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Rapid communication

Inhibitory actions of ropivacaine on tumor necrosis factor-α-induced leukocyte adhesion and tissue accumulation in vivo

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Abstract

We have examined the effect of ropivacaine, a local anesthetic, on leukocyte–endothelium interactions induced by tumor necrosis factor-α (TNF-α) in vivo by the use of intravital microscopy in the mouse cremaster microcirculation. It was found that ropivacaine markedly reduced venular leukocyte adhesion and tissue recruitment in response to TNF-α challenge, whereas leukocyte rolling was unchanged. Thus, treatment with ropivacaine may be a useful pharmacological tool to control acute inflammation.

Keywords: Inflammation; Leukocyte; Ropivacaine

1. Introduction

Local anesthetics have been suggested to have anti-inflammatory properties. For example, local administration of lidocaine and ropavacaine has been reported to be beneficial in colitis (Arlander et al., 1996). However, the mechanism of this protective effect remains elusive. Local anesthetics have been shown to influence a broad spectrum of cellular functions, which are relevant to inflammatory diseases. Thus, it has been reported that local anesthetics reduce phagocytosis (Cullen and Haschke, 1974), superoxide production (Peck et al., 1985), leukocyte–endothelium interactions and release of proinflammatory mediators from leukocytes (Martinsson et al., 1997). Tumor necrosis factor-α (TNF-α) is a pleiotrophic cytokine, which may play a fundamental role in inflammatory conditions by triggering leukocyte activation and tissue accumulation. Leukocyte recruitment in response to TNF-α is a multistep process, in which P-selectin-mediated rolling is a precondition for the subsequent firm adhesion and recruitment (Mayadas et al., 1993; Frenette et al., 1996). The objective of the present study was to examine the impact of clinical doses of locally administered ropivacaine on TNF-α-induced leukocyte rolling, adhesion and accumulation in postcapillary venules in vivo.

2. Materials and methods

We used male NMRI mice (23–28 g) anaesthesized with 7.5 mg ketamine hydrochloride (Hoffman-La Roche, Basel, Switzerland) and 2.5 mg xylazine (Janssen Pharmaceutica, Beerse, Belgium) per 100 g body weight intraperitoneally and the cremaster muscle was prepared for intravital microscopy as previously described (Baez, 1973). The preparation was performed on a transparent pedestal to allow transillumination and microscopic observation (BX50WI, Olympus Optical, Hamburg, Germany). The microscopic image was televised using a charge-coupled device videocamera (FK 6990 Cohu, Pieper , Berlin, Germany) and recorded on videotape for subsequent off-line analysis. Analysis of leukocyte rolling and adhesion were made in venules (20–40 μm) with stable resting blood flow. Rolling leukocyte flux was determined by counting the number of rolling leukocytes per 30 s, passing a reference point in the microvessel and expressed as cells per minute. Leukocyte adhesion (stationary for >30 s) was counted in 200–400-μm-long venular segments and expressed as number of adherent cells per millimeter. Intrascrotal injection of 0.5 μg of recombinant TNF-α (R&D Systems Europe, Abingdon, Oxon, UK) in 0.15 ml of phosphate buffer solution (PBS) or ropivacaine (65 and 650 μM, Narop, Astra, Södertälje, Sweden) was performed 3–4 h prior to microscopic observation. Samples of cremaster muscle tissue were fixed, stained with giemsa

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and extravascular leukocytes were counted (expressed as cells/mm^2). Statistical evaluations were performed using Kruskal–Wallis one-way analysis of variance on ranks for unpaired samples. The results are presented as mean values ± S.E.M. and n represents number of animals.

3. Results and discussion

It was found that ropivacaine dose-dependently reduced TNF-α-induced leukocyte adhesion. In fact, at 650 μM of ropivacaine, the level of leukocyte adhesion was reduced by 63% (Fig. 1b, $P < 0.05$ vs. TNF-α alone). In contrast, administration of ropivacaine had no effect on leukocyte rolling in response to TNF-α stimulation (Fig. 1a). Thus, these data suggest that the decreased adhesion was not attributable to changes in leukocyte rolling. This lack of effect of ropivacaine on leukocyte rolling is in contrast to a previous study by Martinsson et al., (1997). One explanation may be related to the differences in species and tissues, i.e. here, we studied striated muscle in mice and Martinsson et al., (1997) examined the effect of ropivacaine in the hamster cheek pouch. In addition, it is important to note that we investigated TNF-α-induced rolling, whereas Martinsson et al., (1997) applied ropivacaine in untreated animals. It has been reported that TNF-α-induced rolling may be more complex than spontaneous rolling in untreated animals and supported by multiple adhesion molecules, including P- and E-selectin (Kunkel et al., 1996), while rolling in untreated tissues is exclusively mediated by P-selectin (Mayadas et al., 1993). Thus, it may be possible that ropivacaine may inhibit P-selectin-dependent rolling, but such inhibition is not sufficient to interfere with TNF-α-induced leukocyte rolling. Notably, herein, we observed that 650 μM of ropivacaine also abolished tissue accumulation of leukocytes in response to TNF-α activation, i.e. leukocyte recruitment was reduced by 79% (Fig. 1c, $P < 0.05$ vs. TNF-α alone). This decrease in leukocyte accumulation was similar in extent to the reduction in leukocyte adhesion, suggesting that ropivacaine does not inhibit the transmigration process of inflammatory cells. These studies suggest that the main target of ropivacaine in the extravasation process of leukocytes is the firm attachment to the vascular endothelium. This notion is supported by previous findings showing that TNF-α-induced leukocyte adhesion is mediated by β_2-integrins (our unpublished data) and that ropivacaine inhibits the expression of β_2-integrins on leukocytes triggered by TNF-α (Martinsson et al., 1997).

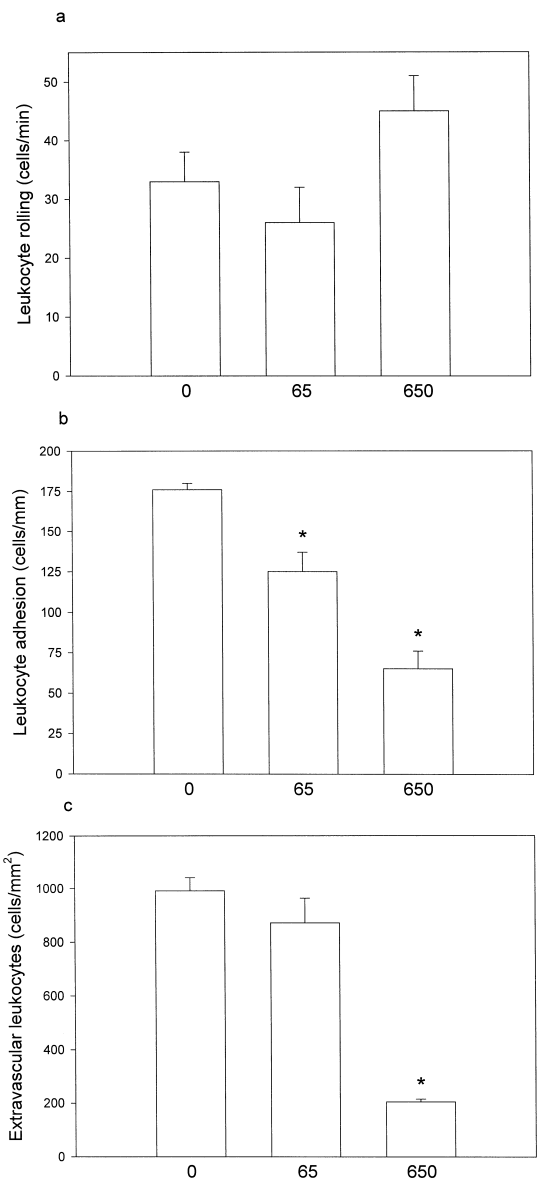

Fig. 1. TNF-α induced leukocyte (a) rolling, (b) adhesion, and (c) recruitment in the mouse cremaster muscle. TNF-α was administered intrascrotally together with 0.15 ml of PBS (0) or ropivacaine (65 or 650 μM) and analyzed 3–4 h later. Data represent mean ± S.E.M. Asterisks indicate significant difference ($P < 0.05$, $n = 5$ vs. TNF-α alone).

4. Conclusion

Our data indicate that ropivacaine attenuates TNF-α-provoked recruitment of leukocytes via inhibition of leukocyte adhesion to the endothelium. Thus, local administration of ropivacaine may be an effective pharmacological intervention in inflammatory diseases characterized by TNF-α activation and leukocyte infiltration.

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