks (CLEA Japan, Inc.) were housed in a 22 °C air-conditioned room with a 12:12-h light–dark cycle and freely provided with food (CLEA Japan, Inc.) and water. The Kanazawa Medical University animal experiment committee approved all animal experimental procedures in advance.

^{125}I-hIGF-1 nasal administration in normal mice

^{125}I-hIGF-1 saline solution (^{125}I-hIGF-1, human recombinant; 185 kBq/mL) was obtained from the Japan Radioisotope Association. Ten microliters was carefully instilled into the left nasal cavity of each normal mouse (N = 25) *via* a microinjection pipette; sneezing was prevented by using anesthesia (intraperitoneal administration of pentobarbital sodium, 0.05 mg/g). To determine the time at which maximum uptake of ^{125}I-hIGF-1 in the cerebrum occurred, the mice were allocated to 5 groups and sacrificed under ether anesthesia at different times after ^{125}I-hIGF-1 nasal administration (10, 30, 60, 180, and 360 min). Tissue samples were obtained from the left cerebrum and the blood. Sample radioactivity was measured by gamma spectrometry by using the Auto Well Gamma System (model ARC-380; Aloka) after weight measurement. The uptake (% dose) of the isotope in each sample (cerebrum or blood) was calculated as a percentage of the radioactivity of each sample per the radioactivity of 10 μL of ^{125}I-hIGF-1 solution. ^{125}I-hIGF-1 uptake was then calculated as the percentage uptake per gram of wet weight of sample (% dose/g).

^{125}I-hIGF-1 uptake in the cerebrum as a percentage of that in the blood was then calculated as the percentage isotope uptake per gram of wet weight (% dose/g) in the left cerebrum divided by the percentage isotope uptake per gram of wet weight (% dose/g) in the blood.

Unilateral olfactory bulb resection

The olfactory bulb was resected unilaterally by using the following method. Under anesthesia (intraperitoneal administration of pentobarbital sodium, 0.05 mg/g), we resected a portion of the left frontal bone of the mouse and exposed the left olfactory bulb. The left olfactory bulb was carefully resected with a sharp curette and totally removed by aspiration with a vacuum pump. Concurrently, the left olfactory nerve was resected between the left olfactory bulb and the frontal skull base. The skin incision was closed with a nylon suture without replacement of the frontal bone.

^{125}I-hIGF-1 uptake assessment in mice subjected to unilateral olfactory bulb resection or sham surgery

We measured ^{125}I-hIGF-1 uptake in the cerebrum and blood 30 min after nasal administration of ^{125}I-hIGF-1. Fourteen days after surgery, we carefully instilled 10 μL of a ^{125}I-hIGF-1 saline solution (185 kBq/mL) into the left nostril of each mouse by microinjection pipette, preventing sneezing by using anesthesia (intraperitoneal administration of pentobarbital sodium, 0.05 mg/g). We waited until 14 days after surgery to allow the acute postsurgical inflammation in the injured base of the frontal cranium to settle down before we performed the procedure.

A blood sample was drawn from the tail vein of the mouse 30 min after nasal administration of the ^{125}I-hIGF-1; the mouse was then perfused through the heart with saline under ether anesthesia before tissue samples were obtained from the left cerebrum. In sham-operated mice, the left olfactory bulb was exposed but not resected.

Phosphorylation of ERK 1/2 in the mouse cerebrum

Thirty minutes after nasal administration of 5 mg hIGF-1 (human recombinant; Cell Signaling), we used an ELISA system (Bio-Plex; Bio-Rad Laboratories) to compare the phospho-ERK 1/2 (Thr202/Tyr204)/(Thr185/Tyr187) content as a percentage of the total ERK 1/2 in the left cerebrum in male ICR mice with resected left olfactory bulbs with that in sham-operated male ICR mice. Lysate was prepared by using a Bio-Plex Cell lysis kit (Bio-Rad Laboratories). Cerebral tissue samples in lysing solution were frozen at −70 °C and then thawed. The samples were centrifuged at 4500 × g for 4 min and the supernatant was collected. Duplicate the Bio-Plex assessments were performed by using 22.5 μg of total protein sample for each well on a 96-well plate. Untreated HeLa lysate (Bio-Rad Laboratories) was used as a positive control for ERK 1/2. Phosphatase-treated HeLa lysate (Bio-Rad Laboratories) was used as a negative control for phospho-ERK 1/2, and Epidermal growth factor-treated HEK293 lysate (Bio-Rad Laboratories) was used as a positive control for phospho-ERK 1/2.

Statistical analysis

We statistically compared mean values by using an unpaired *t*-test or Mann–Whitney *U*-test. All *P* values were 2-tailed. The Kruskal–Wallis test was used to compare nasal ^{125}I-IGF-1 transport to the cerebrum in the 5 groups (Prism 5; GraphPad). A *P* value of <0.05 was considered significant.

Results

^{125}I-hIGF-1 nasal administration in normal mice

To examine the rate of ^{125}I-hIGF-1 nasal transport to the cerebrum, we assessed ^{125}I-hIGF-1 uptake percentage (left cerebrum/blood) 10, 30, 60, 180, and 360 min after instillation of ^{125}I-hIGF-1 into the left nostril in normal mice. ^{125}I-hIGF-1 uptake percentage (left cerebrum/blood) was significantly higher 30 min after nasal administration than at the other time points after administration (Figure 1; $N = 5$ for each group; Kruskal–Wallis test for comparison among 5 groups, $P = 0.002$; unpaired *t*-tests for comparing 2 groups; 10 vs. 30 min, $P = 0.03$; 30 vs. 60 min, $P = 0.04$; 30 vs. 180 min, $P = 0.04$; 30 vs. 360 min, $P = 0.03$). ^{125}I-hIGF-1 uptake percentage (left cerebrum/blood) 60, 180, and 360 min after nasal administration did not differ significantly from that 10 min after administration (Figure 1; $N = 5$ for each group; unpaired *t*-test; 10 vs. 60 min, $P = 0.22$; 10 vs. 180 min, $P = 0.17$; 10 vs. 360 min, $P = 0.42$). In our subsequent experiment, we therefore assessed mice 30 min after ^{125}I-hIGF-1 administration.

Unilateral olfactory bulb resection and changes in ^{125}I-hIGF-1 uptake percentage (left cerebrum/blood)

To determine the effects of unilateral olfactory bulb resection on ^{125}I-hIGF-1 uptake percentage (left cerebrum/blood), we first exposed and resected the left olfactory bulb in model mice *via* resection of a panel in the left frontal bone; in the control mice we simply exposed the left olfactory bulb.

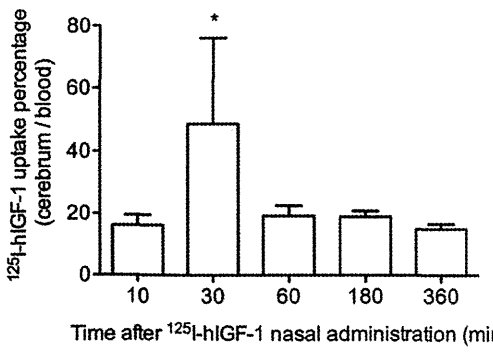

Figure 1 ^{125}I-hIGF-1 uptake percentages (left cerebrum/blood) 10, 30, 60, 180, and 360 min after instillation of ^{125}I-hIGF-1 into the left nostril in normal mice ($N = 5$ for each group; unpaired *t*-test, $*P = 0.03$; 10 vs. 30 min). Bars indicate means ± standard deviation.

We then compared the ^{125}I-hIGF-1 uptake percentage (left cerebrum/blood) in the mice subjected to left olfactory bulb resection with that in the sham-operated mice. Thirty minutes after administration of the isotope, the percentage was significantly higher in the sham-operated mice than in the mice with left olfactory bulb resection (Figure 2; $N = 6$ for each group, Mann–Whitney *U*-test, $P = 0.005$).

Phosphorylation of ERK 1/2 in the cerebrum of mice with unilateral olfactory bulb resection or sham-operated mice

To determine the effects of unilateral olfactory bulb resection on the phosphorylation of ERK 1/2 in the mouse cerebrum, we compared phospho-ERK 1/2 (Thr202/Tyr204)/(Thr185/Tyr187) as a percentage of total ERK 1/2 in the left cerebrum in mice subjected to left olfactory bulb resection with that in sham-operated mice 30 min after intranasal instillation of hIGF-1. The percentage in the left cerebrum was significantly higher in sham-operated mice than in mice with unilateral olfactory bulb resection (Figure 3; $N = 6$ for each group, Mann–Whitney *U*-test, $P = 0.009$; raw data used in the Bio-Plex analysis are shown in Supplementary Table 1).

Discussion

Thirty minutes after nasal administration of hIGF-1, we found significant differences in ^{125}I-hIGF-1 uptake percentage (left cerebrum/blood) and phospho-ERK 1/2 (Thr202/Tyr204)/(Thr185/Tyr187) as a percentage of total ERK 1/2 in the cerebrum between sham-operated mice and mice with unilateral olfactory bulb resection. In mice, olfactory nerve resection between the olfactory bulb and frontal skull base results in reduced intranasal olfactory transport of anterograde neuronal tracer to the olfactory bulb (Kinoshita et al. 2008). These results indicate that nasally administered IGF-1 is transported to the cerebrum along afferent fibers of the

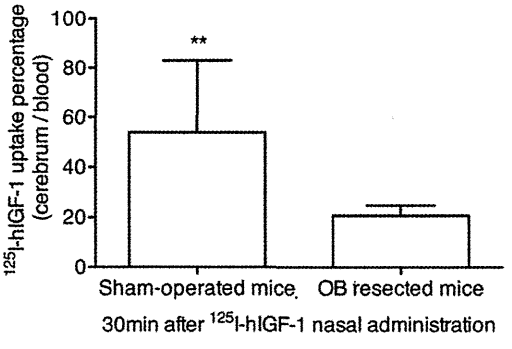

Figure 2 Unilateral olfactory bulb resection and changes in ^{125}I-hIGF-1 uptake percentage (left cerebrum/blood). ^{125}I-hIGF-1 uptake percentage (left cerebrum/blood) was significantly higher in sham-operated mice than in mice with left olfactory bulb resection ($N = 6$ for each group, Mann–Whitney *U*-test, $**P = 0.005$). Bars indicate means ± standard deviation. OB, olfactory bulb.

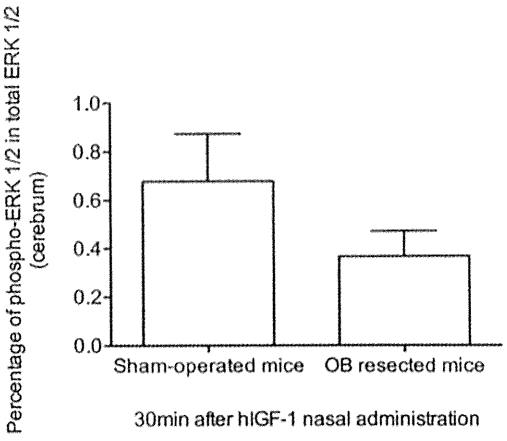

Figure 3 Phosphorylation of ERK 1/2 in the cerebrum in mice with unilateral olfactory bulb resection and in sham-operated mice. Phospho-ERK 1/2 (Thr202/Tyr204)/(Thr185/Tyr187) as a percentage of total ERK 1/2 in the left cerebrum was significantly higher in sham-operated mice than in mice with unilateral olfactory bulb resection ($N = 6$ for each group, Mann–Whitney U-test, $^{**}P = 0.009$). Bars indicate means ± standard deviation. OB, olfactory bulb.

olfactory nerve. Nasal administration of ^{125}I-hIGF-1 may be thus useful for analyzing olfactory bulb damage in animal models of traumatic olfactory impairment.

^{125}I-hIGF-1 uptake percentage (left cerebrum/blood) was significantly higher 30 min after nasal administration than at other time points in normal mice, even though the error bar for the result was relatively large 30 min after nasal administration. Intranasal transport of IGF-1 to the cerebrum is faster than that of manganese ion, which takes 24 h to be transported to the brain after nasal administration in normal mice (Kanayama et al. 2005). Manganese ion enters the nerve terminals *via* calcium channels (Narita et al. 1990). Intranasal transport of IGF-1 to the cerebrum may depend on mechanisms different from those for manganese ion.

Meredith and O'Connell (1988) used histochemistry to investigate horseradish peroxidase (HRP) uptake from the nasal cavity to the brain in mammals. In contrast, ^{125}I-hIGF-1 uptake in samples can be counted by gamma spectrometry; therefore, using ^{125}I-hIGF-1 may be simpler and more quantitative than HRP. Furthermore, if ^{125}I-hIGF-1 uptake by the cerebrum could be visualized with a gamma camera in live animals, follow-up would be available *in vivo* without sacrifice after olfactory nerve transection.

Currently, we can use ^{201}Tl imaging to visualize the connection between the olfactory epithelium and olfactory bulb *in vivo* (Shiga et al. 2009). Nasally administered ^{201}Tl migrates to the olfactory bulb 24 h after administration, as has been shown in healthy volunteers by using a combination of single-photon emission computed tomography (SPECT), X-ray computed tomography (CT), and MRI (Shiga et al. 2011). The rate of nasal ^{201}Tl migration to the olfactory bulb is lower in patients with olfactory impairment from major causes (head trauma, upper respiratory tract infection, or chronic rhinosinusitis) than in healthy volunteers (Shiga et al. 2013). Patients have to return to hospital for SPECT-CT analysis 24 h after nasal administration of ^{201}Tl. ^{201}Tl migrates to the olfactory bulb, where it decays. It is thus difficult to assess the disconnection between the olfactory bulb and the central nervous system in patients with olfactory damage by using ^{201}Tl-based imaging. If we could adapt an isotope-conjugated hIGF-1 nasal administration technique for use in patients with head injuries resulting in anosmia, the decrease in odor sensitivity due to disconnection of the olfactory tract could be validated when damage to the brain is assessed with MRI.

Here, we used commercially available ^{125}I-hIGF-1 for our analysis. We are planning to test a new isotope-conjugated hIGF-1 instead of nasal administration of ^{125}I-hIGF-1, because the half-life of iodine-125 (60.1 days) is too long for clinical application. Unlike intravenous administration of radiopharmaceutical agents, which delivers only small amounts of radiation to the nasal cavity, nasal administration of an isotope delivers a high radiation dose. Iodine-123, which has a short half-life (13.2 h), could be used with the hIGF-1–conjugated agent; its use warrants further investigation in an animal study.

Recovery from chemosensory deficits can occur up to 12–18 months after a traumatic event (Reiter et al. 2004). However, the recovery rate in humans with traumatic olfactory impairment is less than 30% (Fujii et al. 2002). Patients who have intact olfactory nerves and are candidates for long-term treatment of olfactory dysfunction may be selected accurately by using new isotope imaging techniques. Molecular imaging *via* nasal administration of isotope-conjugated molecules may be useful for analyzing the efficacy of treatment with new medications in patients with posttraumatic olfactory impairment.

In conclusion, unilateral olfactory bulb resection in mice results in reduced delivery of nasally administered ^{125}I-hIGF-1 to the cerebrum and prevents phosphorylation of ERK 1/2 in the cerebrum after intranasal hIGF-1 administration *in vivo*.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

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References

Bohannon NJ, Corp ES, Wilcox BJ, Figlewicz DP, Dorsa DM, Baskin DG. 1988. Localization of binding sites for insulin-like growth factor-I (IGF-I) in the rat brain by quantitative autoradiography. Brain Res. 444(2):205–213.

Fujii M, Fukazawa K, Takayasu S, Sakagami M. 2002. Olfactory dysfunction in patients with head trauma. Auris Nasus Larynx. 29(1):35–40.

Kanayama Y, Tsuji T, Enomoto S, Amano R. 2005. Multitracer screening: brain delivery of trace elements by eight different administration methods. Biometals. 18(6):553–565.

Kinoshita Y, Shiga H, Washiyama K, Ogawa D, Amano R, Ito M, Tsukatani T, Furukawa M, Miwa T. 2008. Thallium transport and the evaluation of olfactory nerve connectivity between the nasal cavity and olfactory bulb. Chem Senses. 33(1):73–78.

Meredith M, O'Connell RJ. 1988. HRP uptake by olfactory and vomeronasal receptor neurons: use as an indicator of incomplete lesions and relevance for non-volatile chemoreception. Chem Senses. 13:487–515.

Narita K, Kawasaki F, Kita H. 1990. Mn and Mg influxes through Ca channels of motor nerve terminals are prevented by verapamil in frogs. Brain Res. 510(2):289–295.

Reiter ER, DiNardo LJ, Costanzo RM. 2004. Effects of head injury on olfaction and taste. Otolaryngol Clin North Am. 37(6):1167–1184.

Shiga H, Washiyama K, Hirota K, Amano R, Furukawa M, Miwa T. 2009. Use of thallium transport to visualize functional olfactory nerve regeneration in vivo. Rhinology. 47(4):460–464.

Shiga H, Taki J, Yamada M, Washiyama K, Amano R, Matsuura Y, Matsui O, Tatsutomi S, Yagi S, Tsuchida A, *et al.* 2011. Evaluation of the olfactory nerve transport function by SPECT-MRI fusion image with nasal thallium-201 administration. Mol Imaging Biol. 13(6):1262–1266.

Shiga H, Taki J, Washiyama K, Yamamoto J, Kinase S, Okuda K, Kinuya S, Watanabe N, Tonami H, Koshida K, *et al.* 2013. Assessment of olfactory nerve by SPECT-MRI image with nasal thallium-201 administration in patients with olfactory impairments in comparison to healthy volunteers. PLoS One. 8(2):e57671.

Thordarson G, Slusher N, Leong H, Ochoa D, Rajkumar L, Guzman R, Nandi S, Talamantes F. 2004. Insulin-like growth factor (IGF)-I obliterates the pregnancy-associated protection against mammary carcinogenesis in rats: evidence that IGF-I enhances cancer progression through estrogen receptor-alpha activation via the mitogen-activated protein kinase pathway. Breast Cancer Res. 6(4):R423–R436.

Thorne RG, Pronk GJ, Padmanabhan V, Frey WH 2nd. 2004. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience. 127(2):481–496.

Yousem DM, Geckle RJ, Bilker WB, McKeown DA, Doty RL. 1996. Posttraumatic olfactory dysfunction: MR and clinical evaluation. AJNR Am J Neuroradiol. 17(6):1171–1179.

JOHNS 特集○神経をどう扱うか

再生・再建の工夫

神経再生に有効な薬剤

三輪高喜*
Takaki Miwa

● Key Words ●神経再生，成長因子，漢方薬，ドラッグデリバリーシステム●

はじめに

耳鼻咽喉科領域の神経性疾患として，感音難聴，顔面神経麻痺，嗅覚障害，味覚障害，反回神経麻痺などの疾患が挙げられ，その治療には副腎皮質ステロイド薬があらゆる場面で活躍しているが，果たして副腎皮質ステロイド薬に神経再生作用はあるのであろうか。現時点で副腎皮質ステロイド薬の神経再生作用については明らかにされてはいない。また，これらの神経の中で，実際にニューロンが再生するのは嗅神経のみである。多くの神経疾患の回復は，生き延びたニューロンの回復あるいはその軸索の進展による効果器との再接合によるものである。

そこで，本稿では純粋な神経再生のみならず，神経機能の回復に影響を及ぼす薬剤あるいは因子について概説する。ただし，医薬品として製品化されていないものや，製品化されていても耳鼻咽喉科疾患の治療薬として認められていないものも含まれているので注意が必要である。

成長因子

1．塩基性線維芽細胞増殖因子（basic fibroblast growth factor：bFGF）

FGF ファミリーの１つであり，FGF2 とも呼ばれる。元来，分裂促進因子として，成熟組織では血管新生，創傷治癒の過程に関与するとされていたが，神経系にも作用し，脳やニューロンの生存にとって主要な決定因子とされている。遺伝子組み換え製剤トラフェルミンがフィブラストスプレーとして，褥瘡，皮膚潰瘍治療薬として製品化されている。

Hato ら[1]は，顔面神経麻痺に対する顔面神経減荷術の際に，bFGF をゼラチンハイドロゲルと含浸させ徐放化させたものを，露出した顔面神経周囲に留置させることにより，従来の減荷術および保存治療例よりも高い治癒率を得たと報告している。また，動物実験ではあるが，Nota ら[2]は，メチマゾールで嗅上皮障害を起こしたマウス鼻腔に bFGF とゼラチンハイドロゲルとを含浸させたものを留置することにより，行動学的にも組織学的にも嗅覚の再生が促進されることを報告した。

2．インスリン様成長因子-1（insulin-like growth factor-1）

インスリンと類似の構造を持つポリペプチドであり，生体では肝臓で分泌される。IGF-1 と IGF-2 が存在し，前者はインスリン様の作用に加えてほとんどの細胞，特に神経細胞の成長に関与している。IGF-1 も遺伝子組み換え技術により製剤化され，ソマゾン（一般名メカセルミン）として販売されている。効能は，インスリン受容体異常症 A 型および B 型，ラブソン・メンデンホール症候群，脂肪萎縮性糖尿病，妖精症患者における高インスリン血症，高血糖，黒色表皮腫，多毛の改善ならびにラロン型小人症，成長ホルモン抵抗性の成長ホルモン単独欠損症 Type 1A の成長障害の改善など，ホルモン製剤としての効果を狙うものであり，神経再生に関しての適応はない。しかし，近年，IGF-1 による脳梗塞，アルツハイマー病などの中枢性疾患に対する効果が指摘されており，その投与経路として，嗅神経を介した鼻腔内投与が注目されている[3]。

耳鼻咽喉科領域では，Nakagawa ら[4]がステロ

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イド無効の突発性難聴患者にIGF-1を用いた臨床試験を行った。IGF-1とゼラチンハイドロゲルを含浸させて鼓室内，正円窓窩に留置したところ，半数例で聴力改善効果が認められ，投与4週経過してから効果が得られたことから，何らかの再生作用が考えられると報告している。一方，同グループのHayashiら[5]は，動物実験の結果として，IGF-1が蝸牛の支持細胞に作用して，有毛細胞の保護作用を持つことを報告しており，ニューロンの傷害からの保護と再生の両面からの効果が示唆されている。

3．肝細胞増殖因子（hepatocyte growth factor：HGF）

成熟肝細胞の増殖因子として同定された蛋白質で，肝臓をはじめ，肺，腎などさまざまな実質臓器の損傷で発現が上昇する。神経系ではコモンマーモセットを用いた実験で，脊髄損傷後にヒト組み換えHGF蛋白を髄腔内に投与することにより，運動機能の回復を認めたと報告されており[6]，rhHGFを用いた筋委縮性側索硬化症に対する臨床試験が開始されている。

耳鼻咽喉科領域では，Esakiらが顔面神経を圧迫挫滅したマウスモデルに単純ヘルペスウイルスベクターを用いてHGFを導入し，麻痺の回復が促進することを報告した[7]。

4．神経栄養因子

神経栄養因子は，神経成長因子（nerve growth factor：NGF），脳由来神経栄養因子（brain derived neurotrophic factor：BDNF），neurotrofin 3（NT3），neurotrophin 4/5（NT4/5）によって構成される蛋白質ファミリーの総称である。中でもNGFは代表的な神経栄養因子で，1950年代にイタリア人のLevi Montalcini女史により発見された。Montalciniはその業績により，1986年に上皮成長因子（epidermal growth factor：EGF）の発見者であるStanley Cohen博士とともにノーベル医学生理学賞を受賞した。NGFは中枢神経系では大脳皮質，海馬ならびに嗅球に豊富に含まれ，大脳基底核のコリン作動性ニューロンを栄養し，逆にそれらの領域からアセチルコリンによる刺激を受けている。筆者らはNGFが嗅球に豊富に存在することに着目し，嗅球のNGFと嗅細胞の再生についてマウスを用いた一連の研究を行ってきたので簡単に紹介する。

NGFには，他の神経栄養因子すなわちBDNF，NT-3，NT-4/5と共通する低親和性受容体（LNGFR）と，NGFに独自の高親和性受容体TrkAが存在する。嗅上皮においては，LNGFR，TrkAともに通常状態では発現は低いが，嗅神経切断後の再生時ならびに胎生期の最も嗅細胞の新生の強い時期に両受容体の発現が増強し，再生ならびに新生完了時には発現は再び低値となった。また，嗅球にアイソトープラベルしたNGFを投与したところ，投与側の嗅粘膜に高い集積を認めたことから，嗅球のNGFが逆行性に嗅神経へと輸送されていることが推測された。さらに，嗅球のNGFの機能を解明するために，嗅球内にNGFに対する抗体を持続的に投与したところ，嗅細胞の変性が生じ，同時に嗅上皮でのNGF受容体の発現が亢進した。以上の結果から，嗅球に存在するNGFが嗅上皮での嗅細胞の再生，維持に重要な役割を有していることが判明した[8]。

嗅細胞は上皮内の基底部に存在する基底細胞が嗅細胞へと分化し，成熟し，そしてアポトーシスにより細胞死を起こすいわゆるターンオーバーを繰り返しており，その過程にはさまざまな成長因子や接着因子が関与していることが判明している。筆者らが観察したNGFはその1つであるが，それ以外にも基底細胞の増殖と生存には白血病阻止因子（leukemia inhibitory factor：LIF）やFGF2が関与し，基底細胞から嗅細胞への分化にはトランスフォーミング増殖因子β2（transforming growth factorβ2：TGFβ2）ならびにBDNF，そして嗅細胞の生存維持にはNGFの他，NT-3，血小板由来成長因子（PDGF），骨形成タンパク質4（BMP4）とさまざまな因子が関与していることが判明している。このように嗅細胞は元来，嗅上皮内に存在する基底細胞（幹細胞）から再生するという特殊な環境下にあるため，再生，新生のために自己分泌および傍分泌される多種多様な因子が複雑に関与しているものと思われる。

漢方薬

漢方薬による治療は，体質や体調などの全身状態，すなわち証に基づいた随証治療が基本であるが，近年，生薬成分の解析により，個々の薬理作用が明らかになり，西洋薬的な使い方もされるようになってきた。有名なものとして，小青竜湯に含まれる麻黄には，エフェドリン，プソイドエフェドリンが含まれており，鎮咳，気管支拡張，鼻閉改善などの効果があるため，上気道炎やアレルギー性鼻炎の治療薬として用いられている。

神経再生の観点からは，生薬の中に神経突起作用や，NGF の産生促進を促すもの，あるいは神経保護作用を持つものが数多く知られている。耳鼻咽喉科領域で使用されている当帰芍薬散，加味帰脾湯，人参養栄湯にもそれらの生薬が多く含まれている。筆者らのマウスを用いた実験では，当帰芍薬散ならびに加味帰脾湯を投与したマウスにおいて，嗅球内の NGF 量の増加が認められた。また，加味帰脾湯の予防投与により，抗がん薬のパクリタキセルによるマウス嗅神経傷害を予防することが報告されている[9]。加味帰脾湯を構成する生薬の中で，遠志，蒼朮は NGF 合成促進作用，地黄，人参は神経突起進展作用，陳皮は髄鞘形成促進作用をそれぞれ有していることが知られており，これらの成分が，神経の再生を促進していることが予想される。一方，当帰芍薬散にも蒼朮が含まれており，さらに芍薬も中枢神経に作用して記憶障害改善作用を有することから，加味帰脾湯同様，神経再生作用を有しているものと思われる。また，Ito ら[10]は，ラットを用いた動物実験において，当帰芍薬散による顔面神経保護作用を報告している。

筆者らは，感冒後嗅覚障害に対して当帰芍薬散を投与し，副腎皮質ステロイド薬投与よりも高い改善度を認めることを報告した[11]。感冒後嗅覚障害は中高年の女性に圧倒的に発生頻度が高いことが知られているが，当帰芍薬散は，元来，女性の月経困難症や更年期障害に用いられている漢方薬である。本剤が，卵巣ホルモンであるエストラジオール様の効果を有すること，そして卵巣摘出ラットにエストラジオールを投与することにより，脳内のアセチルコリン合成酵素活性と NGF の発現が亢進することが報告されており[12,13]，感冒後嗅覚障害に対する当帰芍薬散の作用機序として，NGF，エストラジオールのいずれかあるいは両者の関与が考えられる。今後，NGF の標的機関への到達経路を考える場合，NGF は分子量が大きく，消化管からの吸収度は低く，また，脳に対しては血液脳関門が存在するため，実際に NGF を神経系に作用させる経路の確保が問題となる。このような場合に，漢方薬を用いて内在性の NGF の活性を亢進させることは，漢方薬が持つ神経再生効果のみならず，ドラッグデリバリーシステムの観点からもユニークな活用法と言えよう。

おわりに

本稿で示した以外にも，神経再生を促進する薬物，因子は多数あり，今後も増えるであろう。その作用は神経再生のみならず，軸索進展，細胞死からの保護などさまざまである。今後はそれらをどのように製剤とするか，さらにどのような形で神経に作用させるかというドラッグデリバリーシステムの開発も不可欠である。冒頭に述べたように，これらの薬剤，因子はいまだ製剤として存在しないものや，耳鼻咽喉科領域の疾患治療薬としての適用を取得していないものばかりである。したがって，このまま使用することはできないが，動物実験あるいは臨床試験のためのヒントとして，研究に役立てていただければ幸いである。

文　献

1) Hato N, Nota J, Komobuchi H, et al：Facial nerve decompression surgery using bFGF-impregnated biodegradable gelatin hydrogel in patients with Bell palsy. Otolaryngol Head Neck Surg **146** (4)：641-646, 2012.
2) Nota J, Takahashi H, hakuba N, et al：Treatment of neural anosmia by topical application of basic fibroblast growth factor-gelatin hydrogel in the nasal cavity. JAMA Otolaryngol Head Neck Surg **139** (4)：396-400, 2013.
3) Shiga H, Nagaoka M, Washiyama K, et al：Reduced Nasal Transport of Insulin-Like Growth Factor-1 to the Mouse Cerebrum With Olfactory Bulb Resection. Chem Senses Jul 23, Epub ahead of print, 2014.
4) Nakagawa T, Ogino-Nishimura E, Hiraumi H, et al：

Audiometric outcomes of topical IGF1 treatment for sudden deafness refractory to systemic steroids. Otol Neurotol **33**：941-946, 2012.
5) Hayashi Y, Yamamoto N, Nakagawa T, et al：Insulin-like growth factor 1 inhibits hair cell apoptosis and promotes the cell cycle of supporting cells by activating different downstream cascades after pharmacological hair cell injury in neonatal mice. Mol Cell Neurosci **56**：29-38, 2013.
6) Kitamura K, Iwanami A, Nakamura M, et al：Hepatocyte growth factor promotes endogenous repair and functional recovery after spinal cord injury. J Neruosci Res **85**：2332-2342, 2007.
7) Esaki S, Kitoh J, Katsumi S, et al：Hepatocyte growth factor incorporated into herpes simplex virus vector accelerates facial nerve regeneration after crush injury. Gene Ther **18**（11）：1063-1069, 2011.
8) Miwa T, Moriizumi T, Horikawa I, et al：Role of nerve growth factor in the olfactory system. Microsc Rec Tech **58**：197-203, 2002.
9) 山本純平：パクリタキセルによる嗅神経傷害に対する加味帰脾湯の予防効果に関する実験的研究．金医大誌 **37**：28-34, 2012.
10) Ito M, Ohbayashi M, Furukawa M, et al：Neuroprotective effects of TJ-23（Tokishakuyakusan）on adult rat motoneurons following peripheral facial nerve axotomy. Otolaryngol Head Neck Surg **136**（2）：225-230, 2007.
11) 三輪高喜，塚谷才明，池野幸子，他：感冒罹患後ならびに外傷性嗅覚障害に対する当帰芍薬散の治療効果．味と匂誌 **12**（3）：523-524, 2005.
12) 朴　正福，鳥居塚和生，矢部武士，他：卵巣摘出マウス脳内のChAT活性，NGFおよびNGF-mRNAの変動に関する当帰芍薬散およびエストラジオールの作用．J Trad Med **18**：210-216, 2001.
13) Chung MH, Suzuki S, Nishihara T, et al：Estrogenic effects of kampo formula, Tokishakuyakusan, in parous ovariectomized rats. Biol Pham Bull **31**（6）：1145-1149, 2008.

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