Short communication

Detection of landiolol using high-performance liquid chromatography/fluorescence: A blood esterase-sensitive ultra-short-acting β1-receptor antagonist

Manabu Suno [a], Takayuki Kunisawa [b], Akio Yamagishi [b], Takashi Ono [a], Joe Yamamoto [a],
Takehiro Yamada [a], Yoshikazu Tasaki [a], Keiko Shimizu [c], Hiroshi Iwasaki [b], Kazuo Matsubara [a,*]

[a] Department of Hospital Pharmacy & Pharmacology, Asahikawa Medical College, Asahikawa 078-8510, Japan
[b] Department of Anesthesiology and Critical Care Medicine, Asahikawa Medical College, Asahikawa 078-8510, Japan
[c] Department of Legal Medicine, Asahikawa Medical College, Asahikawa 078-8510, Japan

ARTICLE INFO

ABSTRACT

Landiolol hydrochloride, a new adrenergic β1-selective antagonist having an ultra-short half-life, is used to prevent tachyarrhythmia during surgery. Since landiolol is thought to be rapidly hydrolyzed to an inactivate metabolite by esterases, quantification of the drug concentration in the blood is impractical. The landiolol concentration in blood was halved within 5 min after blood sampling. This degradation was effectively prevented by pre-treatment with neostigmine (100 μg) in the sampling tube, but not by EDTA pre-treatment, indicating that landiolol could be metabolized by pseudocholinesterase in plasma. After the one-step solid-phase extraction, fluorescence detection of landiolol reduced chromatographic background signals and then improved assay sensitivity to the lower limit of 10 ng/ml in blood; this reproducible approach yielded coefficient variation of less than 6%. The blood concentration-time profile of landiolol hydrochloride in patients of the present investigation afforded more practical assessment than previously reported studies, thus improving accuracy and facilitating detailed pharmacokinetic study in relation to the pharmacological action of drug.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Adverse cardiovascular changes, including hypertension and tachycardia, often occur during general anesthesia. Since tachycardia may carry a higher risk in terms of induction of myocardial ischemia compared with hypertension during surgery, the control of tachycardia is an important factor in the anesthetic management of patients. Many different anesthetic drugs and techniques have been used for the prevention and treatment of acute hemodynamic responses during surgery. Of the many anesthetics, the administration of adrenergic β1-receptor antagonist is known to be an effective approach in the treatment of tachycardia [1–4]. The use of this type of drugs offers significantly more rapid recovery by decreasing the anesthetic dose required to maintain an adequate depth of anesthesia and reduces postoperative analgesic requirement [5,6]. Landiolol hydrochloride, (−)-[(S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl 3-[4-[(S)-2-hydroxy-3-(2-morpholinocarbonylamino) ethylamino] propoxy] phenylpropionate monohydrochloride, is a newly developed ultra-short-acting β1-receptor antagonist with a

structural resemblance to esmolol [7]. The potency selective ratio of β1/β2 of this drug is 5 times higher than that of esmolol [7,8], thus providing better control of cardiac conditions of the patient during general anesthesia [9,10]. However, the use of cardiovascular agents during anesthesia induces hemodynamic changes. In cases where a short-acting β1-receptor antagonist is combined with anesthesia induction agents and with continuous infusion, there is a dose-dependent risk of hypotension and bradycardia [11].

Prior knowledge of the pharmacokinetic parameters of a drug facilitates prediction of its adverse reactions. However, the pharmacokinetics of landiolol hydrochloride has not been well documented, since accurate determination of the blood concentration of this drug is not practical due to its esterase-sensitive structure. As landiolol hydrochloride has an ester bond in its chemical structure (Fig. 1), this drug is thought to be rapidly hydrolyzed to an inactive metabolite by the esterase in blood. Although an analytical method for quantitative determination of landiolol in blood has recently been published [12,13], studies probing on enzymatic degradation after blood sampling have yet to be attempted. Furthermore, a previous method that relies on high-performance liquid chromatography (HPLC) separation with UV detector in quantitative determination of blood landiolol concentrations requires extremely complicated sample preparation procedures with poor
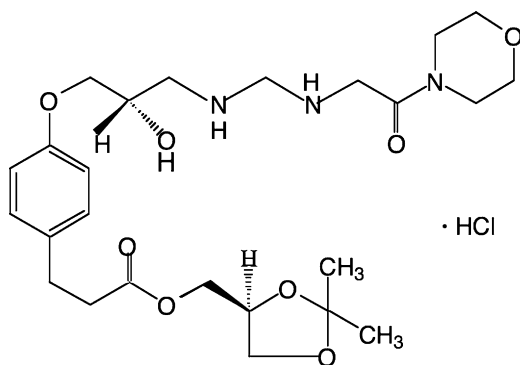
Fig. 1. Chemical structure of landiolol hydrochloride.

detection sensitivity limits exceeding 50 ng/ml [12]. In this study, we improved the assay method of lindiolol hydrochloride with added fluorescence detection, which also prevented drug degradation after blood collection and provided data for predicting drug metabolisms and pharmacokinetics.

2. Material and methods

2.1. Chemicals and materials

Authentic landiolol hydrochloride and ONO-SA-137 (2,2-dimethyl-1,3-dioxolan-4RS-ylmethyl 3-[4-{S-2-hydroxy-3-(2-(4-morpholinecarboxamide)-ethylamino) propoxy}-3-methyl-phenyl] propionate hemioxalate) were gifts from ONO Pharmaceutical Co., Ltd., (Osaka, Japan). ONO-SA-137 was used as an internal standard (IS). Bond Elut C_{18} cartridges (200 mg/3 ml type; Varian, Lake Forest, CA, USA) were used for sample-cleanup. Citric acid monohydrate, sodium acetate, neostigmine methyl sulfate and sodium 1-octane sulfonate (Nacalai Tesque, Kyoto, Japan) and all other chemicals were products graded for HPLC or of reagent grade (Wako, Osaka, Japan). Redistilled water was used throughout the study.

2.2. Blood samples

Samples of antecubital venous blood were obtained from 3 healthy adult volunteers (age range: 38–53 years) and 5 intraoperative inpatients (age range: 37–70 years). This research protocol had been approved by the Institutional Review Board of Asahikawa Medical College, and written informed consent from participants after proper briefing of the study has been obtained accordingly.

Blood (1 ml) was withdrawn in a tube containing EDTA (1.6 mg) and neostigmine (100 μg) from patients and healthy volunteers before prompt treatment with ice-cold ethanol (6 ml). The supernatant was collected after centrifugation at 1600 × g for 10 min and stored at −20 °C until analysis.

2.3. Sample preparation for quantification of landiolol hydrochloride

An aliquot of 100 μl of 1000 ng internal standard in methanol was added to the deproteinized sample before drying under a stream of nitrogen at 50 °C. The residue was dissolved in 1% acetic acid (3 ml), and then was passed through a Bond Elut C_{18} cartridge sequentially pre-washed with methanol (3 ml), redistilled water (3 ml) and 1% acetic acid (3 ml). After rinsing redistilled water (6 ml) and acetonitrile (2 ml), the cartridge was vacuum-dried for 5 min. The target chemicals were eluted with 1% ammonia in methanol (3 ml), and the eluant was dried under a steam of nitrogen at 50 °C. The dried residue was re-resolved in the mobile phase solution
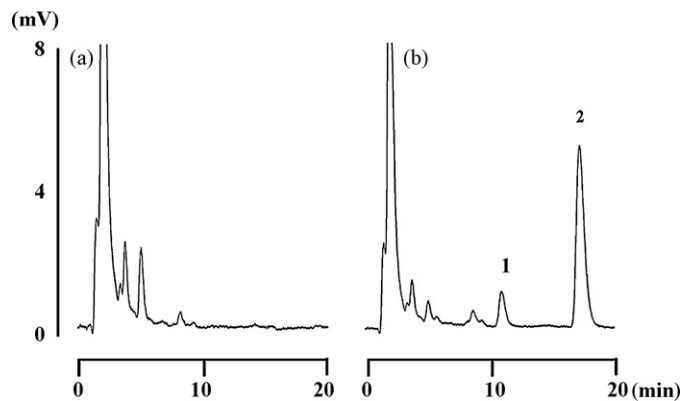

Fig. 2. Typical chromatographic separations of landiolol hydrochloride using blank human whole blood (a) and patient samples (b) with internal standards (IS; 1000 ng). The concentration of landiolol in the patient sample was determined to be 115 ng/ml. Peaks 1 and 2 represent landiolol hydrochloride and IS, respectively.

(100 μl) before a 25-μl sample aliquot was subjected to HPLC analysis (Shimadzu LC-10A, Kyoto).

2.4. HPLC analysis

Landiolol was measured by HPLC equipped with a fluorescence detector (Jasco FP-920S; Tokyo) at excitation and emission wavelengths of 280 nm and 310 nm, respectively. Separation of the respective compounds was achieved on a TSK gel ODS-100V column (150 mm × 2.0 mm, Tosoh, Tokyo, Japan). The mobile phase of 0.1 M citric acetate buffer (pH 3.0) containing of 0.2 mM sodium 1-octanesulfone and acetonitrile in the ratio of 75:25 (v/v) was delivered at a flow rate of 0.3 ml/min at 40 °C.

2.5. Calibration curve

A known amount of landiolol hydrochloride in the range of 10–5000 ng/ml was spiked into 1.0 ml of drug-free human whole blood from volunteers and carried through the same workup procedure (in triplicate) as described above. The calibration curve of landiolol hydrochloride was constructed by plotting peak-area ratios of the authentic compounds to IS.
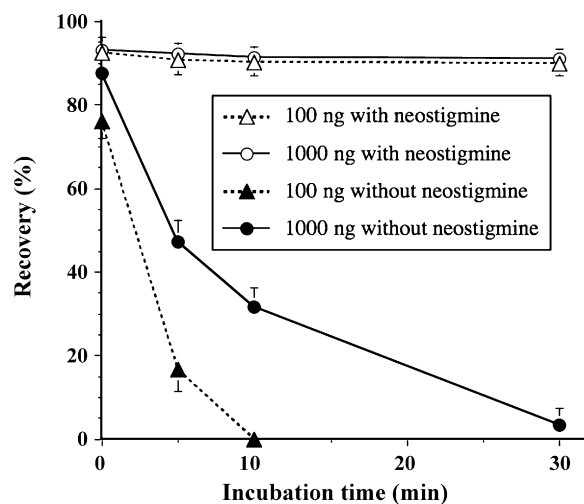

Fig. 3. Effect of neostigmine on landiolol degradation in blood. Landiolol hydrochloride was spiked into the blood sample (1 ml) with or without neostigmine (100 μg). EDTA was added to each sample. After incubation at 37 °C, landiolol remaining in the blood was analyzed. Data represent the mean ± SEM of triplicate analyses.

Table 1
Recovery of landiolol from human whole blood.

Added concentration (ng/ml)	Recovery (%)	CV (%)
10	93.6	3.8
100	93.8	4.6
1000	93.6	3.4
2000	93.0	3.2

The data are expressed as the mean ± SD of 6 analyses.

3. Results

3.1. Chromatographic separation

Chromatographic separation of landiolol hydrochloride was achieved within 18 min without any interference peak (Fig. 2). The retention time of landiolol hydrochloride and IS on the column were 10.7 min and 17.1 min, respectively.

3.2. Calibration curve

The standard curves were expressed as the regression equation of a straight-line ($y = ax + b$), where y is the concentration, a is the slope, x is the peak-area ratio against internal standard and b is the ordinate intercept, by the weighted liner regression analysis. The equation describing the calibration curves obtained was $y = 769.2x - 7.5$ with a correlation coefficient of 0.999 (data not shown). The lower detection limit of landiolol hydrochloride was 10 ng/ml (where the signal to noise (S/N) ratio was >3) spiked in drug-free whole blood.
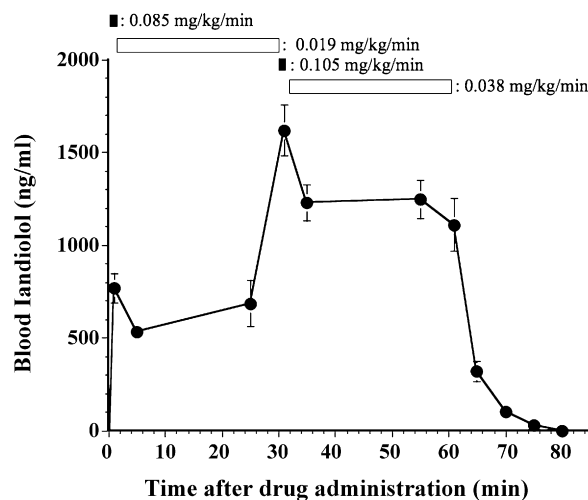
3.3. Recovery and precision studies

Degradation of landiolol in the blood was examined. Landiolol hydrochloride (100 or 1000 ng) was spiked in the blood sample (1 ml) with or without neostigmine (100 μg). After incubation at 37 °C for 0–30 min, landiolol remaining in the blood was analyzed. Landiolol degraded rapidly in the blood (Fig. 3); the drug was more likely to be affected by the relevant enzymes in blood at lower than higher concentrations. A considerable portion of landiolol was rapidly degraded without neostigmine pre-treatment of the sample. However, degradation of the landiolol sample was effectively prevented by neostigmine pre-treatment.

The recovery of landiolol after subjection to the present solid-phase extraction procedure was evaluated using four concentrations expressed as the hydrochloride salt; i.e., 10 ng/ml, 100 ng/ml, 1000 ng/ml and 2000 ng/ml (each $n = 6$). The efficiency of landiolol extraction from whole blood was 93.6%, 93.8%, 93.6% and 93.0% at 10 ng/ml, 100 ng/ml, 1000 ng/ml and 2000 ng/ml, respectively (Table 1). The accuracy and precision study of the assay method was conducted at 7 different concentrations of landiolol hydrochloride (Table 2). The method was reproducible with coefficients of variation (CV) ranging within less than 6%.

Fig. 4. A typical blood concentration-time curve of landiolol hydrochloride in patients. Blood landiolol concentrations were determined in 5 intraoperative inpatients who were a administered with landiolol hydrochloride intravenously by the dose-escalation method: an initial dose administered by 29-min continuous infusion (0.019 mg/kg/min) after 1-min loading with 0.085 mg/kg/min was followed by a second loading of 0.105 mg/kg/min for 1 min with 29-min constant infusion (0.038 mg/kg/min). The concentration is expressed as the hydrochloride salt. The plots represent the mean ± SEM of triplicate analyses.

3.4. Application of the method

Blood landiolol concentrations were determined in 5 intraoperative inpatients to whom landiolol hydrochloride was given intravenously by the dose-escalation method: an initial dose administered by 29-min continuous infusion at 0.019 mg/kg/min after 1-min loading at 0.085 mg/kg/min was followed by a second loading of 0.105 mg/kg/min for 1 min with 29-min constant infusion at 0.038 mg/kg/min. The blood concentration-time curve of landiolol illustrated the concentration of landiolol immediately established a steady state level after loading (Fig. 4); the steady state levels (as hydrochloride salt) registered 562.9 ± 145.1 ng/ml and 1248.6 ± 273.4 ng/ml (mean ± SEM) at respective infusion rates of 0.085 mg/kg/min and 0.038 mg/kg/min. The elimination half-life was derived as 2.8 min.

4. Discussion

Short-acting β_1-adrenoceptor antagonists, which are easier to control during surgeries, attenuate hemodynamic changes during anesthesia even for patients with a higher risk for side-effects [1,2,14]. If the typical side-effects of β-adrenergic blockers should develop, infusion of the drug can be discontinued or adjusted to establish rapid diminution or reversal of induced side-effects. However, as the pharmacokinetic parameters of drugs are essential for predicting relevant adverse reactions, an accurate method for quan-

Table 2
Accuracy and precision study on the landiolol assay.

Prepared (ng/ml)	Run-to-run				Day-to-day		
	Detected (ng/ml)	Accuracy (%)	CV (%)		Detected (ng/ml)	Accuracy (%)	CV (%)
10	10.3 ± 0.39	−3.8	4.28		9.6 ± 0.41	4.4	3.66
20	20.0 ± 0.75	4.3	4.25		20.1 ± 0.76	4.2	4.52
100	101.1 ± 7.21	−5.6	3.88		98.0 ± 4.73	−3.2	5.22
500	497.6 ± 6.78	4.2	3.95		490.2 ± 7.76	4.8	4.65
1000	986.6 ± 45.3	6.7	5.25		980.5 ± 38.9	−5.0	5.56
2000	2034.5 ± 41.5	−5.2	4.25		1988.3 ± 77.8	−5.5	4.56
5000	4879.8 ± 173.5	5.0	4.98		5026.2 ± 127.1	7.2	4.23

Data are expressed as the mean ± SD of 6 analyses.

titative detection in blood is warranted. In this study, we have demonstrated a simple and accurate assay method for quantitative monitoring of landiolol concentrations in blood.

The sample preparation method developed in this study was very simple compared with the previously reported approach [13]. The fluorescence intensity of landiolol was weak and was not much different from that of UV absorption when the authentic compound was analyzed using the previously reported method. However, the employment of fluorescence detection in our present method dramatically reduced the chromatographic background signals when the blood sample was analyzed, resulting in the achievement of a sample preparation with 5 times higher sensitivity using a simple purification procedure. For UV detection in the previous method, further complicated purification steps for sample preparation were required [13]; a process which most likely would yield quantitative errata because of the rapid continuous degradation of landiolol after blood sampling (even in cases where blood samples were immediately mixed with ice-cold ethanol after sampling, 10–30% of landiolol were degraded). Thus, the use of enzyme inhibitors, such as neostigmine in the present study, is essential to prevent landiolol degradation, especially when blood is collected in the operation theater. In addition, neostigmine is easy to handle in the operation room, since it is used to reverse the effects of non-depolarizing muscle relaxants at the end of an operation.

Esterases are members of the hydrolase family of enzymes that primarily hydrolyze endogenous and exogenous esters, with substrate specificity overlapping with lipases [15]. They are ubiquitously expressed in mammalian blood and various organs [16]. The blood esterase mediating the hydrolysis of chemically landiolol-like esmolol in human blood can be inhibited by sodium fluoride, EDTA, and p-hydroxymercuribenzoate, but not by echothiophate, physostigmine and acetazolamide. This tendency, therefore, indicates that an arylesterase in erythrocyte cytosol mediates the hydrolysis of drugs, while an aliphatic esterase mediates the hydrolysis of esmolol in guinea pig and rat plasma [17]. Arylesterase in human serum is a calcium-dependent hydrolase, and this category of enzymes specifically acts on the carboxylic ester bonds [18]. However, degradation of landiolol was effectively prevented by neostigmine, but not by EDTA in the present study. Neostigmine potently inhibits acetylcholinesterase in blood cell membranes and pseudocholinesterase in serum [19]. In addition, landiolol has been demonstrated to inhibit physostigmine in human serum as well [20]. Taken together, the hydrolysis of landiolol is most likely mediated by pseudocholinesterase in human blood. However, our results could not exclude the possible involvement of erythrocyte membrane acetylcholinesterase in the landiolol degradation. The fact that landiolol hydrochloride is hydrolyzed by pseudocholinesterase in blood could be an important issue in critically ill patients with hepatic failure.

The elimination half-life of landiolol hydrochloride (2.8 min) in this study was shorter than the previously reported values in healthy male volunteers (3.5 min) [12] and patients with arrhythmia (3.0–3.3 min) [1]. This discrepancy may be ascribed to the analytical method, where underestimation of blood concentrations due to the neglect of drug degradation after blood collection could have contributed to inflated elimination half-life values in previous assays. Thus, future studies on the pharmacokinetics are warranted to ascertain proper quantitative monitoring of landiolol hydrochloride.

In conclusion, we improved the analytical method for determination of landiolol hydrochloride in blood using the HPLC/fluorescence detection method with neostigmine pretreatment of blood samples. The rapid degradation of landiolol was prevented by neostigmine, indicating that landiolol hydrochloride might have been hydrolyzed by pseudocholinesterases. The improved analytical method most likely allows and facilitates quantitative and accurate pharmacokinetic/pharmacodynamic studies of landiolol hydrochloride in clinical laboratories.

References

[1] H. Atarashi, A. Kuruma, M. Yashima, H. Saitoh, T. Ino, Y. Endoh, H. Hayakawa, Clin. Pharmacol. Ther. 68 (2000) 143.
[2] R. Gray, T.M. Bateman, L.S.C. Czer, C.M. Conklin, J.M. Matloff, J. Am. Coll. Cardiol. 5 (1985) 1451.
[3] S. Saito, F. Nishihara, A. Tomioka, K. Nishikawa, H. Obata, F. Goto, N. Yuki, Can. J. Anesth. 52 (2005) 1027.
[4] B.M. Biccard, Anaesthesia 59 (2004) 60.
[5] A. Kitamura, A. Sakamoto, T. Inoue, R. Ogawa, Eur. J. Pharmacol. 51 (1997) 467.
[6] T. Kurita, K. Morita, K. Fukuda, K. Takata, M. Uraoka, Y. Sanjo, S. Sato, Br. J. Anaesth. 96 (2006) 602.
[7] S. Iguchi, H. Iwamura, M. Nishizaki, A. Hayashi, K. Senokuchi, K. Kobayashi, K. Sakaki, K. Hachiya, Y. Ichioka, M. Kawamura, Chem. Pharm. Bull. 40 (1992) 1462.
[8] K. Muraki, H. Nakagawa, N. Nagano, S. Henmi, H. Kawasumi, T. Nakanishi, K. Imaizumi, T. Tokuno, K. Atsuki, Y. Imaizumi, M. Watanabe, J. Pharmacol. Exp. Ther. 278 (1996) 555.
[9] J.W. Johansen, R. Flaishon, P.S. Sebel, Anesthesiology 86 (1997) 364.
[10] J.W. Johansen, G. Schneider, A.M. Windsor, P.S. Sebel, Anesth. Analg. 87 (1998) 671.
[11] E. Figueredo, E.M. Garcia-Fuentes, Acta. Anaesthesiol. Scand. 45 (2001) 1011.
[12] M. Murakami, H. Furuie, K. Matsuguma, A. Wanibuchi, S. Kikawa, S. Irie, Drug Metab. Pharmacokinet. 20 (2005) 337.
[13] Y. Hashimoto, S. Nago, M. Tsunekawa, K. Shibakawa, Y. Miyata, Biol. Pharm. Bull. 32 (2009) 121.
[14] D.T. Duane, S.R. Redwood, R.M. Grounds, Anesthesia 51 (1996) 474.
[15] R. Mentlein, H. Rix-Matzen, E. Heymann, B. B. A. 964 (1988) 3198.
[16] T. Satoh, M. Hosokawa, Annu. Rev. Pharmacol. Toxicol. 38 (1998) 257.
[17] C.Y. Quon, H.F. Stampfli, Drug Metab. Dispos. 13 (1985) 420.
[18] K. Lorentz, B. Flatter, E. Augustin, Clin. Chem. 25 (1979) 1714.
[19] Y. Iwanaga, T. Kimura, N. Miyashita, K. Morikawa, O. Nagata, Z. Itoh, Y. Kondo, Jpn. J. Pharmacol. 66 (1994) 317.
[20] K. Tsunekawa, H. Imawaka, K. Yamamoto, K. Shibakawa, S. Hiraku, Drug Metab. Pharmacokinet. 12 (1997) 31 (in Japanese).