Abstract: Administration of local anesthetics with adrenaline can cause tachycardia and hypertension. This study assessed whether combined administration of lidocaine with adrenaline and landiolol would induce local anesthesia without causing hemodynamic changes. Normal saline (NS), lidocaine with adrenaline (LA), and lidocaine with adrenaline and landiolol (LLA) were injected into Wistar Kyoto (WKY/Izm) or spontaneously hypertensive (SHR/Izm) rats, followed by measurement of the pulse rate (PR), systolic, diastolic and mean blood pressures (SBP, DBP and MBP). In the LLA group, the increase in PR was significantly suppressed in both SHR/Izm and WKY/Izm rats relative to those given NS or LA, it was elevated in SHR/Izm rats compared with LA. Although SBP was significantly reduced in WKY/Izm rats given LLA, relative to those given NS or LA, it was elevated in SHR/Izm rats given LLA. Landiolol-induced changes in PR may be due to blockade of adrenaline-induced β-receptor stimulation, which suppresses cardiac hyperactivity, whereas the early surge of blood pressure in SHR/Izm rats given LLA may be due to the dominant alpha-adrenergic effects of β1-receptor inhibition. The anti-adrenergic effects of LLA were safe and effective in WKY/Izm rats, although the unexpected early hypertensive surge in SHR/Izm rats indicates the need for caution.

Keywords: adrenaline, beta blocker, landiolol, SHR, WKY

Introduction

Local anesthetics are absorbed by the surrounding capillaries after administration into the oral cavity and their duration of action is extremely short. Surgical invasion after administration of a local anesthetic into the oral mucosa, which is rich in blood vessels, can lead to difficulty with bleeding control [1]. On the other hand, when adrenaline is added to the local anesthetic, blood vessels are constricted through stimulation of α receptors, thus inhibiting migration of the anesthetic into the bloodstream [2]. This enhances the effect of the anesthetic, prolongs its duration of action, and reduces blood loss. Because of vasoconstriction, however, adrenaline also elevates blood pressure and increases the heart rate and myocardial contractile force via stimulation of β1 receptors [3,4]. Therefore, administration of a local anesthetic with adrenaline during dental treatment in patients with cardiovascular diseases, such as hypertension or ischemic heart disease, can affect hemodynamics by causing an increase in blood pressure and/or tachycardia [5,6].

When adrenaline is administered to patients with combination of non-selective beta blockers, monoamine oxidase inhibitors, or tricyclic antidepressants, α-receptor stimulation dominates and causes an increase in blood pressure [7-11]. Conversely, when adrenaline is used in combination with α-receptor blockers, such as phenothiazines and butyrophenone antipsychotics, β-receptor stimulation dominates, causing a decrease in blood pressure [12,13]. Therefore, local anesthetics, such as 3% propo- caine containing the vasoconstrictor felypressin and mepivacaine, which does not contain a vasoconstrictor, are selected occasionally. However, these may produce undesirable effects such as myocardial ischemia and reduced cardiac function. Moreover, these local anesthetics have a duration of anesthetic action shorter than that of 2% lidocaine with 1/80,000 adrenaline [14].

This study was conducted to test the hypothesis that administration of a selective β1-blocker with a local anesthetic + adrenaline would attenuate the increase in heart rate and myocardial contractility, thus reducing hemodynamic changes.

This was an animal study employing spontaneously hypertensive (SHR/Izm) rats, which were originally created by selective breeding of Wistar rats with hypertension by Okamoto and Aoki of Kyoto University in 1959. These rats develop hypertension spontaneously between 7 and 15 weeks of age without any artificial treatment. Therefore, they are considered to be an optimal animal model for studies of essential hypertension [15-17]. The Wistar Kyoto (WKY/Izm) rat is widely used as a control for the SHR/Izm rat because it has the same genetic background, but normal blood pressure.

The aim of this study was to examine whether the selective β1-blocker, landiolol, would suppress the hemodynamic changes induced by administration of local anesthetic with adrenaline in both normal and hypertensive rats.

Materials and Methods

Experimental animals

This study was approved by the Animal Care and Use Committee of The Nippon Dental University School of Life Dentistry at Tokyo (16-24-3).

Experiments were performed on 42 rats. The test animals were 11-week-old SHR/Izm (Sankyo Labo Service, Tokyo, Japan) or Wistar-Kyoto rats (WKY/Izm: Sankyo Labo Service). The mean weights of the SHR/Izm and WKY/Izm rats were 292.3 ± 19 and 314.9 ± 12.6 g, respectively (mean ± standard deviation: S.D.). Rats were divided into three groups based on the type of drug administered (n = 7 per group): normal saline (NS: Otsuka Pharmaceutical Factory, Tokushima, Japan); lidocaine with adrenaline (LA; 1.75% lidocaine + 37.5 μg/kg adrenaline); and lidocaine with adrenaline + landiolol (LLA; 1.75% lidocaine + 37.5 μg/kg adrenaline + 300 μg/kg landiolol).

Drug preparation

LA was prepared by mixing 875 μL of 2% lidocaine (Xylocaine Injection Polyvamp 2%, Aspen Japan, Tokyo, Japan) with 125 μL of 0.1% adrenaline (Bosmin, Daichi Sankyo, Tokyo, Japan). The final concentration of adrenaline was 1/8,000. LLA was prepared by dissolving 1 mg landiolol (Onoaco, Ono Pharmaceutical, Osaka, Japan) into 1 mL LA.

Anesthesia and measurement of hemodynamics

Rats were immobilized with 5% isoflurane in an inhalation anesthesia box, and an anesthesia mask was attached to their heads to maintain anesthesia with 1.5% isoflurane under spontaneous respiration. A measurement cuff was attached to their tails for non-invasive, automatic blood pressure monitoring (BP-98A-L, Softron, Tokyo, Japan). A small animal-warmer (THC-31, Softron) and a heat insulating cylinder (TC-60, Softron, Tokyo, Japan) were used to maintain the body temperature at 37°C (Fig. 1). After anesthesia, the rats were allowed to rest for 30 min, after which pulse rate (PR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and...
mean blood pressure (MBP) were measured.

Four measurements were performed after 30 min of rest, and the middle value was used as the 0 min value. Subsequently, the tongue was pulled out from the mouth with forceps to administer the test drugs slowly over 5 s at 0.3 mL/kg into the center of the tongue muscles on the left side via a micro-syringe attached to a 31 G needle. Next, the PR, SBP, MBP and DBP of the rats were measured at 1, 2.5, 5, 7.5, 10, 15, and 20 min after drug administration.

Statistical analysis
SPSS Statistics Version 22 (SPSS, IBM Japan, Tokyo, Japan) was used for statistical analysis. Because the data were confirmed to be parametric using a histogram, two-way analysis of variance followed by Bonferroni post-hoc test were used for data comparison. Differences were considered significant at \( P < 0.05 \).

Results

Changes in pulse rate
WKY/Izm rats
The PR in the LLA group was significantly lower than that in the LA group. No significant difference in the PR was observed between the LLA and NS groups up to 10 min after drug administration. In the NS and LA groups, PR increased at 1 min after drug administration. In addition, no fluctuation in PR was observed in the LLA group until 10 min after drug administration. These data indicate that the addition of landiolol suppressed the adrenaline + lidocaine-induced rise in PR (Fig. 2A).

SHR/Izm rats
PR in the LLA group was significantly lower than that in the LA group at all time points. There was no significant difference in PR between the LLA and NS groups. PR increased at 1 min after drug administration. In addition, no fluctuation in PR was observed in the LLA group until 10 min after drug administration. These data indicate that the addition of landiolol suppressed the adrenaline + lidocaine-induced rise in PR (Fig. 2B).

Changes in systolic blood pressure
WKY/Izm rats
SBP in the LLA group was significantly lower than that in the NS and LLA groups at all time points. Furthermore, SBP in the LLA group was significantly decreased between 5 and 20 min after administration, relative to that at 0 min (Fig. 3A).
SBP in the LLA group was significantly higher than that in the LA and NS groups at 1 min after drug administration. However, SBP in the LLA group was significantly lower at 5 and 10 min after drug administration than that in the NS group.

In addition, SBP was significantly higher at 1 min after drug administration compared with that at 0 min in all groups. In the LLA group, SBP was significantly decreased between 5 and 10 min relative to that at 0 min (Fig. 3B).

Changes in diastolic blood pressure

WKY/Izm rats
Overall, no significant difference in DBP was observed among the groups. However, there was a significant decrease in DBP in the LLA group at 7.5 min after drug administration relative to that at 0 min (Fig. 4A).

SHR/Izm rats
DBP in the LLA group was significantly higher than that in the NS group at 1 min after drug administration. There were no significant differences in DBP between the LA and LLA groups. Compared with the value at 0 min in all groups, DBP was increased significantly at 1 min after drug administration. Furthermore, there was a significant decrease in DBP in the LLA group at 5 min after drug administration (Fig. 4B).

Changes in mean blood pressure

WKY/Izm rats
MBP in the LLA group was significantly lower than that in the NS and LA groups between 1 and 7.5 min after drug administration. No significant differences were observed in MBP between the NS and LA groups. Furthermore, MBP in the LLA group was significantly decreased between 5 and 10 min relative to that at 0 min (Fig. 5A).

SHR/Izm rats
MBP in the LLA group was significantly higher than that in the NS and LA groups at 1 min after drug administration. In addition, MBP was significantly lower in the LLA group than in the NS group at 5 min after drug administration. Further, there was a significant increase in MBP in all groups at 1 min after drug administration relative to that at 0 min (Fig. 5B).

Discussion

Beta-blockers are categorized as selective β₁ blockers, selective β₂ blockers, and non-selective blockers on the basis of their receptor binding capacity [18,19]. When adrenaline is administered to patients taking non-selective beta-blockers, the vasodilative effect due to stimulation of the beta receptors is not expressed, and abnormal elevation of blood pressure may occur due to vasoconstriction as a result of alpha receptor stimulation. This study was designed to investigate whether a combination of selective β₁ blockers and adrenaline would suppress cardiac hyperactivity, while sparing the vasodilative effect, thereby inhibiting hemodynamic fluctuations [20]. The β₁/β₂ blocking ratio of landiolol hydrochloride is 277:1, making it an extremely selective β₁ blocker [21]. In clinical practice, landiolol is administered at 0.01-0.06 mg/kg/min [22]. In this study, the lowest amount of landiolol that had suppressed hemodynamics in a preliminary study was employed. Clinically, adrenaline with lidocaine causes a large change in hemodynamics immediately after administration. Sanjay Byakodi et al. reported that there were no significant hemodynamic changes 10 min
after injection of lidocaine with adrenaline relative to that in the absence of adrenaline [23]. In this study, therefore, measurement was performed until 20 min. This study revealed that administration of lidocaine in combination with adrenaline and landiolol hydrochloride to WKY/Izm and SHR/Izm rats inhibited the increase in PR. Therefore, this may have been attributed to the inhibitory effect of landiolol hydrochloride on the binding of adrenaline to β₂ receptors in the heart. Moreover, the increase in PR was suppressed for a longer period in the SHR/Izm rats when compared with the WKY/Izm rats. Although the reason for this remains unclear, it is possible that β₁ receptors are down-regulated in the blood vessels of SHR/Izm rats, which have a higher level of catecholamines in the blood [24]. This would result in relatively higher concentrations of landiolol hydrochloride, which may have lengthened the duration of receptor blockade. It is thought that in SHR/Izm rats there is increased progression of receptor down-regulation with increased progression of hypertension, possibly resulting in longer suppression of the PR increase by landiolol hydrochloride [25].

LLA suppressed the PR in both normal and hypertensive rats, but early surges in blood pressure were observed in hypertensive rats. In general, patients with hypertension may have comorbidities, such as arteriosclerosis. The early surges in blood pressure can be explained by the relative adrenergic dominance under arteriosclerotic conditions caused by down-regulation of β₁ and β₂ receptors [26]. In addition, the higher catecholamine concentration in the blood in SHR/Izm rats could induce this effect [26].

Changes in DBP are associated with elasticity of the blood vessel walls. Therefore, blockade of β₁ receptors may not significantly affect the DBP. In this study, the DBP of WKY/Izm rats was not significantly changed by addition of landiolol hydrochloride, which is in accord with the above assumption, and moreover there was no difference between SHR/Izm rats that had been administered LA and those that had received LLA.

The non-invasive automatic blood pressure monitor used in this study attaches a manchette to the tail of rats using the tail-cuff method. A caudal vein pulse wave was detected using the photoelectric pulse wave method. This method enables measurement of blood pressure and PR without causing stress to the animal [27].

The rats were immobilized with isoflurane to allow the test drugs to be administered sublingually. The minimum required alveolar concentration of isoflurane is 1.15%; however, preliminary testing revealed that hemodynamics could not be measured with isoflurane concentrations of <1.5% due to body movement. Therefore, a concentration of 1.5% was used, which did not significantly suppress hemodynamics, but prevented body movement [28].

The aim of this study was to examine whether a selective β₂ blocker would suppress the accelerated hemodynamics induced by adrenaline. In the preliminary study, 12.5 μg/kg adrenaline was administered for measurements of hemodynamics. However, this reduced the blood pressure. Therefore, the present study employed the amount of adrenaline (37.5 μg/kg) that had elicited a change in hemodynamics, a dose that would not be administered in general dental clinical practice. In spite of this high concentration of adrenaline, the hemodynamics of WKY/Izm rats were well controlled by addition of the selective β₂ blocker, landiolol. However, the concentration of landiolol (300 μg/kg) used in this study produced a transient early surge in the SHR/Izm rats. Further investigations are required to assess the appropriate concentration with addition of landiolol hydrochloride. Furthermore, future studies should consider the effect of landiolol hydrochloride on anesthetic action.

Acknowledgments
We thank Emeritus Professor Yukio Miyagawa from The Nippon Dental University for his helpful suggestions on the statistical analyses for this study.

Conflict of interest
The authors declare that they have no conflict of interest.

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