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Article CopyRight	The Author(s), under exclusive licence to Springer-Verlag GmbH, DE part of Springer Nature (This will be the copyright line in the final PDF)	
Journal Name	Archives of Toxicology	
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Schedule	Received	30 July 2020
	Revised	
	Accepted	4 January 2021

Abstract

Hyperbaric 2% prilocaine is increasingly used for spinal anesthesia. It is the only local anesthetic metabolized to o-toluidine, a human bladder carcinogen. Increase of o-toluidine hemoglobin adducts, a

marker of o-toluidine ability to modify the DNA structure, was described following subcutaneous injection. In this prospective cohort study we aimed to assess and quantify o-toluidine hemoglobin adducts and urinary o-toluidine after a single intrathecal dose of hyperbaric prilocaine.
10 patients undergoing surgery received 50 mg of hyperbaric prilocaine intrathecally. Blood and urine samples were collected before injection and up to 24 h later (Hospital Braine l'Alleud-Waterloo, Belgium). Urinary o-toluidine and o-toluidine hemoglobin adducts were measured by tandem mass-spectrometry after gas-chromatographic separation (Institute of the Ruhr-Universität, Bochum Germany). The trial was registered to ClinicalTrials.gov (NCT03642301; 22-08-2018)
Intrathecal administration of 50 mg of hyperbaric prilocaine leads to a significant increase of o-toluidine hemoglobin adducts (0.1 ± 0.02–11.9 ± 1.9 ng/g Hb after 24 h, $p = 0.001$). Peak of urinary o-toluidine was observed after 8 h (0.1 ± 0.1–460.5 ± 352.8 µg/L, $p = 0.001$) and declined to 98 ± 66.8 µg/L after 24 h (mean ± SD)
Single intrathecal administration of hyperbaric prilocaine leads to a systemic burden with o-toluidine and o-toluidine hemoglobin adducts. O-toluidine-induced modifications of DNA should be examined and intrathecal hyperbaric prilocaine should not be proposed to patients chronically exposed to o-toluidine.
Clinical trial number and registry URL
NCT03642301.

Keywords (separated by '-')	Local anesthetic - Hyperbaric prilocaine - o-toluidine - Hemoglobin adducts - Spinal anesthesia
Footnote Information	Emmanuel Guntz and Andrea Carini contributed equally to this work as first co-authors.

Archives of Toxicology
https://doi.org/10.1007/s00204-021-02973-w

TOXICOKINETICS AND METABOLISM

Quantification of systemic o-toluidine after intrathecal administration of hyperbaric prilocaine in humans: a prospective cohort study

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Received: 30 July 2020 / Accepted: 4 January 2021

Abstract

Hyperbaric 2% prilocaine is increasingly used for spinal anesthesia. It is the only local anesthetic metabolized to o-toluidine, a human bladder carcinogen. Increase of o-toluidine hemoglobin adducts, a marker of o-toluidine ability to modify the DNA structure, was described following subcutaneous injection. In this prospective cohort study we aimed to assess and quantify AQ1 o-toluidine hemoglobin adducts and urinary o-toluidine after a single intrathecal dose of hyperbaric prilocaine.

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Clinical trial number and registry URL NCT03642301.

Keywords Local anesthetic · Hyperbaric prilocaine · o-toluidine · Hemoglobin adducts · Spinal anesthesia

Introduction

Prilocaine, an amide-type local anesthetic, has been used for decades in different composition and formulations, such as EMLA gel for venipuncture in children, gel for the treatment of premature ejaculation, as well as for subcutaneous injections for tumescent liposuction or head and neck surgery. Spinal administration of prilocaine was proposed in 1965 (Crankshaw 1965) but withdrawn from the market in 1978 in England due to stability problems related to the production procedure (Hillmann 1978; Robertson 1978). Since 2009, a new and stable formulation of 2% hyperbaric prilocaine for intrathecal administration has been developed and increasingly used in day-case surgery setting. The ever-growing use of this drug led to several studies defining the optimal doses for different types of surgery (Gebhardt et al. 2013, 2014; Kaban et al. 2014; Guntz et al. 2014). Metabolism of prilocaine and particularly its downstream metabolite o-toluidine was also studied (Fig. 1) (Hjelm et al. 1972). Initially,

Emmanuel Guntz and Andrea Carini contributed equally to this work as first co-authors.

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prilocaine is hydrolyzed by the carboxylesterases CES 1A and CES 2 to o-toluidine (Higuchi et al. 2013). Further on, o-toluidine is activated to metabolites which can induce the formation of methemoglobin, hemoglobin and DNA adducts (Käfferlein et al. 2014; Böhm et al. 2011; Skipper et al. 1990; Ringe et al 1988) Several phase I and II enzymes, some of which exhibit polymorphisms, are involved in prilocaine and o-toluidine metabolism.

Prilocaine is the only local anesthetic whose metabolism leads to the formation of o-toluidine, an aromatic amine which was classified as a human carcinogen some years ago (IARC 2010; DFG 2010). Indeed, o-toluidine exposure has been linked to an increased risk of bladder cancer by epidemiological studies among workers from rubber chemicals and azo dyes factories (Ward et al. 1996). Moreover, Gaber et al. also reported that a single 100 mg subcutaneous injection of prilocaine induces a 40-fold increase of hemoglobin adducts of o-toluidine (Gaber et al. 2007).

Formation of systemic o-toluidine can be detected by measuring its presence in urine as the sum of free and conjugated urinary o-toluidine and by measuring hemoglobin adducts of o-toluidine in blood. Consecutive values of urinary o-toluidine can provide useful information on quantitative metabolism and kinetics of prilocaine and its metabolite o-toluidine. As hemoglobin adduct formation of aromatic amines needs the same metabolic activation as DNA adducts, measuring of hemoglobin adducts can be considered as a quantitative proxy for DNA adducts formation (Skipper et al. 1990).

In the field of anesthesia, to our knowledge, production of hemoglobin adducts after administration of prilocaine was only studied by Gaber et al. after subcutaneous administration (Gaber et al. 2007).

Therefore, in this prospective cohort study, we aimed to assess a single intrathecal dose of 50 mg of hyperbaric prilocaine as a source of systemic o-toluidine: hemoglobin adducts of o-toluidine and urinary o-toluidine were quantified using biological monitoring, before and after administration of the drug.

Materials and methods

Ethics approval was obtained by the local Medical Ethics Committee, (code EC 332, OM 157; B076201836443, Chairperson Dr Etienne Stevens) and the trial was registered in the publicly accessible study register ClinicalTrials.gov (NCT03642301). Ten patients scheduled for non-urgent lower-limb surgery were enrolled in the study and signed informed consent was obtained. Inclusion criteria were aged 18 or older and ASA status I or II. Patients with standard contraindications to neuraxial block, neurological impairment, known allergy to local anesthetics, liver or renal failure, as well as smokers and patients with a history of potential environmental or occupational exposure to o-toluidine were excluded from the study. Surgeries were all performed at Hospital Braine l'Alleud-Waterloo (CHIREC), Belgium.

Anesthesia protocol

Anesthetic management is summarized: first placement of an IV line with a normal saline infusion (500 mL NaCl 9 g/L), followed by standard monitoring application (non-invasive blood pressure, EKG, SpO_2). The patient was placed in a sitting position, the intervertebral space L4-L5 was clinically identified, the injection site was disinfected with alcohol chlorhexidine 0,5%. A local anesthesia of the injection site was performed with 3 mL of 2% lidocaine. Spinal anesthesia was administered with a 25G Whitacre spinal needle (Becton Dickenson, Madrid, Spain), 50 mg of hyperbaric prilocaine 2% (Nordic Pharma, Wilrijk, Belgium) were injected and the patient was positioned supine for the surgery.

Blood and urinary tests were performed according to the following scheme:

Blood: Blood samples were collected before intrathecal anesthesia and 24 h after (5 ml of blood in an EDTA tube).

Urine: Urine samples were collected before intrathecal anesthesia and at 8, 16 and 24 h post procedure.

Blood and urinary samples were stored at 6 °C and transported for analysis to a specialized laboratory within 30 h: Department of Human Biomonitoring, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-Universität, Bochum Germany.

Chemicals

o-Toluidine, 2-(*N*-morpholino) ethanesulfonic acid (MES), and heptafluorobutyric anhydride (HFBA) were all purchased from Sigma-Aldrich (Steinheim, Germany). The o-toluidine-d_7 was purchased from Toronto Research Chemicals Inc. (North York, Canada). Deionized water was obtained using a Millipore Advantage A10 with a Quantum® cartridge. Ethanol, diethyl ether, sodium hydroxide and concentrated hydrochloric acid were obtained from Merck (Darmstadt, Germany).

Determination of o-toluidine in urine

The o-toluidine was measured in urine according to Weiss and Angerer, with slight modifications (Weiss et al. 2002). In short, 5 mL urine was hydrolysed with concentrated hydrochloric acid, neutralized with a sodium hydroxide solution and adjusted to pH 6.4 with a 2-(N-morpholino) ethanesulfonic acid buffer system. The buffered solution was

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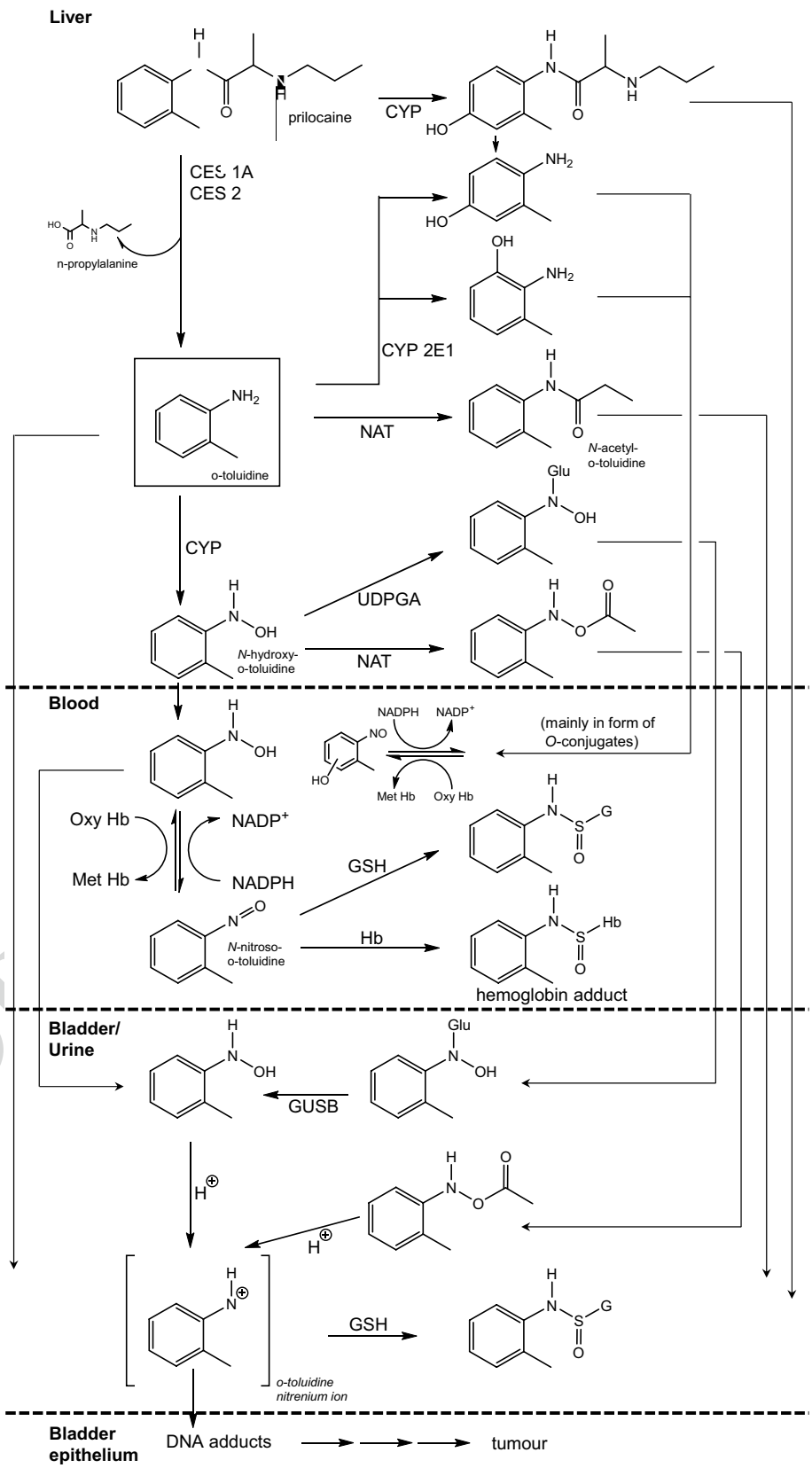

Fig. 1 Proposed simplified metabolic pathway of prilocaine and its downstream metabolite o-toluidine according to Higuchi et al. (2014), Gaber et al. (2007), Lewalter (1994) and Weiß (2005). CES: carboxylesterases 1A and 2; CYP: Cytochrome P450; NAT: N-acetyltransferase; UDGPA: Uridine diphosphate glucuronic acid; GSH: Glutathione; GUSB: Beta-glucuronidase; COX: Cyclooxygenase; NADP: Nicotinamide adenine dinucleotide phosphate; Hb: Hemoglobin; OxyHb: Oxyhemoglobin; MetHb: Methemoglobin

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extracted once with 5 mL n-hexane. After centrifugation, the n-hexane layer was transferred to another vial, vaporized to about 1 mL in a SpeedVac system and derivatized with heptafluorobutyric anhydride. After washing with a phosphate buffer (pH 8), the organic solvent was evaporated to 30 µL in a vacuum centrifuge. Quantification was carried out after gas chromatographic separation with tandem mass spectrometry (GC–MS/MS) using o-toluidine-d_7 -as an internal standard. The within series (1.0 µg/L ± 5.9%; 15.1 µg/L ± 3.7%) and between days (1.0 µg/L ± 7.4%; 15.1 µg/L ± 4.9%) inaccuracies were each < 10%. The creatinine content of the urine samples was determined according to Larsen (Larsen 1972). In clinical terms, urinary creatinine is regularly used as a parameter to verify the kidney function of patients. In occupational and environmental exposure monitoring creatinine adjustment is routinely used to normalize analyte concentrations in spot or consecutive urine samples for urinary dilution (Barr et al. 2005).

Determination of hemoglobin adducts of o-toluidine in blood

As described by Weiss et al. five millilitres of EDTA whole blood were centrifuged at 1200 g for five minutes (Weiss et al. 2002, 2013). The supernatant was carefully pipetted off. The resulting erythrocytes concentrate (approx. 2.5 mL) was made up with 0.9% NaCl solution to a volume of 5 mL and centrifuged for five min at 1200 g. The supernatant was carefully pipetted off again and discarded. This procedure was repeated until the supernatant was no longer yellow. For lyses, the isolated erythrocytes were finally diluted with 2.5 mL deionized water and stored at −20 °C until further processing.

Hemoglobin was isolated from the lysed erythrocytes solution by precipitation according to Lewalter et al. (2001). For 10 min, the red blood cell solution was centrifuged at 1200 g, the supernatant removed and 20 mL ethanol was added to precipitate the hemoglobin. After hemoglobin had settled, the supernatant was decanted and disposed. The precipitated hemoglobin was then transferred to an empty SPE column with PE filter. The hemoglobin was immediately washed with water, followed by an ethanol/water solution, an ethanol/diethyl ether solution and diethyl ether. Finally, the hemoglobin was sucked dry and stored at −20 °C until further processing.

Then 200 mg hemoglobin was dissolved in a 1 N sodium hydroxide solution with the help of an ultrasonic bath. The solution was then shaken for one hour on a laboratory shaker to allow hydrolysis of covalently bound aromatic amines. The solution was then extracted with 5 mL n-hexane and transferred to another vial after centrifugation, evaporated to approx. 1 mL in a vacuum centrifuge and derivatized with heptafluorobutyric anhydride. After washing with a phosphate buffer (pH 8), the organic solvent was evaporated to 30 µL. Quantification was carried out after gas chromatographic separation by tandem mass spectrometry (GC–MS/MS) in NCI mode with methane as a reactant gas. The o-toluidine-d_7 was used as an internal standard. The within series (0.5 ng/g Hb ± 3.8%; 5.3 ng/g Hb ± 5.6%) and between days (0.5 µg/L ± 9.7%; 5.3 µg/L ± 6.3%) inaccuracies were each < 10%.

Data analysis

The quantitative evaluation of the mass spectrometric data was carried out with the Masshunter software (Agilent, Waldbronn, Germany). Further data analysis was performed with Microsoft Excel 2010 and Graph Pad Prism Version 7. Values are expressed as mean ± standard deviation. A Wilcoxon matched pairs signed rank test was used for testing significance levels ($p < 0.05$). The sample size was based on our previous experience with similar occupational exposure study design (Korinth et al. 2007).

Results

Urinary analysis after 8 and 16 h were not recorded and analysed for patient 1; values at 0 and 24 h were included in the statistical analysis.

Intrathecal administration of 50 mg of hyperbaric prilocaine led to a significant increase of hemoglobin adducts of o-toluidine (0.1 ± 0.02 to 11.9 ± 1.9 ng/g Hb after 24 h, $p = 0.001$, Fig. 2, Table 1). Mean peak of urinary o-toluidine was observed after 8 h (0.1 ± 0.1–460.5 ± 352.8 µg/L, $p = 0.001$) and declined to 98 ± 66.8 µg/L after 24 h (Figs. 3, 4a). After adjustment to urinary creatinine (Fig. 4b) all patients showed their maxima in the samples withdrawn 8 h after prilocaine application. In patient five, the unadjusted and creatinine adjusted urinary level of o-toluidine was nearly the same after 8 and 16 h (Fig. 4a,b). In patient two, we observed the lowest Hb adduct concentration, as well as the highest urinary levels (Fig. 4a,b; Table 1; o-toluidine Hb-adducts: 8.9 ng/g Hb, urinary o-toluidine peak at 8 h post exposure: 1319 µg/L).

Discussion

In the current study, a single intrathecal administration of 50 mg of hyperbaric prilocaine led to an increase in both urinary o-toluidine and hemoglobin adducts of o-toluidine.

Human data on the elimination kinetics of o-toluidine are, to our knowledge, not available in the literature. Therefore, information from animal experiments were taken to develop the sample drawing schemes for the present study.

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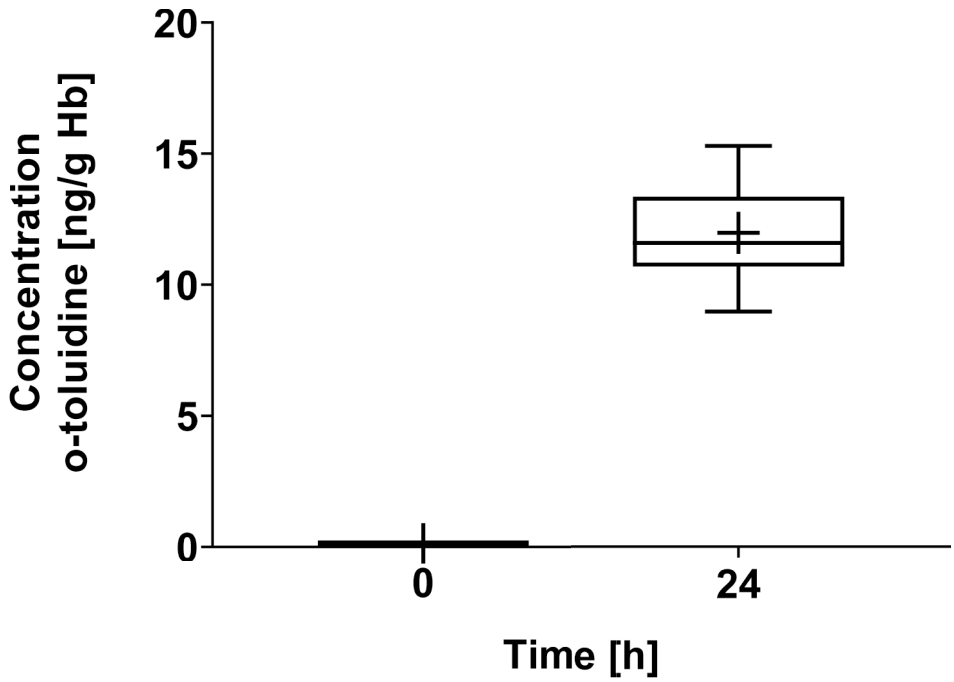

Fig. 2 Blood concentrations of hemoglobin adducts of o-toluidine after intrathecal administration of 50 mg of hyperbaric prilocaine. Samples were collected before administration and after 24 h. o-Toluidine-hemoglobin adducts are expressed as median, mean and whiskers from 5 to 95th percentile

Table 1 Results of analysis o-toluidine as metabolic product of prilocaine in blood as Hb-adducts and urinary o-toluidine

	o-Toluidine Hb-adducts [ng/g Hb]		Urinary o-toluidine [µg/L]			
Sampling point [h]	0	24	0	8	16	24
Number of values	10	10	10	9	9	10
Minimum	0.08	8.97	0.03	174.4	52.96	37.1
25% Percentile	0.10	10.69	0.03	240.5	122.9	41.0
Median	0.11	11.6	0.06	351.6	271.9	80.0
75% Percentile	0.12	13.35	0.13	558.1	462.5	143.5
Maximum	0.12	15.29	0.44	1319	626.2	218.5
Mean	0.10	11.98	0.11	460.5	304	98.0
Std. deviation	0.02	1.85	0.13	352.8	195.5	66.8

In rats, approximately 5% of a subcutaneous administered o-toluidine dose is rapidly eliminated within 24 h into urine, in the form of unchanged or conjugated o-toluidine, within 6 h it was about 3.6% (Son et al. 1980). More than 74% of the subcutaneously administered o-toluidine dose was eliminated in the form of urinary metabolites within 24 h (Kulkarni et al. 1983). As no toxicokinetic data were available concerning the hydrolysis of prilocaine to o-toluidine, we decided to collect urine samples at several points of time (0, 8, 16, 24 h after application). Hemoglobin adduct formation requires hydrolysis of prilocaine to o-toluidine and further activation to o-nitrosotoluidine (Fig. 1). Considering the rapid metabolism of prilocaine, its short half-time in plasma, the observation that hemoglobin adducts of aromatic amines are not repaired and are expected to have the same lifetime as erythrocytes, blood sampling was scheduled 24 h after application (Åkerman et al. 1966; Klein et al. 1994; Skipper et al. 1990).

The German Commission for the investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission), as well as the German Human Biomonitoring Commission, have both set the reference value of 0.2 µg o-toluidine per liter urine, which represents the 95th percentile of the background burden in the general population (DFG (Deutsche Forschungsgemeinschaft Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe 2007). Reference values for hemoglobin adducts of o-toluidine have not been established to date. Weiß et al. measured

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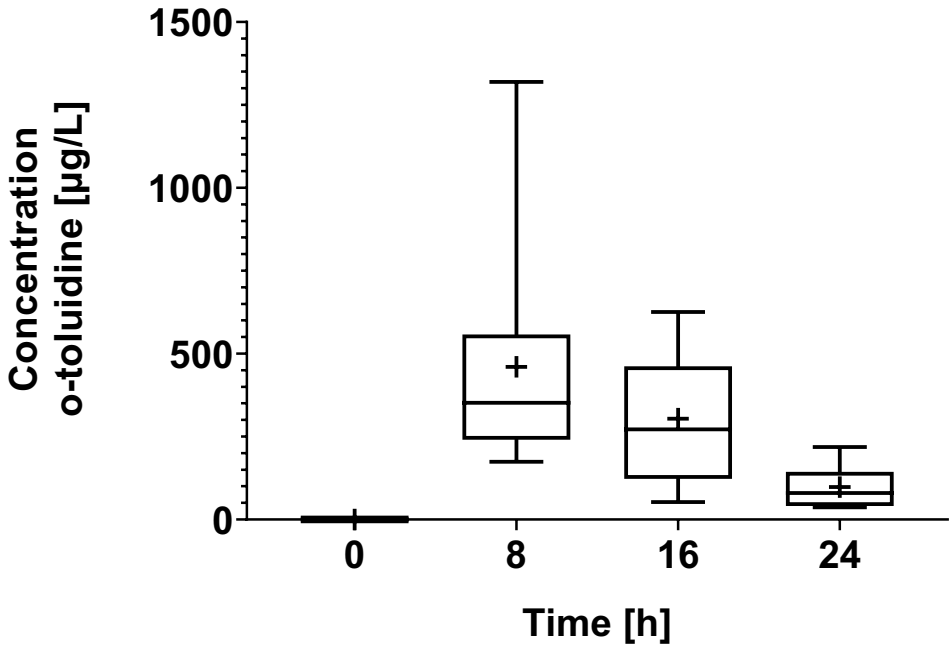

Fig. 3 Urinary concentrations of o-toluidine after intrathecal administration of 50 mg of hyperbaric prilocaine. Samples were collected before injection (0) and after 8, 16 and 24 h. Urinary o-toluidine is expressed as median, mean and whiskers from 5 to 95th percentile

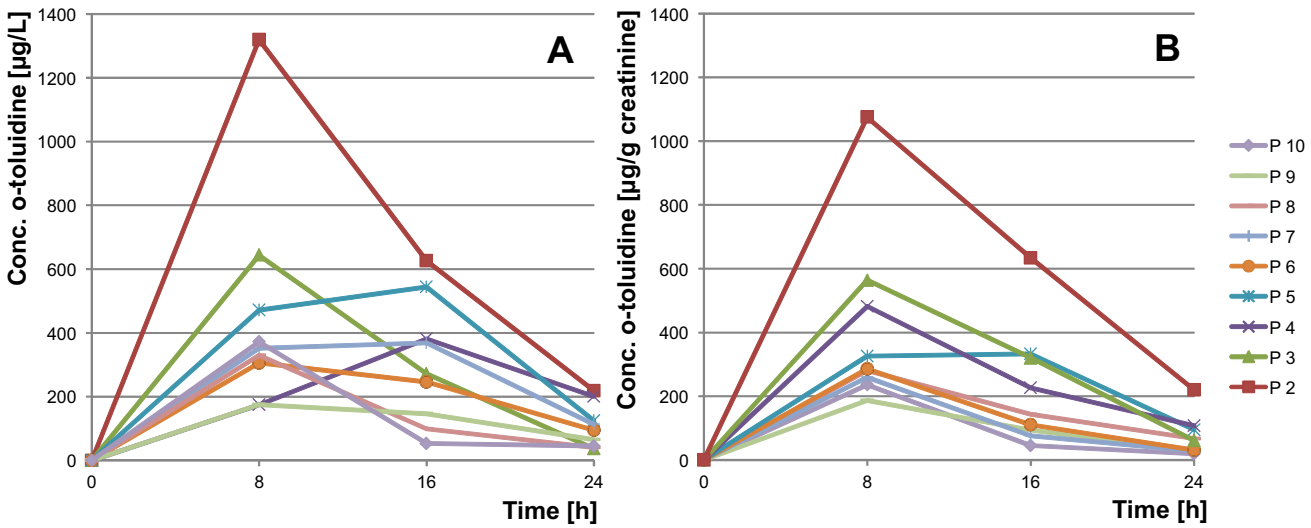

Fig. 4 Values of urinary o-toluidine **a** and creatinine adjusted urinary o-toluidine **b** in 9 patients during 24 h after intrathecal administration of 50 mg of hyperbaric prilocaine (patient 1 was excluded as only two samples were available for anlysis)

o-toluidine adducts in 200 people from the German general population and found median concentrations of 0.14 ng/g Hb in 154 non-smokers and 0.16 ng/g Hb in 46 smokers (Weiss et al. 2002).

Our results have shown that before the administration of prilocaine, patients exhibited concentrations of hemoglobin adducts and urinary o-toluidine in the same concentration range than that regularly found in the general population (Weiß et al. 2000, 2005; Kütting et al. 2009). Two patients in the present study had urinary o-toluidine exceeding the reference value (patient 2: 0.21; patient 5: 0.44 µg/L) before injection of prilocaine.

A significant increase of urinary o-toluidine was observed, with maximum concentrations in samples withdrawn 8 h after intrathecal administration. In the samples that were withdrawn at 16 and 24 h, urinary concentrations gradually decreased but did not reach baseline within 24 h (Fig. 3). These data allowed the calculation of an approximate estimate of the elimination half-life of o-toluidine

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in urine, after prilocaine administration, which was in between 8 and 11 h.

The mean urinary o-toluidine concentrations, 8 h after application of prilocaine, were clearly higher than those reported in occupationally workers exposed during the production of o-toluidine based rubber chemicals or in, both non-smoking and smoking rubber workers (Table 2) (Ward et al. 1996; Korinth et al. 2007). The mean concentrations in the present study were approximately 4.5 times higher than the means reported by Ward et al. and 20 times higher than the means reported by Korinth et al. One worker from Korinth et al. was exposed to an airborne o-toluidine concentration near the former technically based German occupational exposure limit (OEL) and the current binding occupational exposure limit value (BOELV) of the European Union (500 µg/m^3) and presented a urinary o-toluidine concentration of about 100 µg/L. The patients of the current study had a mean cumulative exposure of about 3.5 times more than a rubber worker during one single 8 h exposure at the BOELV.

In regard to hemoglobin adducts of o-toluidine, we found a sharp increase, which is comparable to values reported by Gaber et al. In this study, authors had injected subcutaneously in the highly vascularized head and neck area, the double dose of prilocaine (100 mg), compared to the one we used. Mean hemoglobin adduct concentrations were found nearly double of the mean shown by our results. It is noteworthy that the values of hemoglobin adducts in the present work, as well as the values in Gaber's et al. study, are of the same order of magnitude as the levels of adducts measured among 46 workers in the production of rubber chemicals. These workers have been reported to present an increased risk of bladder cancer (Ward et al. 1996; Gaber et al. 2007).

Finally, similar dosages of hemoglobin adducts of o-toluidine following different routes of administration suggest to consider EMLA application as a potential source of hemoglobin adducts of o-toluidine. In the particular setting of repeated use in pediatric population, dosages of metabolites, as in the present work, would allow a data-based discussion.

When comparing hemoglobin adduct values deriving from exposure or application of a single dose with values of persons who are continuously exposed the lifespan of human erythrocytes has to be taken into account. Human erythrocytes have a regular lifespan of about 120 days and are then eliminated from the body. Consequently, erythrocytes which are exposed continuously will have a larger fraction of exposure (over 120 days) compared to short-term exposures (e.g., fraction of 1-day-old erythrocytes for 1 day only). Theoretically, 120-day-old erythrocytes with an equal daily exposure could contribute 120 times more to the adduct concentration than the fraction of 1-day-old erythrocytes. This means that a single dose contributes to 1/60 of the hemoglobin adduct concentration than the same recurrent dose over a period of 120 days (Neumann et al. 1993; Bader and Wrbitsky 2006; DFG 2000). Taking this into account, patients in the present study were exposed to the same mean dose that workers were cumulatively exposed to on approximately 17 working days in the study from Ward et al. (1996).

However, ambiguity exists about o-toluidine carcinogenicity classification: different international societies have not classified o-toluidine equally: in 2006, the MAK Commission classified o-toluidine as a proven human bladder carcinogen (DFG 2007). In 2008, the International Agency for Research on Cancer followed announcing o-toluidine as a proven carcinogen for humans (IARC 2008). Both, MAK Commission's and IARC's decisions were based on epidemiological investigations and follow-up studies in a North

Table 2 Comparison of exposure, urinary o-toluidine and Hb adducts with data from the literature

	Dose/ Exposure	Urinary o-toluidine	o-Toluidine Hb adducts (continuous exposure)	o-Toluidine Hb adducts (single or mean daily exposure)
General population (Weiss 2002)	–	< 0.2 µg/L	< 0.6 ng/g Hb	–
Present study	50 mg (intrathecal)	460.5 µg/L (SD ± 352.8)	–	11.9 ng/g (± 1.9)
Gaber et al. (2007)	100 mg (s.c.)	–	–	21.7 ng/g Hb (± 12.6)
Ward et al. (1996)	412 µg/m^3 (± 366)	98,7 µg/L (± 119,4)	41 ng/g Hb (± 32)	0.7 ng/g Hb (± 0.5)
Korinth et al. (2007) Smokers	11,0 µg/m^3	14,5 µg/L	1.8 ng/g Hb	0.03 ng/g Hb*
Korinth et al. (2007) Non-smokers	61.4 µg/m^3	38.6 µg/L	2.7 ng/g Hb	0.05 ng/g Hb*

*Hemoglobin adduct concentrations for the mean daily exposure were calculated from adduct values after continuous exposure by a factor of 1/60

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American plant in which rubber chemicals have been produced using aniline and o-toluidine as educts (Ward et al. 1996; Markowitz et al. 2004, 2005). However, these studies were largely criticized in the literature as co-exposure to considerably more potent bladder carcinogens than o-toluidine (e.g., 2-naphthylamine and 4- aminodiphenyl) was observed in nearly all bladder cancer cases. Afterwards, in 1996, the American Conference of Governmental Industrial Hygienists (ACGIH) downgraded the o-toluidine classification from "A2: Suspected human carcinogen" to "A3: Confirmed animal carcinogen with unknown relevance to humans" (ACGIH 1996). The European Chemicals Agency currently lists o-toluidine as a substance whose carcinogenic potential is presumed but based primarily on animal data (Carc 1B) (European Chemicals Agency 2008). Nevertheless, recently Carreon et al. confirmed previous studies which described a link between o-toluidine and bladder cancer and recommended to re-examine occupational exposures limits (Carreón et al. 2014).

Moreover, a relevant difference between exposure to o-toluidine among workers of chemical industries and patients in this study is the origin of the compound. Occupational exposure to o-toluidine is derived mainly from combustion of organic materials, coal tar and its products, manufacturing of chemicals, rubber and azo dyes, as well as substances and mixtures that contain many other chemical products with carcinogenic potential. Occupational exposure is either via inhalation and/or direct dermal contact. In the present study, o-toluidine derives exclusively from the metabolism of prilocaine. Indeed, of outmost importance is that although urinary o-toluidine and o-toluidine hemoglobin adducts were detectable in all patients before administration, huge increases were recorded in both parameters after intrathecal injection. Consequently, the burden with o-toluidine is strictly related to the intrathecal administration of prilocaine.

The cumulative dose and the mean time of exposure are also major factors which should be considered. Even if hemoglobin adducts and urinary o-toluidine in patients exposed to intrathecal prilocaine are comparable to occupational exposure to o-toluidine, a single high-dose exposure is of far less concern than the same cumulative exposure to lower doses of o-toluidine over many years (Neumann 2007; Ehrenberg et al. 1974). Nonetheless, hemoglobin adducts have been described as a biochemical marker of exposure to o-toluidine and a surrogate of DNA adducts (Richter et al. 2002). Therefore, the present results underline the ability of intrathecal prilocaine to generate a mutagenic metabolite, which may affect the structure of DNA. Taking into account that no threshold was ever determined for this potential initiating carcinogenic property, it is certainly worrying that a single administration of a frequently used drug can expose patients to comparable high levels of a presumed carcinogenic substance. Therefore, intrathecal injection of prilocaine should be considered as a factor of overexposure to o-toluidine with patients chronically exposed to this agent in the setting of their work. However, for precautionary reasons other local anesthetics such as bupivacaine or chloroprocaine can be considered as alternatives for spinal anesthesia.

Moreover, the fact that the DNA structure could be affected by the administration of prilocaine raises the ethical question of its the use in pregnant women especially during the organogenesis period.

Finally, the fact that the urinary elimination of o-toluidine and the extent of hemoglobin adduct formation is subject to inter-individual variability might be of clinical relevance. Indeed, the faster o-toluidine and its conjugates are excreted via urine, the shorter o-toluidine will be available to enzymatic activation for binding to DNA. Particularly, polymorphisms of the phase II enzymes *N*-acetyltransferase 1 and 2 (NAT 1, NAT 2) may modulate bladder cancer risk in humans after exposure to aromatic amines. Early studies indicated that the extent of hemoglobin adduct formation is a balanced process involving *N*-oxidation and *N*-acetylation as two competitive metabolic steps, e.g., in the case of smokers with regard to 4-aminobiphenyl (Bartsch et al. 1990). In the present study, especially one patient (Fig. 4, Patient 2), was identified with differences in the velocity of prilocaine metabolization. This patient presented the highest concentration of o-toluidine in urine and simultaneously the lowest concentration of hemoglobin adducts. Taking into account these results, the influence of enzyme polymorphisms on metabolic profiles of patients that might correlate to increased or reduced risk relative to o-toluidine exposure should be the object of further studies.

The present study confirms and quantifies the presence of high level of hemoglobin adducts of o-toluidine and urinary o-toluidine after a single intrathecal injection of hyperbaric prilocaine. Patients' DNA modification and their metabolic profiles should be investigated and specific populations suffering from occupational chronic exposure to o-toluidine should be considered.

Funding This study received fundings from BARA, the Belgian association for Regional Anesthesia, BARA vzw, UZ Leuven – Departement Anesthesie, Herestraat 49, 3000 Leuven (BE), and Association Vésale, Maison des Infirmières – CHU St. Pierre – 322 Rue Haute – Bruxelles (BE).

Compliance with ethical standards

Conflict of Interest None.

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Ethical approval Ethics committee registration number: EC 332, OM 157; B076201836443. This study was presented as a poster at the 38th annual ESRA Congress in Bilbao, Spain, 11–14 September 2019.

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