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Research Article

Oxaliplatin enhances gap junction-mediated coupling in cell cultures of mouse trigeminal ganglia

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ABSTRACT

Communications between satellite glial cells and neighboring neurons within sensory ganglia may contribute to neuropathic and inflammatory pain. To elucidate the role of satellite glial cells in chemotherapy-induced pain, we examined the effects of oxaliplatin on the gap junction-mediated coupling between these cells. We also examined whether the gap junction blocker, carbenoxolone, can reverse the coupling.

Primary cultures of mice trigeminal ganglia, 24–48 h after cell isolation, were used. Satellite glial cells were injected with Lucifer yellow in the presence or absence of oxaliplatin (60 μM). In addition, the effect of carbenoxolone (100 μM) on coupling, and the expression of connexin 43 proteins were evaluated.

Dye coupling between adjacent satellite glial cells was significantly increased (2.3-fold, $P<0.05$) following a 2 h incubation with oxaliplatin. Adding carbenoxolone to the oxaliplatin-treated cultures reversed oxaliplatin-evoked coupling to baseline ($P<0.05$). Immunostaining showed no difference between expression of connexin 43 in control and oxaliplatin-treated cultures.

Our findings indicated that oxaliplatin-increased gap junction-mediated coupling between satellite glial cells in primary cultures of mouse trigeminal ganglia, and carbenoxolone reversed this effect. Hence, it is proposed that increased gap junction-mediated coupling was seen between satellite glial cells in TG. This observation together with our previous data obtained from a behavioral study suggests that this phenomenon might contribute to chemotherapy-induced nociception following oxaliplatin treatment.

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Introduction

A large proportion of patients (80%) undergoing treatment for cancer with the chemotherapeutic agent oxaliplatin (third generation platinum analog) experience peripheral sensory side effects, e.g. dysesthesias (abnormal sensation) and pain [18,2]. For up to 40% of patients, a certain degree of sensory neuropathy may persist, which may develop into chemotherapy-induced neuropathic pain (CINP) [18]. CINP is characterized by mechanical allodynia, cold allodynia or loss of heat sensitivity [6]. The pathobiology of CINP is complex and the mechanisms underlying these phenomena are not fully elucidated. Disruption of axonal transport, mitochondrial dysfunction, and reentering into the cell cycle followed by apoptosis have been suggested as possible mechanisms [18,2]. It is known that platinum-based chemotherapeutic drugs (e.g. oxaliplatin) affect the structure and function of sensory neurons [11,5,2]. Neural damage can occur at the cell body, axon, and myelin sheath [6]. Neuronal cell bodies within sensory ganglia are surrounded by non-neuronal cells, satellite glial cells (SGCs). Little is known about potential effects of oxaliplatin and other chemotherapeutic agents on SGCs or their cell–cell communication properties (e.g. glia–glia and neuron–glia communications). It has been proposed that abnormal communication between cells within sensory ganglia contributes to the ectopic neuronal activity that results from nerve injury, inflammation, or treatment with certain chemotherapeutic agents. Ectopic neuronal activity is believed to be a major factor in chronic pain following these insults [3,13].

Studies have recently shown that following nerve injury or inflammation, SGCs undergo activation similar to that seen for astrocytes in the CNS. Both activated astrocytes and activated SGCs exhibit increased expression of glial fibrillary acidic protein (GFAP), which is considered a marker for glial activation [9,19]. The communication between SGCs and neurons within sensory ganglia is under extensive investigation to understand SGC contribution in chronic pain [9,14,12,7,17]. Glia–glia and neuron–glia communications are mediated through gap junctions and by chemical messengers such as adenosine triphosphate (ATP) [20,16,12]. It was suggested that these communications are augmented following neuronal injury or inflammation and may contribute to chronic pain [15,20,7]. Therefore, disruption of such communication under pathological conditions can be beneficial to control neuropathy and pain. In fact, blockade of gap junctions has analgesic effects in several pain models in rodents [15,10,13]. Augmented communication by gap junctions among SGCs in DRG of mice was observed in a model of chemotherapy-induced pain, caused by oxaliplatin or taxol, and administration of gap junction blockers reduced behavioral responses to mechanical stimulation in these animals [17]. In order to elucidate the underpinning mechanisms of the observations seen in the behavioral studies, further investigation is required. Hence, the aims of the present study were (1) to investigate whether oxaliplatin increases dye coupling between adjacent SGCs in short-term cultures of mice trigeminal ganglia, (2) whether carbenoxolone, a gap junction blocker, would be able to reduce such coupling, and (3) whether oxaliplatin increased the expression of connexin 43 (Cx43). Findings from this study may help to understand how oxaliplatin would eventually alter SGC gap junction communication, which may be a potential underlying mechanism of CINP.

Materials and methods

Animals

The experiments were carried out on male and female Balb/c mice 2–4 months old. Animals were housed in small unisex groups in a temperature-controlled room (22–24 °C) on a 12 h light/dark cycle, with access to food and water ad libitum. The procedures were approved by the Animal Care and Use Committee of the Hebrew University-Hadassah Medical School and the study was conducted according to the guidelines set by the International Association for the Study of Pain (IASP).

Cell isolation

Animals were euthanized by CO_2 inhalation followed by decapitation. The trigeminal ganglia (TG) on both sides were surgically removed and care was taken to minimize the amount of connective tissue attached to the isolated ganglia. The ganglia were transferred to a solution of 1.5 mg/ml collagenase (C9891, Sigma-Aldrich, MO, USA) in MEM-Alpha growth medium (01-043-1A, Biological Industries, IL) supplemented with 1% Penicillin-Streptomycin-Amphotericin B (03-033-1C, Biological Industries). The tissue was then incubated at 37 °C for 60 min in a shaking water bath (100 strokes/min), followed by 5 min centrifugation at 1000 RPM, before the collagenase was replaced with fresh medium, supplemented with 10% fetal bovine serum (04-001-1A, Biological Industries) and 1% Penicillin–Streptomycin–Amphotericin B. The ganglia were then mechanically triturated, by repeated pipetting until a homogenized mixture was obtained. The mixture was centrifuged and triturated to a homogenized mixture twice, before the cell isolate was allocated to glass bottom dishes (In Vitro Scientific, CA, USA). The dishes were maintained in an incubator at 37 °C (95% air/5% CO_2). After 24 h, the dishes were washed in fresh growth medium to remove dead cells. Cultures were used for experiments within 48 h after culturing.

Oxaliplatin treatment

Oxaliplatin (2623, Tocris Bioscience, UK) was diluted to a 0.6 mM stock solution in MEM-Alpha growth medium and stored at −80 °C. Before adding oxaliplatin, the stock solution was diluted 10-fold, in growth medium to a 60 μM. Cultures (30 h and 48 h) were incubated for 2 h with the oxaliplatin-medium prior to cell injections. Half an hour prior to the injections, the fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, D9542, Sigma-Aldrich), was added to the medium to stain the cell nuclei.

Connexin 43 expression

The cultures were fixed in 4% paraformaldehyde for 20 min at room temperature; they were then washed in PBS, 3 times for 5 min each. Blocking was carried out using 5% bovine serum albumin (BSA) in PBS with 0.4% Triton X-100, for 1 h at room temperature. Primary antibody against Cx43 (rabbit anti-connexin 43, C6219, Sigma-Aldrich) was diluted 1:400 in PBS with 1% BSA and 0.4% Triton X-100, and incubated overnight at 4 °C. Cultures were washed in PBS and incubated with the secondary antibody, donkey anti-rabbit conjugated to Alexa 594 (ab150068, Abcam, UK), diluted 1:400 in PBS with 10 μmol/L