ORIGINAL ARTICLE

Influence of high dose tumescent local anaesthesia with prilocaine on systemic interleukin (IL)-6, IL-8 and tumour necrosis factor-α

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Abstract

Background and objective Tumescent local anaesthesia (TLA) with high prilocaine doses leads to formation of methemoglobin (MHb) which is known to be a potent activator of pro-inflammatory endothelial cell response *in vitro*. As TLA is widely used for large dermatological resections, the aim of this study was to investigate the effects of high prilocaine doses on the systemic inflammatory response *in vivo* and its clinical relevance.

Methods This prospective study examines the influence of MHb on serum interleukin (IL)-6, IL-8 and tumour necrosis tumour necrosis (TNF)-α levels up to 72 h after application of TLA with prilocaine in doses higher than 600 mg.

Results A total of 30 patients received prilocaine in a median dose of 1500 mg (range: 880–4160 mg) for large resections. Peak prilocaine serum concentration was reached 4 h (0.72 ± 0.07 μg/mL), the maximum concentration of MHb (7.43 ± 0.87%) and IL-6 (28.4 ± 4.1 U/L) 12 h after TLA application. TNF-α and IL-8 release were not found significantly increased. Three patients developed MHb concentrations >15%.

Conclusions This clinical study shows for the first time that a high prilocaine serum concentration leads *in vivo* to elevated systemic levels of IL-6 but not of IL-8 and TNF-α because of initial high MHb levels. Because of possible and unpredictable high MHb concentrations, TLA should only be performed with prilocaine in doses of 2.5 mg/kg. In general, new solutions of TLA are necessary to achieve adequate anaesthesia for large dermatological resections to decrease the risk of methemoglobinaemia.

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Keywords

anaesthetic techniques, cytokines, dermatological surgery, methemoglobin, prilocaine, tumescent local anaesthesia

Conflict of interest

None declared.

Introduction

Tumescent local anaesthesia (TLA) is an anaesthesia technique where high fluid volumes containing diluted local anaesthetics are infused subcutaneously. Initially used to facilitate liposuction,[1] TLA is carried out for plastic, dermatological and proctological surgery also in ambulatory settings.[2–4] Reports of adverse side-effects after liposuction in TLA with lidocaine made prilocaine to become the preferred substance.[5] Prilocaine is an amide-type local anaesthetic with an extremely high distribution volume. However, prilocaine is known as a trigger for methemoglobinaemia by its metabolites *o*-toluidine and nitrosotoluidine.[6,7] Although an upper limit of 2.5 mg/kg prilocaine was recently recommended, some authors reported the use of up to 43 mg/kg.[8–10] Thus, elevated methemoglobin (MHb) serum levels are presumable, especially in extended resections in dermatological surgery, when large amounts of TLA are applied.[11] There is a high interindividual variability in the amount of MHb formed for any given dose of prilocaine administered but MHb is more likely to happen with higher doses.[12] This effect may also be potentiated by other oxidative drugs.[8] The production of MHb has clinical consequences: it causes a shift of the oxygen-dissociation curve to the left,[13] it can result in respiratory failure[14] and a variety of physically symptoms.

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Healthy individuals without anaemia have few symptoms at 15% MHb, but levels of 20–30% can cause mental status changes, headache, fatigue, exercise intolerance, dizziness and syncope and levels of 50% result in dysrhytmias, seizures, coma and death.[15] Furthermore, MHb is a critical mediator of the inflammatory response, as a product of NO and nitrate production, e.g. as a mediator of oxydative stress, and as the source of haeme and iron with additional proinflammatory and oxidative proclivities.[16]

Mainly known from sepsis research, endothelial cells act as direct targets for free MHb.[17,18] *In vitro* studies demonstrate that MHb is a potent activator of endothelial cells through NF-kB-mediated up-regulation of cell adhesion molecule expression, chemokine and cytokine production.[19]

Most patients treated with TLA-procedures are day surgery patients and therefore, a detailed knowledge about side-effects of prilocaine is necessary to guarantee high safety standards. So far, there are no clinical data published, describing *in vivo* inflammatory response as a result of high prilocaine doses under clinical conditions. This fact might be of significant importance for an early hospital discharge.

The present prospective study examines the influence of prilocaine-induced MHb on serum levels of interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α up to 72 h after application of TLA with prilocaine in doses higher than 600 mg in extended dermatological surgery and investigates clinical symptoms.

Methods

This prospective, single-centre, observational study was performed according to the guidelines of the Ethic Commission II, Faculty for Clinical Medicine Mannheim, Germany (Vote: 2007-258N-MA) and was registered internationally (International Standard Randomised Controlled Trial Number Register, ISRCTN: 19821978). From January until September 2008, 42 patients received in-house dermatological procedures in TLA. Eight patients did not meet the inclusion criteria whereas four declined participation. Verbal and written information was given to each of the 30 adult patients before informed written consent was obtained.

Inclusion and exclusion criteria

All patients [16 male/14 female, 18–85 years; American Society of Anaesthesiologists physical (ASA) status I–III] undergoing in-house dermatological procedures in TLA were eligible to take part in the study. Exclusion criteria were an allergy against prilocaine, patients considered to be ASA status IV–VI and pregnancy.

Patients and procedures

Prior to the scheduled operation, all patients had to see an anaesthesiologist for premedication and were informed verbally and in written form about the study before consenting to participate. All patients received 7.5 mg midazolam (Dormicum; Roche Pharma, Grenzach-Wyhlen, Germany) for oral premedication. Venous cannulation with a 16-gauge peripheral needle was performed in all patients undergoing the procedure (Liniest Purr; KLINIKA Medical, Singen, Germany) and an infusion with a maximum of 500 mL balanced crystalloid solution (Deltajonin; Delta Select, Dreieich, Germany) was started. ECG, non-invasive arterial blood pressure and SO_2 were measured at 5-min intervals throughout the entire operation.

Tumescent local anaesthesia

The TLA solution consisted of 2 mg prilocaine + 0.5 mg epinephrine (Xylonest 0.5% with Adrenalin 1 : 250, Astra Zeneca, Wedel, Germany) solved in 500 mL of balanced crystalloid solution (Deltajonin; Delta Select) and was manufactured directly before the start of surgery. Each patient received at least 600 mg prilocaine, which is equal to 150-mL of TLA solution via a TLA-pump (Cutanest, Liposat, Moeller Medical, Fulda, Germany). Optionally, propofol (Propofol 1%; Fresenius Kabi, Bad Homburg, Germany) was injected until a light level of sedation was reached, referring to an observer's assessment of alertness/sedation of 4–5 (OAA/S).[20] Oxygen was applied at a flow rate of 8 L/min via an oxygen mask. Respiration was monitored by measuring oxygen saturation and by semiquantitative CO_2 detection.

Blood samples and analysis

Blood samples were drawn before application of TLA solution (basal) as well as 1, 2, 4, 12, 24, 48 and 72 h. All blood samples (15 mL/sample) were drawn via a 16-gauge cannula inserted into the forarm vein and collected in heparinized syringes. MHb was immediately analysed on an ABL 835 (Radiometer, Willich, Germany) in haemolysed venous blood. Hb, lactate dehydrogenase (LDH), cytokeratin (CK), C-reactive protein (CRP) and procalcitonin (PCT) were analysed in the Institute of Clinical Chemistry of University Medical Centre Mannheim. After centrifugation at 20 °C (4000 U/min, 5 min), separated serum samples were stored at −20 °C until essayed. Using commercially available ELISA kits, serum levels of IL-6, IL-8 and TNF-α (using Immunotech EIA IL-6, IM1120, EIA IL-8, IM2237 and EIA TNF-α, IM1121, Marseille, France) were measured. Serum prilocaine levels were measured using liquid chromatography-mass spectrometry (Agilent 1200 HPLC-System, Waldbronn, Germany; HCT-Ultra, Bruker Daltonics, Bremen, Germany). Sample preparation contained protein precipitation via TCA, and dilution of supernatant 1 : 2 with buffer A (H2O LC-MS Chromasolv; Fluka, Steinheim, Germany; 0.2% Acetic acid Merck, Darmstadt, Germany; 2 mM Ammonium acetate; Merck, Darmstadt, Germany). High performance liquid chromatography was performed in buffer A using an analytical Synergie 4 U POLAR-RP 80A column (Phenomenex, Aschaffenburg, Germany) applying a linear gradient from 0% to 60% buffer B (Methanol Fluka, Steinheim, Germany; 0.2% acetic acid) over 10 min. Prilocaine was identified by his [MH] + ion with *m/z* 221.2 and quantified by the area of the base peak of this signal.

Cycloheximide treatment

To study if the upregulation of IL-6 production was resulting from *de novo* synthesis, whole blood samples were stimulated or not with prilocaine in the presence or absence of cycloheximide (CX). Whole blood (1.0 mL) was either unstimulated or stimulated for 24 h with prilocaine or *o*-toluidine in concentrations ranging from 0.2 to 20 μg/mL in the presence or absence of CX. CX-concentration (10 μg/mL) was optimized in the previous studies and a possible toxic effect of CX could be excluded. Serum was harvested and IL-6 production was measured using enzyme-linked-immunosorbent serologic assay.

Statistical analysis

Quantitative data are presented as mean ± SD. To evaluate the course of serum concentrations of prilocaine, MHb and cytokines by time, ANOVA for repeated measurements have been performed. Differences between two time points have been tested with *t*-tests for two paired samples. Test results with $P < 0.05$ were considered as statistically significant. For all statistical calculations, the SAS System (release 9.01; SAS Institute, Cary, NC, USA) has been used.

Results

A total of 30 patients (16 male/14 female, age 54.9 ± 18.1 years, body weight 85.0 ± 20.0 kg) received prilocaine in an average dose of 19.8 mg/kg (±5.6 mg/kg, median total amount: 1500, range: 880–4160 mg). Of all the patients, 20 had a malignant melanoma, eight suffered from acne inversa and two patient had malignant skin tumours (Table 1). Table 2 shows the serum concentrations of all measured parameters.

Table 1 Demographical and clinical characteristics

	(*n* = 30)
Gender (M/F)	16/14
Age (years)	54.9 ± 18.1
Body mass index (kg × m^2)	28.8 ± 6.0
Prilocaine (mg/kg)	19.8 ± 5.6
Malignant melanoma/acne inversa/malignant skin tumours	20/8/2

For quantitative data (except prilocaine dose), characteristic values are presented as mean ± SD.

Prilocaine serum concentration

Serum prilocaine (Fig. 1) had its peak with 0.72 ± 0.07 μg/mL 4 h after TLA application. Only in two patients prilocaine was detected 48 h after TLA in marginal increased concentrations ($P < 0.0001$ for changes in concentration over time; ANOVA).

MHb serum concentration

Basal MHb serum levels (Fig. 2) were 0.62 ± 0.03%. The level increased to 1.56 ± 0.13% after 1 h, to 3.06 ± 0.33% after 2 h, to 6.31 ± 0.8% after 4 h with a peak of 7.43 ± 0.87% 12 h after TLA (each $P < 0.0001$ after 2, 4 and 12 h compared with basal). MHb serum levels decreased rapidly to 1.36 ± 0.18% after 24 h and to 0.51 ± 0.05% after 48 h ($P < 0.0001$ for MHb serum level changes over time; ANOVA).

Table 2 Serum concentrations of all measured parameters

	Prilocaine (μg/mL)	MHb, % (range)	CRP (mg/L)	LDH (U/L)	PCT (ng/mL)
Basal	0	0.62 ± 0.03 (0.4–0.9)	5.4 ± 1.6	200.3 ± 7.2	0.109 ± 0.004
1 h	0.42 ± 0.05	1.56 ± 0.13 (0.6–3.2)	5.4 ± 1.5	197.3 ± 9.7	0.110 ± 0.004
2 h	0.55 ± 0.06	3.06 ± 0.33 (0.5–7.2)	5.4 ± 1.5	199.7 ± 10.1	0.108 ± 0.004
4 h	0.72 ± 0.07	6.31 ± 0.80 (1.5–17.9)	5.4 ± 1.5	202. 3 ± 9.3	0.108 ± 0.005
12 h	0.17 ± 0.05	7.43 ± 0.87 (1.7–20.7)	7.5 ± 1.9	210.7 ± 11.0	0.105 ± 0.003
24 h	0.04 ± 0.03	1.36 ± 0.18 (0.5–4.5)	14.4 ± 4.4	207.0 ± 10.7	0.105 ± 0.003
48 h	0.02 ± 0.01	0.51 ± 0.05 (0.1–1.3)	24.2 ± 5.4	206.3 ± 9.7	0.105 ± 0.004
72 h	Not available	Not available	Not available	Not available	Not available
	CK (U/L)	**IL-6 (U/L)**	**IL-8 (U/L)**	**TNF-α (U/L)**	
Basal	102.9 ± 10.5	9.5 ± 2.5	15.5 ± 3.6	15.8 ± 1.6	
1 h	102.5 ± 9.6	8.0 ± 2.1	12.5 ± 3.3	15.6 ± 1.6	
2 h	101.3 ± 10.1	9.0 ± 1.5	16.6 ± 4.2	15.4 ± 1.4	
4 h	103.6 ± 8.4	16.1 ± 1.7	11.6 ± 3.5	13.9 ± 1.5	
12 h	114.4 ± 8.8	28.4 ± 4.1	11.3 ± 3.0	13.5 ± 1.6	
24 h	121.3 ± 11.5	21.4 ± 3.7	11.8 ± 2.7	14.0 ± 1.5	
48 h	114.2 ± 11.7	16.1 ± 3.0	14.8 ± 3.6	15.6 ± 1.8	
72 h	Not available	14.8 ± 3.6	14.7 ± 2.9	16.3 ± 2.8	

Characteristic values are mean ± SD.

MHb, methemoglobin; CRP, C-reactive protein; IL, interleukin; TNF, tumour necrosis factor; CK, cytokeratin; LDH, lactate dehydrogenase; PCT, procalcitonin.

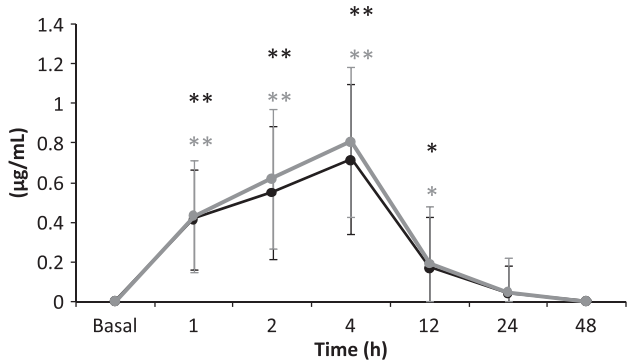

Figure 1 Serum prilocaine concentrations. Presented are mean ± SD of all patients (black line) and only patients with malignant melanoma (grey line). *$P < 0.001$ and **$P < 0.0001$ vs. sample 0 (basal).

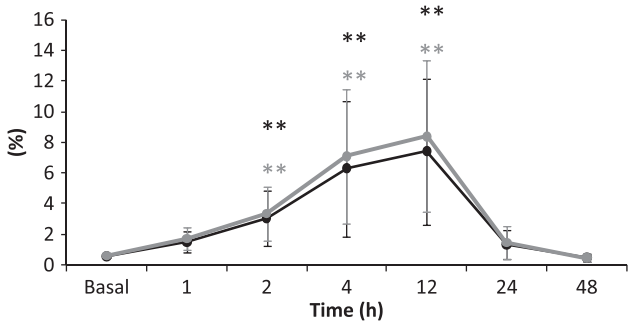

Figure 2 Serum methemoglobin concentrations. Presented are mean ± SD of all patients (black line) and only patients with malignant melanoma (grey line). **$P < 0.0001$ vs. sample 0 (basal).

Special clinical courses

Overall, an MHb concentration >15% was measured in three patients:

1 One 44-year-old male and one 67-year-old female patient developed a methemoglobinaemia (20.7% and 20.0%) with a maximum at 12 h after TLA. No clinical symptoms were observed and no specific therapy was indicated.
2 Four hours after TLA, one 18-year-old female patient suffering from a malignant melanoma developed mild symptoms of cyanosis and hyperventilation at a maximum MHb concentration of 17.9%. She received oxygen via a face mask and a mild sedation. The symptoms disappeared about one hour later. The MHb concentration was found decreased to 8.0%, 12 h after prilocaine application.
3 We conducted the calculations with all patients ($n = 30$) and in addition only with patients, suffering from a malignant melanoma ($n = 22$). It may be speculated that patients with malignant melanoma react differently to prilocaine application. This hypothesis could be excluded (Figs 1–3).

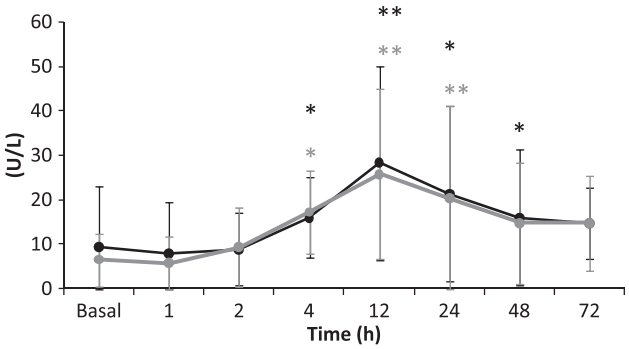

Figure 3 Serum concentrations of interleukin-6. Presented are mean ± SD of all patients (black line) and only patients with malignant melanoma (grey line). *$P < 0.05$ and **$P < 0.0001$ vs. sample 0 (basal).

IL-6 serum concentration

Basal IL-6 serum level (9.5 ± 2.5 U/L; Fig. 3) was altered after 1 and 2 h, but increased significantly to 16.1 ± 1.7 U/L after 4 h ($P = 0.0149$ compared with basal) coinciding with the systemic maximum concentration of prilocaine. IL-6 concentration reached a peak maximum of 28.4 ± 4.1 U/L ($P < 0.001$) 12 h after TLA. After 24 h IL-6 level decreased to 21.4 ± 3.7 U/L ($P = 0.0002$), to 16.1 ± 3.0 U/L after 48 h ($P = 0.0437$) and reached basal level after 72 h ($P < 0.0001$ for changes in response over time; ANOVA).

IL-8 and TNF-α serum concentrations

Serum levels of IL-8 and TNF-α serum did not change significantly in the chronological sequence compared with baseline levels (Table 2; $P = 0.2667$ and 0.3171 respectively). Furthermore, no statistically significant correlation between the applied doses of prilocaine and MHb or prilocaine serum levels and IL-6, IL-8 or TNF-α concentrations could be detected.

CRP serum concentration

From basal level of 5.4 ± 1.6 mg/L, the concentration of CRP increased to 7.5 ± 1.9 mg/L and to 14.4 ± 4.4 mg/L at 12 and 24 h respectively ($P = 0.0030$). A CRP peak maximum of 24.2 ± 5.4 mg/L was reached at 48 h ($P < 0.0001$ compared with basal levels).

Serum concentrations of CK, LDH and PCT

We observed no statistically significant change in serum concentrations of CK, LDH and PCT over the described period of the study ($P = 0.1666$, 0.9590 and 0.8599 respectively). The analysis of PCT was stopped after 20 patients as no correlation was found.

Correlations

At no time statistically significant correlations between the applied prilocaine doses and the serum prilocaine concentrations were found. However, an association between the serum prilocaine concentration and the simultaneously measured MHb concentration at 2 h ($r = 0.38686$, $P = 0.0347$) and at 4 h was detected ($r = 0.49455$, $P = 0.0055$). Indeed, MHb concentration after 4 h and after 12 h seemed to be directly influenced by the serum prilocaine concentration measured earlier ($r = 0.50126$ with $P = 0.0048$ and $r = 0.41852$ with $P = 0.0214$ respectively). No correlation between MHb and IL-6 concentration was found at any time.

In vitro analysis

Interleukin-6 synthesis was completely inhibited under simultaneous CX and prilocaine stimulation, indicating the *de novo* synthesis of this chemokine by immunmodulatory cells (data not shown). The same experiments were performed with *o*-toluidin alone, where also a complete inhibition of IL-6 synthesis was observed. However, no differences compared with the results under prilocaine stimulation were observed.

Discussion

This clinical study demonstrates for the first time that high doses of prilocaine induce MHb generation and the enhanced release of IL-6, but not of IL-8 or TNF-α *in vivo*. IL-6 was *de novo* synthesized upon prilocaine therapy, as no IL-6 could be detected in serum of stimulated or unstimulated blood that was cultured in the presence of CX.

Our study based on the results of a recent manuscript demonstrating the MHb-induced production and release of IL-6 and IL-8, as well as elevated membrane expression of E-selectin *in vitro*.[19] This activation is mediated by mechanisms initiated at the cell membrane level or through intermediates of an MHb-dependent catabolism upstream of hemoxygenase-1 action. These *in vitro* observations indicate that the presence of free MHb in blood or interstitial fluid may contribute to endothelial cell activation.

Interleukin-6 is the main stimulator of the production of acute-phase proteins in the liver.[21] Specifically, the expression of CRP, a preferred marker for the systemic inflammatory reaction[22] is under the direct influence of IL-6. Indeed, the observed CRP serum concentrations (beginning to increase at 12 h after TLA) represent the time lapse of CRP transcription induction in response to increasing systemic IL-6 concentrations. Other authors found a correlation of CRP and IL-6 as an index of oxidative stress in patients with high risk of cardiovascular disease.[23] We interpret the increase of IL-6 and CRP not as a sign of an acute infection but as a prilocaine-induced phenomenon. A positive correlation between serum CRP and IL-6 as well as IL-8 was found during the first year after lung transplantation which was explained by systemic or local inflammation after transplantation.[24] In contrast to the early detectable IL-6, we did not observe increased concentrations of IL-8 at any time. It seems that an acute-phase reaction is initiated but not prolonged over a 12-h period.

Current publications recommend a dose limitation of 2.5 mg/kg.[8] The applied dose in our study was higher than that in the studies of Lindenblatt (6.8 ± 0.8 mg/kg) and Rudlof (6.88 mg/kg), comparable with Sagoo (10–20 mg/kg) but lower than the excessive doses used in the study of Mang *et al.* (28.8–42.6 mg/kg).[3,9,10,25] The high amount of TLA was necessary to achieve an adequate anaesthesia for the large dermatological resections. To our knowledge, there is no other TLA solution published yet, which can be applied with lower risks.

Methemoglobinaemia can induce direct hypoxic injury as well as organ damage through inflammation.[16] Patients can be symptomatic from methemoglobinaemia and require treatment with values as low as 8%.[8,26] This value was exceeded in our study to up to 20.7%.

In general, lidocaine and prilocaine may be used for TLA. Reports about fatal complications after tumescent liposuction with lidocaine prohibit its application.[5,27] Usually a 0.05% prilocaine solution is recommended for liposuction.[9] In the case of larger dermatological procedures, such as extended tumour resections, the anaesthetic potency is usually insufficient. However, prilocaine in a concentration of 0.4% provides adequate analgesia, but in extended resections increased MHb levels were detected.[28]

Taken together, even high doses of prilocaine do not necessarily lead to a clinically significant methemoglobinaemia. MHb concentration after 4 h and after 12 h seemed to be directly influenced by the serum prilocaine concentration measured earlier, while no correlation between MHb and IL-6 concentration was found at any time. MHb is a stimulator for a significant systemic elevation of IL-6, but not for IL-8 or TNF-α. We did not observe acute-phase reactions in response to threefold increased systemic concentrations of IL-6. Therefore, TLA with high dose prilocaine is considered to be safe in case of inflammatory response and provides appropriate anaesthetic effects also in day surgery. As a result of possible and unpredictable high MHb concentrations, TLA can only be recommended with prilocaine in doses of 2.5 mg/kg.[8]

Further investigations are now necessary to explain the difference in cytokine production on molecular level and to improve the development of TLA solutions to reach an adequate analgesia and to minimize side-effects, in extended dermatological resections.

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