Short Communication

Stimulation of Nerve Growth Factor Production by Pyrroloquinoline Quinone and Its Derivatives in Vitro and in Vivo

Kohji YAMAGUCHI, Akeri SASANO, Teizi URAKAMI,* Tomoko TSUJI,† and Kiyosi KONDO

Sagami Chemical Research Center, Sagamihara, Kanagawa 229, Japan
*Biochemical Division, Mitsubishi Gas Chemical Company, Inc., Minato-ku, Tokyo 105, Japan
Received February 4, 1993

Pyrroloquinoline quinone (PQQ), which is a cofactor of microbial quinoprotein enzymes, was found to be a potent enhancer of nerve growth factor (NGF) production in vitro. One of PQQ derivatives, oxazopyrroloquinoline trimethylster, had little activity in vitro, but increased the NGF content in rat brain in vivo.

Nerve growth factor (NGF), a protein composed of 118 amino acid residues, is known to be essential for the development and maintenance of peripheral sympathetic and sensory neurons. It has been found that NGF functions as a neurotrophic molecule for the magnocellular cholinergic neurons in basal forebrain nuclei,2,3 which are specifically lost in Alzheimer's disease and are supposed to be involved in memory and learning processes.4,5 Since this discovery, NGF has been expected to be a potent anti-dementia drug, and numerous efforts6-7 have been made to confirm its therapeutic effects on memory disorders or other diseases, for example, Alzheimer's syndrome. A recent pharmaceutical study on NGF has shown that continuous intracerebral infusion of NGF could partly reverse the cholinergic cell body atrophy and improve retention of a spatial memory task in behaviorally impaired aged rats.8,9 Furthermore, a report that NGF application by intracerebral infusion to an Alzheimer's disease patient showed much effectiveness9 is encouraging not only to the patients but also to the NGF researchers. These reports suggest that exogenously administered NGF works as a neurotrophic factor in the brain to prevent the neurons' death and activates the functions of remaining neurons. However, exogenous NGF administration by intracerebral infusion is difficult and inconvenient. On the other hand, NGF administered by injection via peripheral routes is unable to reach the brain through the blood-brain barrier.

Therefore, low-molecular-weight NGF-inducers that go through the blood-brain barrier would be good candidates as anti-dementia drugs. Some low-molecular-weight-NGF-inducers, for example, catecholamines,10 benzoquinones,11 hericenones,12 and fettulamides13 have been reported. But activities of these compounds were confirmed only in vitro. Most of these compounds has not been tested in the brain in vivo.

During the course of our screening for NGF production enhancers, pyrroloquinoline quinone (4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-γ] quinoline-2,7,9-tricarboxylic acid, PQQ, Fig. 1) was found to be a potent enhancer of NGF synthesis in L-M cells that was known as an NGF productive cell line.14 PQQ has been discovered as a new class cofactor of microbial quinoprotein enzymes, and shown to be widely distributed in microorganisms, plants, and animals.15 Since its first discovery, growth promotive effects for microorganisms,15 vitamin-like activity for mice,16 radical scavenging effects,17 and aldose reductase inhibitory activities18 have been reported as new biological and biochemical functions of PQQ. These potentials of PQQ have been expected to be used as a medicine, and at the same time PQQ derivatives with higher and more selective activity without nephrotoxicity, which is a main side effect of PQQ,19 have been sought. Synthetic modification of PQQ was extensively done, and oxazopyrroloquinoline (OPQ, Fig. 1) was found as a low toxicity PQQ derivative (LD50 > 1000 mg/kg), with higher inhibitory activity against aldose reductase than PQQ.18

Triggered by the discovery that PQQ has a potent NGF-inducing activity, some of its derivatives were also examined in our screening system. In addition to these in vitro experiments, we used some of the PQQ derivatives in in vivo examinations and found that OPQ trimethylster (OPQ-TME) had a NGF-inducing activity in the rat brain. In this paper we report the effects of PQQ and OPQ on production of NGF in L-M cells and in rat brain. This is the first report to show NGF-inducing activity in vivo by measuring the protein level. NGF was measured by enzyme immunoassay (EIA) described previously.13

NGF-inducing activities of PQQs and OPQs in vitro

In L-M cells, PQQ disodium salt (PQQ-2Na) resulted in a maximal increase of about 40-fold in NGF content in the medium cultured for 24 h (Fig. 2). The activity of PQQ-2Na was thought to be the highest of all compounds ever tested by this method. OPQ trimethylster (PQQ-TME) also showed a potent NGF-inducing activity, but the activity was less than that of PQQ-2Na. That means the carboxyl

---

* Corresponding author.

Abbreviations: NGF, nerve growth factor; PQQ, pyrroloquinoline quinone; OPQ, oxazopyrroloquinoline; EIA, enzyme immunoassay; TME, trimethylster.

**Fig. 1.** Chemical Structures of PQQ and OPQ.
groups of PQQ is very important in NGF-inducing activity.

On the other hand, OPQs have only marginal effects on production of NGF (Fig. 2). These observations suggest that the quinone group of PQQ was essential for the activity. The quinone group of PQQ was also reported to be essential for its radical scavenging activity. Moreover, one of the known NGF inducers, idebenone, which has a benzoquinone ring, is also known to be a radical scavenger. From these facts, there supposed to be some unknown relationship between NGF synthesis promotion and radical scavenging.

NGF-inducing activities of PQQ and OPQ in vivo

The effects of PQQ and OPQ on brain NGF production were examined in rats. Considering permeability to the brain, we chose lipophilic derivatives, trimethylesters (TME), of both PQQ and OPQ as the test drugs Twenty-one male Wistar rats (8 week-old) were divided into 7 groups (3 rats/group). Each group of rats were given 4 times intraperitoneal injections of PQQ-TME (0.1, 0.5, 1.0 mg/kg), OPQ-TME (0.1, 0.5, 1.0 mg/kg) or saline, respectively, every 2 days. Two days after the last injection, the rats were killed and their neocortices were separated on dry ice and stored at −80 °C until used. The neocortex samples were made into 5% (w/v) suspensions in phosphate-buffer saline (pH 7.1) and homogenized with a sonicator at 4 °C. The sonicates were centrifuged at 100,000 × g for 30 min and their supernatants were measured by EIA. OPQ-TME caused a maximal increase of about 1.7-fold in NGF content in the neocortex (Fig. 3). Against our expectation, PQQ-TME did not enhance the production of NGF in the neocortex (Fig. 3).

It was reported that PQQ existed as a complex with protein in the body. It is possible that the PQQ-protein complex cannot go through the blood-brain barrier and PQQs could not show the activity in the brain in spite of their strong NGF-inducing activity shown in vitro. To confirm this hypothesis, we examined NGF-inducing activity of PQQs in peripheral nerves in vivo. PQQs administered by intraperitoneal injection enhanced regeneration of sectioned sciatic nerve. These results will be reported elsewhere.

OPQs had little activity in vitro, but increased the NGF content in the brain. This discrepancy can be explained by the existence of an enzyme which changes OPQ to PQQ. This enzyme was found in microorganisms. We suppose a similar enzyme can exist also in mammalian tissues, and OPQ is converted to PQQ, which shows NGF-inducing activity. Detailed experiments will be needed for the explanation of these phenomena.

In our experiments, OPQ was shown to be a possible candidate for an anti-dementia drug. Our results present the possibility that low-molecular-weight NGF-inducers administered by the peripheral route can induce the same effects as intracerebral infusion of NGF.

Acknowledgments. We are grateful to Professor Kyozi Hayashi and Dr. Yoshiko Furukawa of Gifu Pharmaceutical University for valuable advice and for guidance in the technique of purification, bioassay, and enzyme immunoassay of NGF.

References