



CORRESPONDENCE AND COMMUNICATION

Lipolytic effectiveness of phosphatidylcholine in the treatment of 'buffalo hump' of HIV patients

The purpose of this study was to evaluate the lipolytic activity of LIPOSTABIL (Aventis, France) in an *in vitro* model of cultured human primary adipocytes, isolated from the 'buffalo hump' of three lipodystrophy HIV-positive patients under HAART (highly active antiretroviral therapy) treatment undergoing lipectomy.

Peripheral lipodystrophy syndrome in HIV-positive patients under HAART treatment¹ manifests itself as dorso-cervical fat pad enlargement (buffalo hump) consisting of brown adipose tissue (BAT). To relieve functional limitations, such as compression syndrome, especially in the dorso-cervical region, causing discomfort, often neck pain, headaches off and on, as well as important aesthetic manifestations which can drastically affect these patients' lifestyle, they undergo surgical removal of excessive fat pad. The main limitations of lipectomy are the presence of an evident scar in the dorsal area, the expense caused by the need of a surgical room and team, and mandatory use of post surgical compressive medication to reduce the possible formation of a seroma. LIPOSTABIL (formulation of phosphatidylcholine /desoxycholate) has been subject to criticism in many countries because the application of a lipolytic substance by injection in localised lipolysis of adipocytes has never been well standardised in a proper protocol, although it is practiced by over 2000 physicians worldwide.² Therefore, experimental results reported here could assist in development of a specific protocol for treatment of outpatients.

Adipose tissue was surgically excised under sterile aseptic conditions to allow isolation of preadipocytes, expansion and differentiation into mature adipocytes.³ Primary preadipocytes were expanded and maintained in specific culture media containing differentiating factors such as human insulin, dexamethasone and methyl-isobutylxanthine. Cultured preadipocytes during expansion stage demonstrated a characteristic fibroblast-like morphology (Figure 1a, left panel). Following expansion, the cultured cells differentiated after 14 days (Figure 1a,

right panel) into mature adipocytes, characterised by a round shape and lipid-containing vacuoles.

In human adults, BAT, which is specialised in adaptive thermogenesis, is only present in small nests in limited body areas such as the mediastinum, retroperitoneum, axillae and neck. The UnCoupling Protein 1 (UCP1) is a marker of BAT, with a role of uncoupling between oxygen consumption and ATP synthesis in order to promote energy dissipation in the form of heat. Increased *UCP1* mRNA has been associated with non-stavudine (Zerit), PI (protease inhibitor)-containing HAART, and may be an adaptive response of HIV-positive HAART recipients to the increased fatty acid flux associated with PI therapy, contributing to the increased resting energy expenditure.⁴ The other, recently characterised marker of BAT, CD31 (platelet-endothelial cell adhesion molecule-1, PECAM-1),⁵ plays role in neo-angiogenesis and inhibition of vascular cell apoptosis, but also in atrophy of BAT.

Based on these considerations, we verified the expression of the CD31 marker in primary adipocytes isolated from 'buffalo hump' by immunocytochemistry analysis with anti-CD31 primary and fluorescent rhodamine-labelled secondary antibodies. The CD31-positive adipose cells (Figure 1b, right panel) displayed uniformly intense red fluorescence throughout all the cytoplasm. On the contrary, the intense staining (Figure 1b, left panel) was distributed in adhesion foci and, to a lesser extent, also in the cytoplasm of the positive control MVEC endothelial cells. These data confirmed the presence of adipocytes with BAT typology in the 'buffalo hump' tissue of HIV patients.

The lipolytic activity of LIPOSTABIL was evaluated *in vitro* by time-lapse video microscopy on three samples of primary, differentiated adipocytes derived from 'buffalo hump' of three post-antiviral HAART lipodystrophy patients. Figure 1c (left panel) represents contrast phase microscopy images from time-lapse video microscopy analysis of controls untreated, differentiated adipocytes maintained in culture for 24 h. The primary adipocytes treated with 20 µl/ml of LIPOSTABIL were lysed very efficiently (Figure 1, right panel), with only the cellular detritus remaining at the end of treatment of 24 h. The culture medium became opaque and contained a great quantity of lipids released from cellular vacuoles. At this concentration, the activity of LIPOSTABIL was optimal and it acted rapidly. The initial effects were noted just 3–5 h after administration.

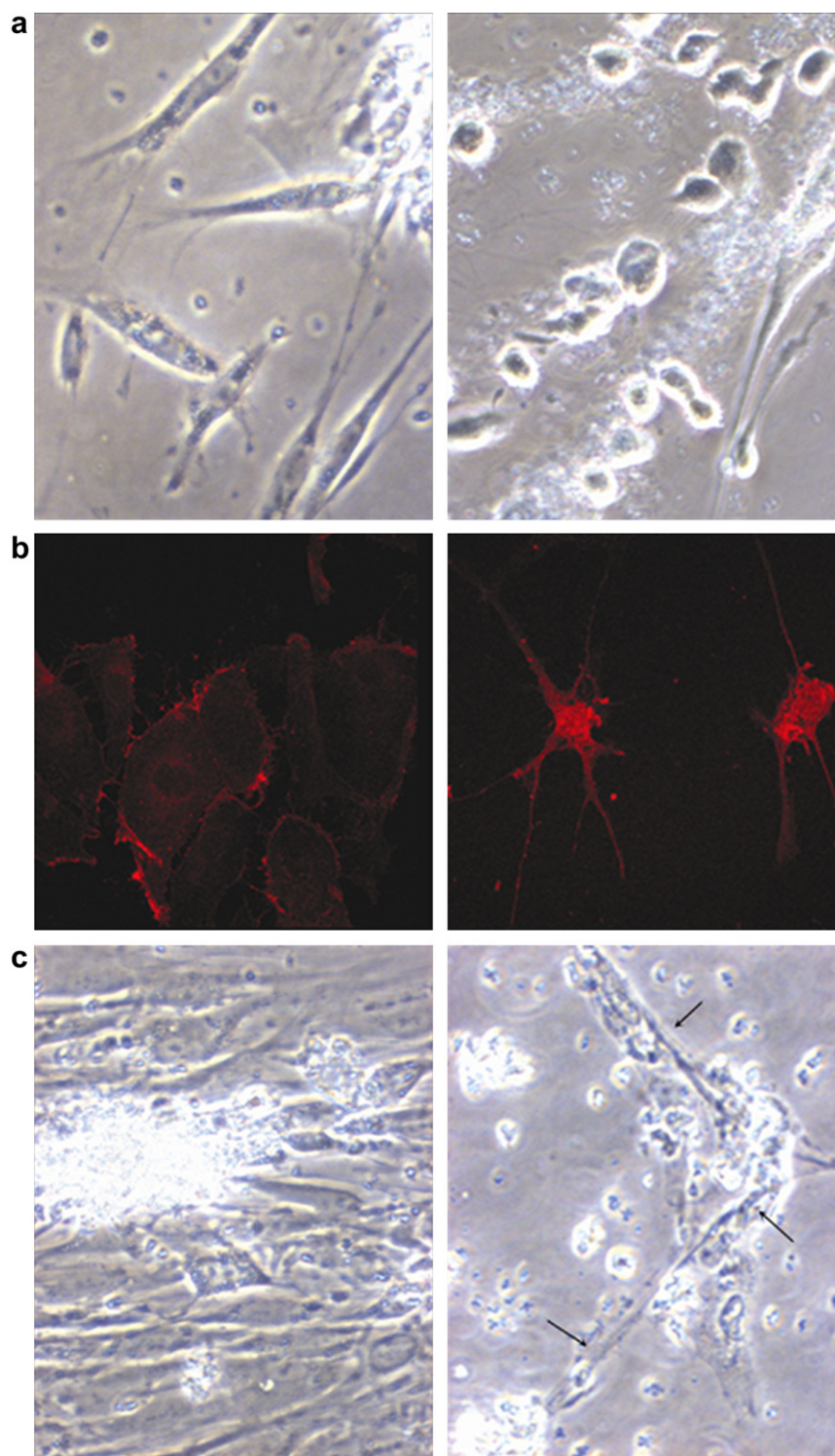


Figure 1 a) Morphology of cultured primary adipocytes. Left panel shows a phase contrast microscopic image of preadipocytes during expansion stage with a characteristic fibroblast-like morphology. Right panel shows differentiated adipocytes, characterised by rounded shape. b) Detection of BAT by immunocytochemistry with anti-CD31 antibody. The intense staining of MVEC endothelial cells used as positive control (left panel) was distributed in adhesion foci and, to less extent, also in the cytoplasm. By contrast, the CD31-positive adipocytes (right panel) displayed uniformly intense red fluorescence distributed throughout the entire cytoplasm. c) Time-lapse video microscopy analysis of live primary adipocytes (left panel) shows micro-image of untreated differentiated control adipocytes maintained for 24 h. The cultured adipocytes at the 5th hour following treatment with 20 µl/ml of LIPOSTABIL were effectively lysed (right panel). The arrows point to a few cells which remained intact.

We conclude that LIPOSTABIL has fast and thorough lipolytic activity *in vitro* against primary human adipose tissue containing BAT. Our results could assist in making of a specific protocol for treatment of patients with less extended 'buffalo hump' so that they can avoid invasive and expensive surgery. Major deformities would still require standard surgical lipectomy. Use of LIPOSTABIL would permit treatment at earlier stages of dorsal fat accumulation, allowing patients to postpone or even to eliminate the need for surgery. We consider LIPOSTABIL a useful, inexpensive and noninvasive alternative to the standard lipectomy for treatment of early and less extended 'buffalo hump'.

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Conflict of interest

None of the authors has a financial interest in any of the products, devices or drugs mentioned in this article.

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