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Original Article

Cell therapy in experimental model of inflammatory bowel disease☆

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ABSTRACT

Inflammatory bowel disease, which mainly involves Crohn's disease and ulcerative rectocolitis, is an inflammatory condition of the mucosa that can afflict any segment of the gastrointestinal tract. Despite the fact that the existing therapies result in improvement in patient's symptomatology and quality of life, there is no curative treatment. Surgical treatment involves complex procedures associated with high morbidity and mortality rates. In this context, cell therapy with stem cells has emerged as a treatment with broad potential applicability. In this study, we intended to verify the efficacy of transplantation of adipose tissue-derived stem cells in rats with intestinal inflammation induced by trinitrobenzenesulfonic acid. The cell population was isolated from the adipose tissue of inguinal region of rats and processed for culture by mechanical dissociation. The animals were evaluated with respect to clinical and biochemical aspects, as well as by macroscopic, microscopic and histological analyses. In the experimental model of bowel inflammation by 2,4,6-trinitrobenzenesulfonic acid, the infusion of adipose tissue significantly reduced the presence of adhesions in the colon and adjacent organs and decreased the activity of myeloperoxidase, a marker of neutrophil infiltration in the injured mucosa. The results suggest that cell therapy with adipose tissue can promote and/or accelerate the regeneration of damaged intestinal mucosa. It is concluded that the presence of adhesions and the determination of myeloperoxidase activity provide indications that adipose tissue can promote and/or accelerate the regeneration of inflammatory bowel mucosa.

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Terapia celular em modelo experimental de doença inflamatória intestinal

Palavras-chave:
Doença Inflamatória Intestinal
Células-tronco
Tecido Adiposo
Terapia Celular

RESUMO

A Doença Inflamatória Intestinal (DII), consistindo principalmente da doença de Crohn e retocolite ulcerativa, é uma condição inflamatória da mucosa que pode acometer qualquer segmento do trato gastrointestinal. Apesar das terapias existentes resultarem na melhora dos sintomas e da qualidade de vida dos pacientes, não há nenhum tratamento curativo. O tratamento cirúrgico envolve procedimentos complexos associados a altas taxas de morbimortalidade. Neste contexto, a terapia celular com células-tronco desponta como opção de tratamento potencialmente promissora. Em função destes aspectos, pretendeu-se, no presente estudo, verificar a eficácia do transplante de células-tronco derivadas do tecido adiposo (ASC) em ratos com inflamação intestinal induzida por ácido trinitrobenzenosulfonico (TNBS). As ASCs foram obtidas por dissociação mecânica do tecido adiposo da região inguinal de ratos e processadas para cultivo. Os animais foram avaliados, considerando-se os aspectos clínicos e bioquímicos, além de análises macroscópica, microscópica e histológica. No modelo de inflamação intestinal induzida por TNBS, a infusão de ASCs reduziu significativamente a presença de aderências entre o cólon e órgãos adjacentes, bem como diminuiu a atividade da mieloperoxidase (MPO), um marcador da infiltração de neutrófilos na mucosa lesada. Os resultados obtidos permitem concluir que a terapia celular com ASCs pode promover e/ou acelerar o processo de regeneração da mucosa intestinal inflamada.

Introduction

Inflammatory bowel disease (IBD) comprises two nosological entities of chronic and recurrent nature: Crohn's disease (CD) and ulcerative rectocolitis (URC).[1] CD is a transmural inflammatory condition of mucosa that can affect any segment of the gastrointestinal tract.[2,3] Unlike CD, URC is a disease characterized by diffuse inflammation of colonic mucosa. URC affects the rectum (95% of cases) and proximal portions of the colon, in a symmetric and continuous manner.[4,5]

Despite the introduction of new clinical therapeutic approaches and their contribution to improve the quality of life of patients with IBD and/or in the prevention of recurrence, there is no definitive clinical curative treatment. The surgical treatment, in turn, involves complex procedures associated with high rates of morbidity and mortality and postoperative complications.[6,7]

Several studies have shown that mesenchymal stem cells (MSC) may be involved in tissue regeneration, since they have the ability to modulate the immune responses of pro-inflammatory cells to induce an environment with a more tolerant phenotype to inflammation.[8–11] Furthermore, it is proposed that these cells act in regulating the immune system in inflammatory tissues, putting into action proinflammatory cytokines and the secretion of chemokines.[12] Thus, it can be postulated that MSC could exert an anti-inflammatory action in response to the induction of injury in experimental models *in vivo* and, thus, acting as a potential therapeutic agent in inflammatory diseases such as URC and CD.

In light of this, in this study we intended to verify the effectiveness of transplantation of MSC derived from adipose tissue (ASC) in rats with intestinal inflammation induced by trinitrobenzenesulfonic acid (TNBS). In addition, with this approach we intend to shed light on pathophysiological aspects of IBD, as well as to suggest and evaluate the effectiveness of new alternative therapies.

Method

Animals

In this study, Wistar albino rats (*Rattus norvegicus*), raised in the Central Bioterium, UNESP – *campus* of Assis, were used under controlled temperature (22 °C) and lighting (12 h of light/12 h of darkness) conditions and fed with a solid diet supplemented with Vitagold® (Tortuga, Sao Paulo, Brazil).

Experimental groups

The animals were divided into the following experimental groups: blank (healthy animals), control (animals with TNBS-induced bowel inflammation and treated with phosphate–PBS buffer) and treated (animals with TNBS-induced bowel inflammation and treated with mesenchymal stem cells) groups.

Isolation by mechanical dissociation and culture of adipose tissue-derived stem cells

The adipose tissue extracted from the groin of rats was mechanically dissociated with the help of two L-shaped syringe needles (BD™, New Jersey, USA) in RPMI 1640 medium (Gibco®, New York, USA) supplemented with 2%