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Protective effect of insulin-like-growth-factor-1 against dopamine-induced neurotoxicity in human and rodent neuronal cultures: possible implications for Parkinson's disease

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Abstract

Parkinson's disease (PD) is characterized by a progressive loss of 70–80% of dopaminergic (DA) neurons in the substantia nigra. High concentrations of DA were suggested to induce oxidative stress and selective neurodegeneration. We evaluated the effect of insulin-like-growth-factor-1 (IGF-1) on DA toxicity in neuronal cultures. IGF-1 (0.5 μg/ml) suppressed cell death induced by exposure to DA (0.3 mM) after 2 and 4 days, in a rat cerebellar culture. Similarly, IGF-1 (0.5 and 1.0 μg/ml) antagonized DA (0.125 and 0.250 mM) neurotoxicity in a human neuroblastoma cell line (SK-N-SH). Flowcytometric analysis of neuroblastoma cells treated with DA (0.5 mM) showed increased apoptosis, which was significantly reduced by IGF-1. The effect of IGF-1 was associated with increased Bcl-2 expression as indicated by flowcytometry and Western blot analysis. We suggest that IGF-1 possesses a neuroprotective effect against DA-induced toxicity, and may have a potential role in the treatment of PD.

Keywords: Insulin-like growth factor; Dopamine; Apoptosis; Parkinson's disease; Neurotoxicity; bcl-2

Parkinson's disease (PD), one of the most common neurodegenerative disorders in the elderly, is characterized by a progressive and massive loss of midbrain dopaminergic (DA) neurons. The mechanism by which these neurons degenerate is still unknown. However, several environmental and genetic factors, such as reactive oxygen species (ROS), mitochondrial dysfunction, excitotoxicity and inflammatory processes, have been suggested to contribute to this selective vulnerability. Postmortem studies performed in the substantia nigra (SN), have yielded evidence of massive lipid peroxidation, protein and DNA oxidation and degradation processes [10]. The precise trigger for the local oxidative stress in PD is not yet clear. It has been indicated however, that high concentrations of DA, neuromelanin and iron in a weak neuroprotective status, blunted the activity of neurotrophic growth factors (glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF)), thus contributing to the vulnerability of the nigral DA neurons [10]. The hypothesis that DA toxicity might be a major trigger for selective neurodegeneration of the DA neurons, is supported by substantial evidence both in-vivo and in-vitro [14,21].

One of the neurotrophic factors that might protect neurons in this harmful microenvironment is insulin-like-growth-factor-1 (IGF-1). The notion that IGF-1 has a role in PD is justified by the following: (a) SN is one of the regions in the human brain where a considerable density of IGF-1 receptors is evident [5]; (b) IGF-1 increases the survival of neurons in the brain stem including the SN [4]; (c) IGF-1 rescues embryonic DA neurons from programmed cell death [20].

The GH-IGF-1 axis activity is reduced with age. Low IGF-1 levels sometimes overlap a similar shortage in growth hormone (GH) and loss of GH and IGF-1 might be related to changes in body structure, metabolic activity and neurodegeneration that appear with the aging process [7]. It is therefore possible that loss of IGF-1 in adulthood influences the development of neurodegenerative diseases such as PD and

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Alzheimer's. Indeed, changes were found in the IGF-1 level or that of its receptors, in brain regions typical of various neuronal disorders [3,6]. Moreover, clinical studies demonstrate the protective effect of IGF-1 in amyotrophic lateral sclerosis (ALS), although not all reports support these conclusions [1]. In the present study, we examined the effect of IGF-1 on DA-induced toxicity in various neuronal cultures.

We used rat cerebellar granular neurons that demonstrated both sensitivity to DA toxicity [20] and responsiveness to IGF-1 [9] and human neuroblastoma cells (SK-N-SH) which also synthesize and secrete dopamine and mimic the damage in DA neurons [18].

Primary rat cerebellar granule cells were obtained from cerebella of 8-day old rats and grown in medium containing 10% fetal calf serum. L-Arabinosylcytosine (ARA-C; 10 μM, Sigma) was added to the culture medium 18–22 h after plating (0.3×10^6/well), to prevent replication of non-neuronal cells. SK-N-SH cells were maintained in RPMI 1640 medium, supplemented with 2% FCS, penicillin (100 μ/ml), streptomycin (100 μg/ml), nystatin (12.5 units/ml) and L-glutamine (2 mM), (Beit-Haemek, Israel). Cells (2000/well) were treated with DA (RBI, USA) and/or IGF-1 (Sigma, USA) and the viability was assessed by measuring 0.1% trypan blue exclusion or by 0.1% neutral red staining using the ELISA reader at 550 nm.

Flowcytometric analysis of apoptotic changes and cell cycle were evaluated in SK-N-SH neuroblastoma cells as described elsewhere [19]. Cell nuclei were isolated and DNA was stained with propidium iodide and analyzed by fluorescence-activated cell sorter (FAXScan, Becton and Dickenson, CA) equipped with an argon ion excitation wavelength of 488 cm and doublet discrimination model (DDM) analyzed by Lysis II software (Becton and Dickenson, USA). FACS analysis of intracellular Bcl-2 was performed on neuroblastoma cells (10^6) by using specific Bcl-2 antibodies (Dako, Denmark), and Intra-Stain Fixation and Permeabilization kit (Dako, Denmark). For Western blot analysis, cell extract (10^6 each sample) proteins were separated by electrophoresis on 12.5% SDS-polyacrylamide gel and electrotransferred to PVDF membranes as recommended by the manufacturer (Amersham, UK). Membranes were probed with anti-Bcl-2 antibody for 1 h at room temperature and washed three times (5 min each) with TBST (50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.05% Tween-20). Membranes were then incubated with horseradish peroxidase conjugated goat anti-rabbit antibodies for 1 h at room temperature, washed and incubated with the enhanced chemiluminescence system (ECL, Amersham). The flowcytometric assays for the determination of apoptotic changes in neuroblastoma cells was expressed as the increment vs. vehicle of three different experiments. The results were expressed as mean ± standard error. The statistical significance was calculated by the Student's *t*-test, and $P < 0.05$ was considered significant.

We found that > 85% of the cerebellar neurons, both untreated or IGF-1- treated (0.5 μg/ml), survived for at least 7 days. DA treatment (0.3 mM) decreased cell viability after 2, 4, and 7 days to 68.3, 29.3 and 11.1 percent of control, respectively. Addition of IGF-1 (0.5 μg/ml) to the DA-treated cells, significantly increased cell survival to 99% after 2 days and 52.5% after 4 days but failed to protect neurons after 7 days of incubation (Fig. 1).

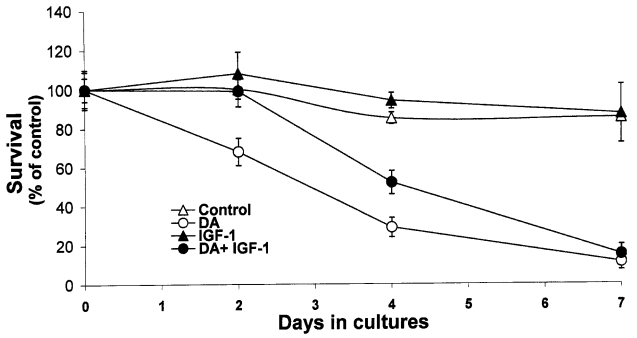

Fig. 1. Effect of IGF-1 (0.5 μg/ml) on survival of newborn rat cerebellar neurons after exposure to DA (0.3 mM) for 2, 4 and 7 days. Each point represents mean ± SE of four determinations conducted in 0.3×10^6 cells. Reference point of 100% was related to viability of control (vehicle treated) cells after 2 days of treatment.

Human SK-N-SH neuroblastoma cells exposed to increasing concentrations of DA (0.06–0.5 mM) for 24 h demonstrated a dose-dependent cell death (23–92% survival rate). Addition of IGF-1 (0.5 and 1.0 μg/ml) to cells slightly increased basal cell viability (5–10%) and significantly enhanced survival of cells exposed to 0.1 mM DA, from 67%, to 101% and 104%, respectively ($P < 0.05$). In cells exposed to 0.25 mM DA, IGF-1 increased cell survival from 28% to 62% and 99%, respectively. At the highest concentration of DA (0.5 mM), only 23% of the cells survived and IGF-1 treatment showed no protection (Fig. 2).

To study whether the protective effect of IGF-1 is mediated via an anti-apoptotic pathway, we examined DNA degradation in neuroblastoma cells under normal medium conditions (2% serum) and when exposed to

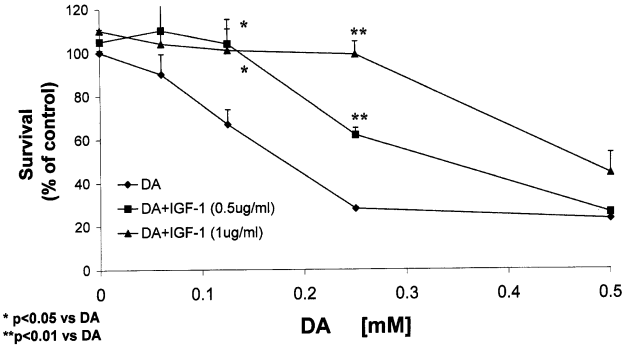

Fig. 2. Effect of IGF-1 (0.5 and 1.0 μg/ml) treatment 1 h prior to DA (0.06–0.5 mM) on viability of human neuroblastoma (SK-N-SH) cells after 24 h. Each point represents mean ± SE of four determinations conducted in 20,000 cells.

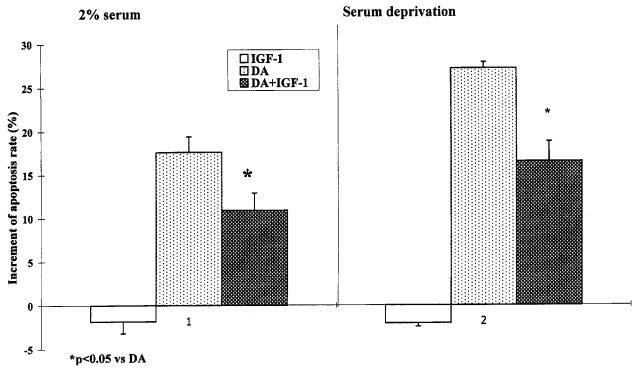

Fig. 3. Effect of IGF-1 (0.5 μg/ml) and DA (0.5 mM), alone and combined, on apoptosis rate 24 h later in neuroblastoma (SK-N-SH) cells (10^6) each, stained with propidium iodide in 2% serum and in serum deprived medium. Each column represents mean ± SE of three independent experiments (10^4 events each).

serum free conditions, using flowcytometry. As compared to vehicle treated cells, DA (0.5 mM) induced increase in typical apoptotic DNA condensation and fragmentations (subG1 phase) by 17.6 ± 1.8 and 27.2 ± 0.7% in the 2% serum and the serum free conditions, respectively. IGF-1 (0.5 μg/ml), slightly decreased basal apoptosis by 1.8 ± 1.4 and 2.0 ± 0.4, and the addition of IGF-1 to the DA-treated cells significantly reduced the DA-induced apoptosis to 10.9 ± 1.6 and 16.5 ± 2.3 ($P < 0.05$ vs. DA) in the two medium conditions, respectively, (Fig. 3). Analysis of cell cycle of the neuroblastoma cells showed that IGF-1 increased DNA synthesis compared to control and DA treated cells (26% vs. 9.9% and 11.7%, respectively), as well as the portion of cells in the G2 + M phase (9% vs. 1.5%, and 1.8%, respectively). In contrast, G1 phase in IGF-1 treated cells was reduced to 54% vs. 76.5% and 60.5% in the controls and in DA-treated cells, respectively.

In previous studies we demonstrated that the protooncogene Bcl-2 is associated with cell rescue, and that overexpression of Bcl-2 protects neurons against DA toxicity both, in-vitro and in-vivo [12,15]. In the present study we found an increase in Bcl-2 expression 24 h following IGF-1 (0.1–0.5 μg/ml) treatment in neuroblastoma cells using Western blot analysis of cells incubated with Bcl-2 antibody (DAKO 1:100) (Fig. 4). Using flowcytometric analysis of intracellular Bcl-2 with antibody (DAKO 1:50), we found a 2.3-fold increase in Bcl-2 expression following IGF-1 (0.5 μg/ml), DA (0.25 mM) treatment moderately increased the expression (ratio 1.5), and the combination of both treatments resulted in an increased ratio of 1.9 compared to vehicle treated cells.

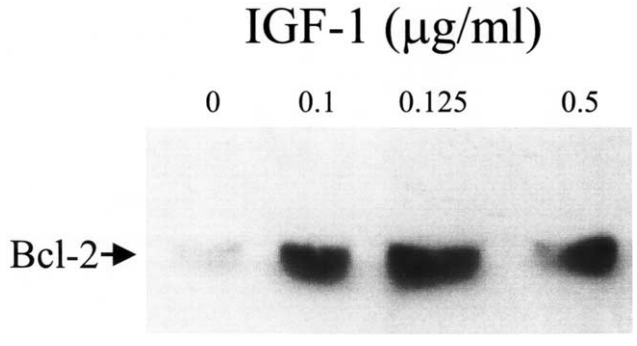

Fig. 4. Western blot analysis of bcl-2 protein expression in neuroblastoma cells (10^6) in controls and after 24 h exposure to IGF-1 (0.1–0.5 μg/ml).

Thus, our data demonstrate that IGF-1 effectively protects neuronal cells of primary rat newborn cerebellar origin, and human neuroblastoma cell-line, from DA-induced toxicity. This protective effect could result from IGF-1 known mitogenic activity and increased DNA synthesis shown previously and in this study. This possibility, however, does not seem to be the sole one, since it cannot explain the protective effect of IGF-1 in the primary cerebellar cells, in which IGF-1 alone did not modify basal cell viability but significantly protected the cells against DA toxicity. Also in the neuroblastoma cells, the protective activity of IGF-1 accounts for a much higher effect than that achieved by its basal enhancement of survival (5–10%).

Previous studies have reported that IGF-1 possesses a marked neurotrophic activity in the brain and in a variety of neuronal cultures [6]. In a recent study in a neuroblastoma (SK-N-SH) cell-line, we also demonstrated that IGF-1 antagonized neuronal cell death induced by serum deprivation and by doxorubicin, through an anti-apoptotic mechanism [8]. In addition, IGF-1 was reported to protect neuroblastoma cells against periosmotic pressure that stimulates apoptosis [11]. IGF-1 was also shown to protect differentiated PC12 cells against death induced by the removal of the NGF and serum deprivation. This effect was accompanied by the elevation of the expression of Bcl-xL protein or inhibition of the anti-apoptotic BAD gene [9,16,17]. Consistent with these reports, we found that high expression of Bcl-2 in PC12 cells provided marked protection against DA apoptosis. Moreover we showed that mice which over-express Bcl-2 in their neurons were resistant to MPTP toxicity [12,15]. Western blot analysis and intracellular staining of neuroblastoma cells with Bcl-2 antibodies indeed revealed that IGF-1 dramatically increased its expression. This finding is consistent with reports on other neurotrophic factors, such as GDNF, brain-derived neurotrophic factor, and vasoactive intestinal polypeptide, that rescue via anti-apoptotic gene regulation [2,13]. The slight increase in bBcl-2 expression following DA (0.25 mM) administration is surprising and suggests that DA-induced apoptosis is not mediated via bcl-2 gene suppression. Such an increase, however, could reflect a compensatory effect of the damaged cells to the apoptotic insult.

Finally, IGF-1 protein is linked to the preservation and homeostasis of the nervous system. Its neurotrophic effect contributes to the notion that IGF-1 has therapeutic potential in different nervous disorders such as PD, amyotrophic lateral sclerosis, Alzheimer's disease, multiple sclerosis, diabetic neuropathy etc. We hypothesize that endogenous

IGF-1 might be relevant to the etiology of PD: loss of IGF-1 in the elderly population might advance the degenerative process, while stimulation of its production might retard progression of the disease.

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