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The effects of strontium chloride on viability of mouse connective tissue fibroblast cells

Fare konnektif doku fibroblast hücre canlılığı üzerine stronsiyum kloridin etkileri

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Abstract

Aim. Strontium salts are effective and selective anti-irritants for chemically induced sensory irritation associated with stinging, burning, or itching. The aim of the present study was to determine the cytotoxic and/or proliferative effects of strontium chloride on fibroblast cell culture. **Method.** A mouse connective tissue fibroblast cell line, L929 (ATCC cell line, NCTC clone 929) was cultured. Fibroblast cell lines were examined with 20%, 10%, 5%, 2.5%, 1.25%, 0.6%, and 0.3% (w/v) concentrations of Strontium chloride hexahydrate ($SrCl_2.6H_2O$). The proliferation assay analyzed the number of viable cells by the cleavage of tetrazolium salts added to the culture medium, using the XTT labeling reagent. The optical density of the samples was compared with that of the control to obtain the percentage viability, as follows: cell viability (%)=[(OD450 (sample)/OD450 negative control))×100]. **Results.** The cytotoxicity value of strontium chloride for all concentrations (w/v) was compared with that of the control, and cytotoxicity levels were not higher than those of the controls ($p>0.05$). The level of viable cell was higher at 1.25% (w/v) than 2.5% (w/v) ($p<0.05$). **Conclusion.** Strontium chloride hexahydrate ($SrCl_2.6H_2O$) had no negative effect on cell viability at all the concentrations.

Keywords: Strontium chloride, fibroblast, cell culture, proliferation, cytotoxicity

Özet

Amaç. Stronsiyum tuzları yanma, batma ya da kaşıntıyla birlikteki kimyasal olarak uyarılmış sensoryal irritasyon için etkili ve seçici antiirritanlardır. Bu çalışmanın amacı fibroblast hücre kültürü üzerine stronsiyum kloridin sitotoksik ve/veya proliferatif etkilerini belirlemekti. **Yöntem.** Fare konnektif doku fibroblast hücrelerinin kültürü (L929 (ATCC cell line, NCTC clone 929)) yapıldı. Fibroblast kültürü %20, %10, %5, %2,5 , %1,25 ve %0,6 ve %0,3 (w/v) konsantrasyonlarda stronsiyum klorid heksahidrat ile muamele edildi. Proliferasyon düzeneğinde XTT işaretli reagen kullanılarak kültür ortamına eklenen tetrazolyum tuzlarının klivajı yoluyla canlı hücre sayısı analiz edildi. Örneklerin optik dansitesi kontrol grubuyla karşılaştırıldı ve şu formül kullanıldı: Hücre canlılığı (%)=[(OD450 (örnek)/OD450 (negatif kontrol))×100]. **Bulgular.** Stronsiyum klorid heksahidrat sitotoksisite açısından kontrol grubuna göre farklılık göstermemiştir ($p>0.05$). %1.25 konsantrasyonda canlı hücre sayısı diğer konsantrasyonlardan daha fazlaydı ($p<0.05$). Tüm konsantrasyonda sitotoksisite değeri kontrol grubundan daha fazla değildi. **Sonuç.** Stronsiyum klorid heksahidratın fibroblast hücre kültüründe, hiçbir konsantrasyonda hücre canlılığı üzerine olumsuz etkisi saptanmamıştır.

Anahtar sözcükler: Stronsiyum klorid, fibroblast, hücre kültürü, proliferasyon, sitotoksisite

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Introduction

Strontium compounds are candidate molecules for the usage in the field of medicine. Strontium ranelate increases bone formation and decreases bone resorption, and can be used in the treatment of osteoporosis [1, 2]. Other strontium compounds have also positive effects on bone formation, and dental resorption (3, 4). Additionally, there are some studies, suggesting that strontium salts can prevent the development of skin irritation [5, 6]. We know that strontium chloride ($SrCl_2$. $6H_2O$) prevents sensorial irritation and may be used in the treatment of irritation according to a human study [5]. The aim of the present study was to determine the cytotoxic and/or proliferative effects of strontium chloride on fibroblast cell culture.

Materials and methods

Chemical

Strontium chloride hexahydrate ($SrCl_2$ $6H_2O$) was purchased from Sigma-Aldrich (255521) and its 20%, 10%, 5%, 2.5%, 1.25%, 0.6%, and 0.3% (w/v) concentrations were prepared in a culture medium.

Cultivation of L929 mouse fibroblast cells

A mouse connective tissue fibroblast cell line, L929 (ATCC cell line, NCTC clone 929) was cultured in Dulbecco's minimum Eagle medium (DMEM) (Sigma, St. Louis, MO,USA) supplemented with 10% fetal calf serum (Sigma, St. Louis, MO,USA) and 2 mM/ml L-glutamine. No antibiotics were added to the cell culture medium. The cultures were cultivated in an incubator at 37°C and 5% CO_2, until the cell monolayer attained confluence, after approximately seven days. Assays were always performed in the exponential growth phase of the cells.

Fibroblast cells were selected because they are the predominant tissue type in the body, and are easy to cultivate and because of their favorable doubling time of 24 hours. Moreover, these cells are recommended by many standard institutions.

Cell proliferation assay

The proliferation assay analyzes the number of viable cells by the cleavage of tetrazolium salts added to the culture medium, using the XTT labeling reagent (Roche, Mannheim, Germany). During the assay, tetrazolium salts are cleaved to formazan by cellular enzymes. An expansion in the number of viable cells results in an increase in the overall activity of mitochondrial dehydrogenases in the sample. This augmentation in enzyme activity leads to an increase in the amount of formazan dye formed, which correlates directly with the number of metabolically active cells in the culture. The formazan dye produced by the metabolically active cells was quantified by a scanning multiwell spectrophotometer by measuring the absorbance of the dye solution at 450nm. Cells were seeded in 96-well microtitre plates at a concentration of 1×105 cells/ml in a final volume of 100µl per well. Strontium chloride cytotoxicity was tested using 20%, 10%, 5%, 2.5%, 1.25%, 0.6%, and 0.3% (w/v) concentrations.

Cells were then incubated for 24, 48, 72, and 96 h with strontium chloride in a humidified atmosphere (37°C, 5% CO_2). After this, 10µl of the XTT labeling reagent were added to 10µl of the culture medium in each well, and the absorbance of the samples was measured at 450nm against the control (the same cells without any treatment) using a micro titre plate reader (Thermo Scientific Microplate Photometer, Multiskan FC, USA). The same volume of the culture medium and XTT labeling reagent (10µl of XTT labeling reagent /100µl of the culture medium) was added to one well to use as a background control (absorbance of culture medium plus XTT in the absence of cells) as a blank position for the ELISA reader. The absorbance was measured after 2 h from the start of the tetrazolium reaction. The experiments were conducted in six replicates. The optical density (OD) of the samples was compared with that of the negative control to obtain the

percentage viability, as follows: cell viability (%)=[(OD450 (sample)/OD450 (negative control))×100].

Statistical analysis

Statistical analysis of the data was carried out using SPSS (SPSS Inc, Chertsey, UK) statistical software for parametric analysis. The results expressed as mean±SD of six replicates, were analyzed statistically by using one-way analysis of variance (ANOVA) at 95% confidence levels for multiple comparisons and Tukey post-test for two-group comparisons. Statistical significance was reported as $p < 0.05$ was considered significant.

Results

Cytotoxicity of strontium chloride was tested for 24, 48, 72, and 96 h by quantitative analysis using the XTT test (Figure 1). Since strontium chloride at high concentrations, 20%, 10%, and 5%, came out of the solution, these solutions were eliminated. The cytotoxicity value of strontium chloride for all concentrations (w/v) was compared with that of the control, and cytotoxicity levels were not higher than those of the controls ($p>0.05$). The level of viable cell was higher at 1.25% (w/v) than 2.5% (w/v) (Figure 1, $p<0.05$).

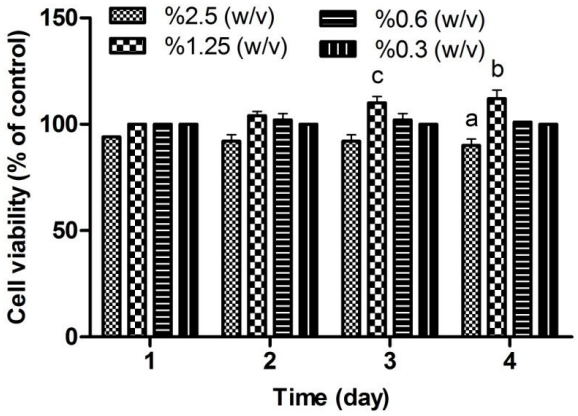

Figure 1. In vitro cytotoxic effect of strontium chloride on L929 fibroblast cells measured by XTT assay; ap<0.05 vs control; bp<0.05 vs strontium chloride at %2.5 (w/v); cp<0.05 vs strontium chloride at %2.5 (w/v).

Discussion

Strontium is an alkaline earth metal. Elemental strontium reacts rapidly with water and oxygen, and so strontium is found in nature only as Sr_2+compounds. Natural strontium is not radioactive [8]. Strontium occurs naturally in Earth's crust (at approximately 0.02-0.03%) in the form of minerals such as celestite (strontium sulfate) and strontianite (strontium carbonate) [9].

Strontium compounds are used in the field of industry and medicine (especially strontium chloride, strontium ranelate) [8].

One of the most common dermatologic disorders is irritant contact dermatitis due to some exogenic factors such as topical agents, soaps, urine and faeces [10]. In particular, strontium chloride, due to its anti-irritant and/or anti-allergic effects on skin and mucous membranes, can be used in the treatment of many disorders in the field of dermatology and cosmetology. The tolerability of Strontium chloride [$SrCl_2$ $6H_2O$] is good, and this chemical form in different formulations can be applicated on the skin and mucosal surfaces.

The skin irritation stimulates the migration and maturation of Langerhans cells, and so irritated skin can easily develop allergic contact dermatitis [11]. Inflammatory cytokines expressed from epidermal cells (keratinocytes, Langerhans cells) have very important contributions to the development of allergic contact dermatitis. Celerier et al. [12] suggested that selenium and strontium salts modulate the levels of these inflammatory cytokines, and thus prevent the maturation and antigen presentation of Langerhans cells. It inhibits TNF-alpha, IL-1 alpha, and IL-6 derived by keratinocytes. Additionally, Hahn et al [5] suggested that strontium salts block the activation of cutaneous C nociceptors responding chemical, thermal, and mechanic stimuli. Strontium chloride might be a candidate molecule to prevent and/or to treat irritant or allergic contact dermatitis.

Strontium can increase osteoblastic cell growth and inhibits bone resorption [13, 14]. Therefore, strontium ranelate can be used in the treatment of osteoporosis.

Skin ulcers may develop due to pressure, inflammation and circulatory disorders. We know well the role of collagen synthesis and fibroblast activities in the healing of wounds or skin ulcers. Basal cell proliferation in the skin also significantly contributes to wound healing [15]. Intracellular adenylate cyclase activity and increased levels of cAMP modulate immune system and cell proliferation. The induction of fibroblast proliferation is associated with early decreases in intracellular cAMP and increases in the cGMP content [16]. Furthermore, Gu et al. [7] showed that strontium reduced the level of intracellular cAMP by inhibiting adenylate cyclase.

For strontium chloride, the oral LD50 was 2.7 and 2.9g/kg body weight as strontium in female and male mice, respectively. No overt toxicity was seen following administration of strontium carbonate at a strontium dose of 14g/kg body weight to rats and mice by stomach tube. Rats (six females) given strontium sulfate at 2g/kg body weight by gavage (950mg/kg body weight as strontium) showed no toxic effects or changes in a “full” macroscopic examination of tissues and organs [8]. According to these data, we can emphasize that strontium sulfate and strontium carbonate do not cause an acute oral toxicity.

Administration of strontium chloride to monkeys by capsule at 2g/kg body weight per day for seven days did not induce any overt toxic effects, but resulted in local irritation, leading to hemorrhagic and erosive lesions in the oesophagus and upper duodenum. Behaviour, growth, food intake, and the weights and microscopic appearance of the liver and kidney were normal in all groups of young adult rats (130-170g; three of each sex per group) fed strontium chloride hexa-hydrate at up to 3000 mg/kg in the diet for two weeks (about 50-100mg/kg body weight per day as strontium, assuming rats ingest food at the equivalent of 5-10% of their body weight daily). There were no clear effects on haematology, and the bones were not examined in this range-finding study [17].

The most comprehensive repeated oral dose study using the lowest doses showing toxic effects involved inclusion of strontium chloride hexahydrate in the diet of weanling rats (40-60g; 10 of each sex per group) at 0, 75, 300, 1200 or 4800mg/kg (strontium doses of 0, 2.5, 10, 40 and 160mg/kg body weight per day, respectively, assuming young rats consume feed at an amount equivalent to about 10% of their body weight per day) for 90 days. Inclusion of this strontium compound at strontium concentrations up to 10mg/kg body weight per day had no effect on behaviour, appearance, growth, food intake, survival, haematology, serum chemistry, blood calcium or phosphorus, liver glycogen, urinalysis, weights of the major organs or microscopic appearance of a fairly wide range of tissues, including bone. The 1200mg/kg dietary concentration of strontium chloride hexahydrate was considered to be NOAEL (no-observed-adverse-effect level) [18].

In a limited study, adult (250g) male rats (12 per group) were given strontium chloride in their drinking-water at a concentration of 0.2 (control), 900, 1900 or 3400mg/L for three years. There were no adverse effects on growth, microscopic appearance of the bone, kidney, lung, adrenal, brain, heart or muscle; organs were not weighed, and tumours were not mentioned [19].

We showed that strontium chloride [$SrCl_2$ $6H_2O$] 1.25% (w/w) had no cytotoxic effect. Furthermore, at the fourth day of cultivation of L929 mouse fibroblast cells, we observed a significantly increased cell proliferation compared with that of the control assay. On the other hand, pharmacological properties and in vivo (human) toxicity should be determined. Based on this information, we suggest that strontium chloride may be an important candidate for treating some inflammatory dermatologic disorders safely.

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