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# Efficacy of adipose tissue-mesenchymal stem cell transplantation in rats with acetaminophen liver injury☆

Federico Salomone[a,*], Ignazio Barbagallo[b], Lidia Puzzo[c], Cateno Piazza[d], Giovanni Li Volti[b,e]

[a] U.O.C. di Gastroenterologia, Ospedale di Acireale, Azienda Sanitaria Provinciale di Catania, Catania, Italy
[b] Department of Drug Sciences, University of Catania, Catania, Italy
[c] Department of Pathology, University of Catania, Catania, Italy
[d] Pharmacokinetic Unit, Unifarm Research Center, University of Catania, Catania, Italy
[e] Istituto EuroMediterraneo di Scienza e Tecnologia, Palermo, Italy

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**Abstract** **Objective** Acetaminophen intoxication is a leading cause of acute liver failure. Liver transplantation for acute liver failure is limited by the availability of donor organs. In this study, we aimed at identifying if the transplantation of adipose tissue-mesenchymal stem cells (ASCs) may exert therapeutic effects on acetaminophen-induced liver injury.
**Methods** ASCs were isolated from human subcutaneous tissue and were transfected with a green fluorescent protein (GFP). Sprague–Dawley rats were administrated 300 mg/kg of acetaminophen intraperitoneally and were transplanted with ASCs or vehicle. After 24 h from acetaminophen administration, rats were sacrificed. Hepatic levels of isoprostanes, 8-hydroxyguanosine (8-OHG), nitrites/nitrates and reduced glutathione (GSH) were determined as markers of oxidative stress; JNK phosphorylation and hepatic levels of inflammatory cytokines and regeneration factors were also assessed.
**Results** Transplantation of ASCs decreased AST, ALT and prothrombin time to the levels observed in control rats. Transplanted animals had normal plasma ammonia and did not display clinical encephalopathy. Liver sections of intoxicated rats treated with vehicle showed lobular necrosis and diffuse vacuolar degeneration; in rats transplanted with ASCs liver injury was almost absent. Transplantation of ASCs decreased liver isoprostanes, 8-OHG and nitrite–nitrates to the levels of control rats, while preserving GSH. Consistently, hepatic levels of TNF-$\alpha$, MCP-1, IL-1$\beta$, ICAM-1 and phospho-JNK were markedly increased in rats treated with vehicle and were restored to the levels of controls in animals transplanted with ASCs. Furthermore, ASC transplantation increased liver expression of cyclin D1 and PCNA, two established hepatocyte regeneration factors, whereas ASCs were not able to metabolize acetaminophen *in vitro*.
**Conclusion** In this study, we demonstrated that ASC transplantation is effective in treating acetaminophen liver injury by enhancing hepatocyte regeneration and inhibiting liver stress and inflammatory signaling.


*Abbreviations:* ALF, acute liver failure; GSH, reduced glutathione; OLT, orthotopic liver transplantation; MSCs, mesenchymal stem cells; ASCs, adipose tissue mesenchymal stem cells; GFP, green fluorescent protein; 8-OHG, 8-hydroxyguanosine; BM-MSCs, bone marrow mesenchymal stem cells; PCNA, proliferating cell nuclear antigen.

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* Corresponding author at: U.O.C. di Gastroenterologia, Ospedale S. Marta S. Venera, Via Caronia, 95024 Acireale (Catania), Italy.
*E-mail address:* federicosalomone@rocketmail.com (F. Salomone).

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## Introduction

Acute liver failure (ALF) is a life-threatening condition in which an acute liver injury can lead to coagulopathy, brain edema and in many cases to multiorgan failure; a variety of etiologies including drugs, viral infections, alcohol, metabolic, autoimmune or genetic disorders may cause acute hepatic dysfunction leading to liver failure (Lee and Stravitz, 2011). The main cause of ALF in industrialized countries is acetaminophen intoxication; although acetaminophen is a safe and widely used analgesic drug, its overdose can lead to ALF and the mortality for acetaminophen intoxication is currently impressive (Lee and Stravitz, 2011).

A number of studies in human and rodents suggest that oxidative stress plays a key role in the pathogenesis of acetaminophen-induced liver injury (Jaeschke et al., 2012). Oxidative stress is the consequence of the acute depletion of reduced glutathione (GSH) that occurs early, after 1.5 to 2 h from acetaminophen overdose, and it is caused by the generation of N-acetyl-p-benzoquinone imine (NAPQI), the toxic metabolite of acetaminophen (Jaeschke et al., 2012). This early event impairs the antioxidant defense of the liver against reactive oxygen species and reactive nitrogen species; the increase of radical species triggers lipid and protein peroxidation, and DNA oxidative damage with consequent death of hepatocytes (Jaeschke et al., 2012).

The treatment of acetaminophen intoxication is based on the use of N-acetyl-cysteine (Lee and Stravitz, 2011). However, in a number of patients with acetaminophen-induced acute liver injury, pharmacological treatment fails and patients need to undergo orthotopic liver transplantation (OLT) (Lee and Stravitz, 2011). Nonetheless, the shortage of donor organs for OLT makes the need of finding alternative therapeutic options. Recently, the transplantation of mesenchymal stem cells (MSCs) has been identified as a therapeutic tool in different types of experimental liver injuries (Sato et al., 2005; Kuo et al., 2008). MSCs are adherent, fibroblast-like, pluripotent and non-hematopoietic progenitor cells, which reside in many tissues and organs investigated so far including the bone marrow (Pittenger et al., 1999), umbilical cord (Bieback et al., 2004), placenta (In 't Anker et al., 2004), amniotic fluid (De Coppi et al., 2007) and subcutaneous adipose tissue (Zuk et al., 2001). Adipose tissue mesenchymal stem cells (ASCs) are abundant in subcutaneous adipose tissue and can be easily obtained by lipoaspiration, thus representing a fast source of MSCs in patients with critical acute diseases. The transplantation of ASCs has demonstrated therapeutic efficacy in $CCl_4$ (Banas et al., 2008), concanavalin A (Kubo et al., 2012) and ischemia–reperfusion (Sun et al., 2012) liver injuries. In this study, we aimed at identifying if the transplantation of ASCs may exert therapeutic effects in rats with acetaminophen-induced liver injury and the underlying molecular events.

## Materials and methods

### ASC isolation, culture and transfection

Subcutaneous adipose tissue was obtained from a 23-year-old man with no significant medical history undergoing umbilical hernioplasty. Written consent was obtained. The choice of a single young and healthy man as source of ASCs was in order to eliminate any bias related to the use of cells from different individuals, which can display different functional activity, and to exclude any situation of disease that could have affect the proliferative properties of ASCs. Adipose tissue was minced with scissors and scalpels into less than 3-mm pieces and isolation of ASCs proceeded as previously described (Banas et al., 2007). Briefly, after gentle shaking with equal volume of PBS, the mixture separated into two phases. The upper phase (containing stem cells, adipocytes and blood) after washing with PBS was enzymatically dissociated with 0.075% collagenase (type I)/PBS for 1 h at 37 °C with gentle shaking. The dissociated tissue was then mixed with an equal volume of DMEM (GIBCO-BRL, Japan) supplemented with 10% FBS and incubated 10 min at room temperature. The solution then was separated into two phases. The lower phase was centrifuged at 1500 rpm for 5 min at 20 °C. The cellular pellet was resuspended in 160 mM $NH_4Cl$ to eliminate erythrocytes and passed through a 40 μm mesh filter into a new tube. The cells were resuspended in an equal volume of DMEM/10% FBS and then centrifuged. Isolation resulted in obtaining $7.7 \times 10^6$ of adherent cells for a primary culture from 5 g of adipose tissue (approximately; $1.0 \times 10^5$ to $4.6 \times 10^6$/1 g) after 7 to 10 days of culture. The cells were suspended in a DMEM/10% FBS plated in concentration $1–5 \times 10^6$ cells/75 $cm^2$. The cells with 70%–80% confluence were harvested with 0.25% trypsin–EDTA and then either repleted at $1.0 \times 10^5$ cells/60-mm dish. The phenotype of ASCs was evaluated by flow cytometry analysis (FC500 Beckman Coulter). Flow cytometry revealed that the cells isolated from the subcutaneous adipose tissue expressed stromal-associated markers CD90 and CD105 but did not express the hematopoietic markers CD34 and CD45 (Suppl. Fig. 1). In an *in vitro* experiment to establish if ASCs metabolize acetaminophen, cells were treated with 2 mM acetaminophen; NAPQI concentration was measured in the cell medium after 2, 8 and 24 h. For the *in vivo* experiment, before transplantation cells were transfected with a baculovirus-mediated transfection system for green fluorescent protein (GFP) (Invitrogen, US).

### Animals and treatments

All procedures fulfilled the Italian Guidelines for the Use and Care of Laboratory Animals. Sprague–Dawley female rats were purchased from Charles River Lab (Calco, Italy). Animals were maintained in a light- and temperature-controlled facility and fed with a standard chow and water *ad libitum*. After an overnight fast, twelve rats weighing about 200 g were administrated a 300 mg/kg body weight dose of acetaminophen (Sigma, Italy) dissolved in PBS. After 2 h from acetaminophen administration, six rats received the infusion *via* the caudal vein of 200,000 cells suspended in 1 mL of saline; six rats were administrated only saline. Four rats were administrated only the vehicles, PBS and saline, and served as healthy control. ASCs were administrated 2 h after acetaminophen administration because this is the time occurring for the formation of NAPQI, and this early time point is widely used in animal studies evaluating the efficacy of a treatment for acetaminophen intoxication (Saito et al., 2010). After 24 h from acetaminophen administration, rats