Vitreoretinal toxicity of basic fibroblast growth factor

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Abstract

Basic fibroblast growth factor (b-FGF) is one of the multifunctional growth factors with important therapeutic potential in the field of ophthalmology. It is also implicated in pathogenesis of vitreoretinal proliferative diseases. In the present study, we evaluated its vitreoretinal toxicity by means of clinical observation, electroretinography (ERG), and histopathology after injection of different doses of b-FGF into the vitreous of rabbit eyes. Doses of b-FGF up to 2 µg per eye caused no toxicity; however, injection of 4 µg or more resulted in sight-threatening vitreoretinal proliferative changes. This information is important for studies aimed at evaluating the therapeutic potential of b-FGF in retinal diseases.

Despite some degree of vitreous organization and opacification, retinal folds, and small areas of traction retinal detachment, the amplitudes of ERGs were normal or even increased (hyperpolarization) in eyes which received 8 µg of b-FGF.

Introduction

Peptide growth factors are multifunctional biological cell regulators. Cells use sets of peptide growth factors as signaling molecules to communicate with each other [30, 31]. Many peptide growth factors with significant action on cells are produced by those cells themselves or by adjacent cells (autocrine or paracrine). Many of the growth factors are regulators of the formation and destruction of extracellular matrix. Furthermore, growth factors play an important role in the response of tissues to injury and the process of tissue repair [30]. The fibroblast growth factor (FGF) family is also multifunctional and some 20–30 different biological activities are the result of the acidic or basic FGF. One of the most important characteristics of FGFs is their affinity for heparin [1, 30]. The FGF family has seven members, including basic and acidic FGF, int-2 and hst-protooncogens, FGF-5, FGF-6, and keratinocyte growth factor (KGF or FGF-7). Various names used in the literature, including brain-derived growth factor, eye-derived growth factor, heparin-binding growth factor, retina-derived growth factor, and retina-derived growth promoting substance are now defined as either basic or acidic FGF. These are produced by numerous cell types and have a wide range of target cells. FGFs are not circulating hormones; rather, they are locally active paracrine or autocrine factors [1, 5, 30, 31]. Basic fibroblast growth factor (b-FGF) is present at a number of specific intracellular and extracellular locations in the normal retina [13, 19]. It has been shown that b-FGF is sequestered and bound to an insoluble fraction of interphotoreceptor matrix between the neural retina and the retinal pigment epi-
thelium (RPE). B-FGF is especially prominent within the cone matrix sheath. It is sequestered in close proximity to surfaces of photoreceptor inner and outer segments and apices of RPE and Müller cells; thus, it has been suggested that it may be synthesized by and play some role in regulating the activity of these cells [13, 16, 20]. B-FGF has been identified in neural cells in addition to vessels and RPE cells of developing and adult rat retina [8]. RPE cells have the capacity to synthesize b-FGF under *in vitro* conditions [27]. In retinal extracts, b-FGF is the principal eye-derived growth factor, both in relative potency and concentration [2]. The b-FGF may have various roles in the eye. It stimulates the healing of injured corneal epithelium [11]. It also promotes wound healing [1]. Some cell types proliferate upon administration of exogenous FGFs [22]. Furthermore, intravitreal injection of b-FGF after argon laser-induced retinal burns accelerates the repair process [26]. Additionally, intracellular injection of b-FGF delays inherited photoreceptor degeneration in RCS rats up to 2 months [9] and prolongs ganglion cell survival after optic nerve transection [10]. There is evidence to indicate that b-FGF is an important neurotrophic factor [1]. Furthermore, b-FGF stimulates photoreceptor differentiation [17, 25] and induces RPE to generate neural retina *in vitro* [24] and causes retinal regeneration *in vivo* [23].

Cells that respond to the FGFs have specific FGF receptors. Under normal condition, the b-FGF receptor is not expressed at high levels.

A large number of tumor cells, including melanomas, are known to synthesize and respond to FGF [1, 14]. This information has led to development of cytotoxic-FGF conjugate (b-FGF-saporin) for treatment of FGF-receptor bearing tumors [3]. These data suggest important therapeutic applications for b-FGF. Therefore, we investigated the vitreoretinal toxicity of b-FGF after its injection into the vitreous of rabbit eyes.

**Materials and methods**

Fourteen eyes of 14 New Zealand white rabbits weighing 2–3 kg were used in this experiment. The animals were treated in conformity with guidelines from the National Institutes of Health [7]. Before intravitreal injection, all eyes were examined by indirect ophthalmoscopy after dilatation of pupils with phenylephrine HCl 2.5% and tropicamide 1%. Animals with ocular abnormality were excluded from the experiment.

To obtain the ERG recordings, the animals were dark adapted for at least 30 minutes and then anesthetized with 0.5 mL of ketamine and xylazine mixture (50 mg ketamine and 5 mg xylazine). Pretreatment electroretinograms (ERGs) were recorded using gold contact lens electrode (Jet) and stainless steel needle electrodes positioned behind the ears (reference) and on the forehead (ground). Scotopic conditions involved a 10 msec Ganzfeld flash (1 per 10 sec) of blue light (Wratten Filter No. 47) at low intensity (Events 1 and 2). The low intensity exposure was repeated without blue filter (Events 3 and 4). The light intensity was increased to medium for Events 5 and 6 and high for Events 7 and 8 using a strobe generator (Model 1539-A, General Radio, Concord, MA).

Human recombinant b-FGF obtained from Sigma Chemical Co. (St. Louis, MO) was dissolved and diluted in sterile saline to desired concentrations (0.5, 1, 2, 4, and 8 μg/mL) and immediately injected via a 30-gauge needle introduced into the midvitreous under direct visualization. Anterior chamber paracentesis and withdrawal of 0.1 mL of aqueous was performed before intravitreal injection. The control eyes received 0.1 mL of sterile saline in the same manner. The eyes were examined 24 hours after injection, every other day for the first week, and every four days for the second and third weeks.

Postinjection ERGs were obtained after three weeks and the rabbits were euthanized by intravenous injection of 100 mg/kg sodium pentobarbital. The eyes were enucleated and processed for paraffin sectioning, stained by hematoxylin-eosin, and examined by light microscope.

**Results**

Twenty-four hours after intravitreal injection, all eyes had mild ciliary injection and vitreous reac-