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Original

The influence of clonidine co-administration on the extent of lidocaine protein binding to rat serum and tissues

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Abstract: Lidocaine is an amide local anesthetic and clonidine is an antihypertensive (α2-adrenergic agonist). The use of these two drugs in combination is recommended to enhance the analgesic effect of lidocaine. The aim of this study was to investigate the influence of clonidine co-administration on the extent of lidocaine binding to rat serum, heart and maxillofacial tissues *in vivo* and *in vitro*. Thirty-two Wistar rats received either lidocaine alone, or lidocaine and clonidine, in the masseter muscle, and were then sacrificed 15 or 30 min after treatment. Serum, masseter, mandible and heart samples were then isolated and incubated in 0.9% NaCl solution for 12 h at 8°C. The extent of binding in the incubation medium and the serum was estimated by ultrafiltration, and the free lidocaine fraction was determined by the radioscopic method in a β-counter. An *in vitro* procedure was also performed. Serum, heart, masseter and mandible samples were incubated at 37°C for 15 or 30 min in Ringer's solution containing either lidocaine or lidocaine and clonidine, and the samples were similarly subjected to ultrafiltration. The percentage binding of lidocaine was again estimated by the radioscopic method. Lidocaine levels were found to be increased by clonidine co-administration *in vivo* and the free lidocaine fraction was enhanced *in vitro* as well in the examined tissues, obviously through mechanisms related to protein binding alterations. (J Oral Sci 53, 61-66, 2011)

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Introduction

Lidocaine, an amide-type local anesthetic, blocks nerve impulse transmission by binding to sodium channels and interfering with the entry of sodium ions *via* the membrane of nerve cells (1). It has been previously demonstrated that lidocaine and propranolol are eliminated *via* hepatic metabolism (2). Lidocaine, after its absorption, is transported from plasma proteins forming a drug-protein complex, which represents a latent store of the drug. This complex can be dissociated very rapidly. Only the unbound drug can diffuse into tissues, because the drug-protein complex is unable to cross the cell membrane. As the unbound drug is removed from plasma, more of the complex becomes dissociated. The drug bound to proteins is in equilibrium with its free fraction, and the interaction between the drug and proteins is reversible (3). The drug binding process involves mainly albumin and alpha-acid glycoprotein (4). Lidocaine, as a cationic molecule (pKa = 7.9), shows stronger binding affinity to alpha-acid glycoprotein than to albumin, and can interact with other cationic drugs, such as propranolol, at the same protein binding site (2).

Only the free drug fraction is pharmacologically active (5,6). Displacement of a drug from its binding sites, and the consequent increase of its free fraction, results in enhancement of its availability for diffusion to sites of action and the increase of its pharmacologic effect. The process of displacement of lidocaine has been demonstrated in various experimental stress models where the presence of elevated free fatty acid (FFA) levels increased the free

lidocaine fraction in serum and tissues (7,8).

The extent of drug protein binding is influenced by factors such as the presence of endogenous substances including hormones and metabolic products, pH, temperature and underlying diseases (malnutrition, liver disease, renal disease, cancer, myocardial infarction etc.). Binding capacity may also be influenced by co-administration of other drugs. The pharmacokinetic interactions between co-administered substances are usually competitive in nature, and are mostly related to drugs that are able to displace, or be displaced, from their binding sites on proteins.

Co-administration of lidocaine and propranolol, for example, results in an interaction between the two substances. Beta-blockers decrease liver perfusion and consequently inhibit the activity of hepatic microsomal lidocaine-metabolizing enzymes, leading to reduction of lidocaine's hepatic metabolic elimination. This results in a marked increase of its serum concentration. Additionally, propranolol significantly reduces the binding of lidocaine to liver proteins through a displacement process. Therefore, co-administration of these two drugs may increase the free fraction of lidocaine excreted by the liver (2).

In addition, the serum concentration of lidocaine is significantly increased after administration of paracetamol or propranolol. Combined administration of propranolol and lidocaine leads to a decrease in the total concentration and the percentage protein binding of lidocaine in tissues, and thus may increase the quantitative importance of this displacement. Additionally, co-administration with paracetamol or propranolol interferes with the metabolic profile of lidocaine, resulting in pharmacokinetic interactions that may be significant when determining the appropriate dose of lidocaine in clinical practice (9).

Clonidine, an α2-adrenergic agonist with a pKa value of 8.25 (10), indicated for the treatment of hypertension, regulates the release of catecholamines, and moreover can improve postoperative analgesia when added to local anesthetic infusions (11-14).

Co-administration of clonidine with local anesthetics increases the duration of anesthesia in situations such as intrathecal, extradural, and peripheral nerve blockade (11). Furthermore, addition of clonidine as an adjuvant analgesic agent for intraarticular local anesthesia enhances the analgesic effect of bupivacaine (12,13). Subcutaneous co-infiltration of clonidine with lidocaine may help to extend the duration of local anesthesia, which is useful after plastic surgery or other superficial procedures (14). Moreover, clonidine is applicable as an adjunct in epidural or spinal morphine treatment for cancer pain (15). It has also been proposed that the combination of lidocaine and clonidine could be useful in dental practice as a safe alternative to lidocaine and epinephrine mixture for intraoral infiltration and block anesthesia (16,17).

Although various studies have addressed the pharmacodynamic effect of clonidine and lidocaine co-administration, there is limited information about the pharmacokinetic interaction between the two drugs. A previous study has indicated that clonidine administration influences the extent of lidocaine binding and enhances free lidocaine in rat serum and heart tissue protein (18).

The aim of the present study was to further investigate the influence of clonidine co-administration on levels of free lidocaine in rat serum and heart, as well as masseter muscle and mandibular bone, in order to clarify any positive interaction that would enhance the anesthetic/analgesic effect of lidocaine in maxillofacial tissues. Moreover, the precise degree of lidocaine binding to serum and tissue proteins was determined *in vitro* (5,19,20).

Materials and Methods

The study design consisted of two parts:

1. *In vivo* intramuscular administration of lidocaine [^{14}C lidocaine and cold (unlabeled, non-radioactive) substance], with and without clonidine, was carried out in order to assess the free fraction of lidocaine in serum, masseter muscle, mandibular bone and heart.

Thirty-two Wistar rats aged 5-6 weeks with a body weight of 210 ± 15 g were divided into 4 groups of 8 animals: Groups I and III received lidocaine (Xylocaine inj.2% 50 ml Astra Zeneca) in the masseter muscle in a single dose of 4 mg/kg. The injection mixture consisted of labeled lidocaine (Carbonyl-^{14}C-lidocaine hydrochloride, specific activity 46 mCi/mmol, DuPont NEN Research Products, Boston, MA, U.S.A.) and cold substance (2.85 μg/ml cold substance + 7 μl labeled lidocaine). Groups II and IV received lidocaine in the same way as above, plus clonidine 1 μg/kg (Catapres amp. 150 μg/ml, Boehringer Ingelheim). The animals were anesthetized with ketamine 100 mg/kg i.p. and sacrificed at 15 (groups I, II) and 30 min (groups III, IV) after initial lidocaine administration. Serum, masseter, mandible and heart samples were then obtained. The tissues were weighed and incubated in 0.9% NaCl solution for 12 h at 8°C. The medium used for tissue incubation, and also the serum, were then filtered through a semipermeable membrane under centrifugation at 700 rpm for 12 h at 4°C in order to obtain the free drug fraction in the ultrafiltrate, in accordance with previous studies (19,21). A visking cellulose membrane 0.028 mm thick with a pore diameter of 15-20 Å was used, forming bags containing 2 ml of serum or tissue incubation medium.

The levels of the free lidocaine fraction were determined

by the radioscopic method in a β-scintillation Packard counter, using 200 μl of ultrafiltrate added to 15 ml of scintillation solution.

2. The *in vitro* procedure was conducted following euthanatization and exsanguination of 28 Wistar rats aged 5-6 weeks with a body weight of 205 ± 8 g divided into 4 groups as above. Heart, masseter and mandible samples were isolated and weighed, then incubated in duplicate at 37°C for 15 and 30 min in 2 ml of Ringer's solution containing either lidocaine or lidocaine and clonidine. The lidocaine and clonidine concentrations in the incubation samples, as described in Table 1, were comparable to the levels obtained with therapeutic doses. To prevent metabolic activity by enzymes possibly remaining in the tissues, 2% NaN_3 solution was added to the incubation fluid. The tissue dry weight was determined once the aliquot had been dried at 105°C in order to obtain a constant weight. The lidocaine mixture consisted of labeled lidocaine hydrochloride [carbonyl-^{14}C] with a specific activity of 46 mCi/mmol, appropriately diluted with a cold solution (2.85 μg/ml cold substance + 7 μl labeled lidocaine). At the end of the incubation period, the samples were collected and ultrafiltered through visking membranes (size 30/32) at 4°C, as described previously by Kurz et al. (20) and Tesseromatis et al. (19). Similarly, 1 ml of serum was incubated in the above drug solutions. The percentage of lidocaine binding was estimated in the ultrafiltrate using the radioscopic method described above. The free fraction of lidocaine was calculated from the percentage of the total drug that was bound to the tissue proteins (B%) in the ultrafiltrate:

$$B\% = [(Cin - Co) \times W \times Wtis] \times 100 / [(Cint-B)-(Cin-Co)] \times V$$

Where Cin represents the concentration of lidocaine in the incubation medium expressed as the number of counts,
Co is the blank (control) value of the incubation medium (Ringer's solution),
W is the percentage of water in the tissues,
Wtis is the wet weight of each examined tissue,
Cint is the concentration of lidocaine in the incubation medium before incubation,
B is the value of the count in the initial solution, and
V represents the volume of the incubation fluid.

The displacement of lidocaine through the presence of clonidine in the incubation procedure was determined by measuring the difference in free lidocaine concentration in the ultrafiltrate with and without clonidine.

The significance of differences between groups was evaluated by one-way analysis of variance with Scheffe or Dunnett correction and *t* test. Differences were considered significant at the level of $P < 0.05$.

The animals received care in accordance with the "Guide for the care and use of laboratory animals" (22), taking into consideration the directives of 24 November 1986 regarding the protection of animals used for experimental purposes (86/609/EEC) (23). All efforts were made to minimize animal suffering and to use the minimum number of animals required in order to reach reliable conclusions.

Results

As shown in Table 2, the levels of free lidocaine in serum and the examined tissues *in vivo* were increased when clonidine was co-administered. The free lidocaine fraction in serum and heart was significantly increased ($P < 0.05$) under the influence of clonidine at both 15 and 30 min after initial lidocaine administration, in comparison to the concentration of free anesthetic at the same time points in the absence of clonidine. Similarly, a very significant ($P < 0.01$) enhancement of lidocaine was observed in masseter muscle at 15 and 30 min, as well as in the mandible at 15 min, in the presence of clonidine. Finally, the free lidocaine fraction was very significantly ($P < 0.0001$) increased in the mandible at 30 min after initial administration of the anesthetic in the presence of clonidine.

As shown in Table 3, the *in vitro* experiment demonstrated enhancement of the free lidocaine fraction in the presence of clonidine in the incubation medium of the tissues tested, and also in serum. Furthermore, the binding of lidocaine decreased in serum ($P < 0.5$) and increased in tissues (NS) as the incubation time was prolonged.

Discussion

The process of binding of a pharmaceutical agent to plasma proteins results in an increase of the drug's plasma solubility, facilitating its transport to sites of action. The agent becomes gradually released from the drug-protein complex and the resulting free faction is distributed to the target tissues, where it exerts its pharmacological effect.

Table 1 Drug concentration in the incubation medium

Drug mg/ml 10^{-4}	Heart	Masseter	Mandible	Serum
Lidocaine+clonidine	0.234 + 2.3	0.234 + 2.3	0.234 + 2.3	0.234 + 2.3
Lidocaine	0.234	0.234	0.234	0.234

Table 2 Free fraction of lidocaine in serum and tissues in the *in vivo* experiment

	Serum μg/ml	Masseter μg/gr^{+}	Mandible μg/gr^{+}	Heart μg/gr^{+}
I. 15 min lidocaine	$(1.645 \pm 0.711) \times 10^{-3}$	1.756 ± 0.96	0.534 ± 0.169	1.048 ± 0.499
II. 15 min lidocaine+clonidine	$(2.523 \pm 0.883) \times 10^{-3}$ *	7.707 ± 4.032 **	1.216 ± 0.46 **	1.924 ± 1.028*
III. 30 min lidocaine	$(2.133 \pm 0.665) \times 10^{-3}$	1.156 ± 0.737	0.886 ± 0.44	1.179 ± 0.328
IV. 30 min lidocaine+clonidine	$(3.499 \pm 1.187) \times 10^{-3}$ *	15.923 ± 10.385 **	3.736 ± 0.532 ***	2.059 ± 0.836 *

$^{+}$ μg/gr of tissue weight; Serum *I/II $P < 0.05$ and *III/IV$P < 0.05$; Masseter **I/II$P < 0.01$ and **III/IV$P < 0.01$; Mandible **I/II$P < 0.01$ and ***III/IV$P < 0.0001$; Heart *I/II$P < 0.05$ and *III/IV$P < 0.05$

Table 3 Percentage (%) of free lidocaine fraction in serum and tissues in the *in vitro* experiment

	Serum	Masseter	Mandible	Heart
15 min lidocaine	9.7 ± 1.7	16.3 ± 1.2	17.9 ± 2.7	16 ± 0.9
15 min lidocaine+clonidine	11.3 ± 1.07*	18.17 ± 0.14*	18.45 ± 1.7	21.62 ± 0.28**
30 min lidocaine	12.8 ± 2.3	13.25 ± 0.14 $^{\#}$	15.67 ± 2.6$^{\#}$	15.1 ± 0.1$^{\#}$
30 min lidocaine+clonidine	16.61 ± 1.2**	15.35 ± 0.16*	16.33 ± 4.2	18.5 ± 0.2*

Serum *I/II $P < 0.05$, **III/IV $P < 0.01$, I/III $P < 0.5$; Masseter *I/II and *III/IV $P < 0.05$, $^{\#}$I/III NS; Mandible I/II and III/IV $P < 0.4$ NS, $^{\#}$I/III NS; Heart **I/II $P < 0.01$,* III/IV $P < 0.05$, $^{\#}$I/III NS

Lidocaine and clonidine have the ability to compete with one other for the same binding sites, displacing each other and leading to an increase in the free drug concentration, which normally enhances the pharmacological action of the displaced substance. However, this phenomenon cannot be generalized, since the consequences of displacement *in vivo* depend on other factors as well, such as the drug's distribution and elimination profile (24).

The results of the present study showed that clonidine interferes with the pharmacokinetics of lidocaine. Co-administration of lidocaine and clonidine resulted in a significant increase of the free lidocaine fraction at 15 and 30 min. As both drugs are cationic (clonidine pKa = 8.25 and lidocaine pKa = 7.9), lidocaine is probably displaced from its binding sites in serum and tissue proteins. Both drugs are known to possess a higher affinity of binding to alpha-acid glycoprotein than to albumin, but in clinical practice only the amount of the free active fraction is important, since this is responsible for the pharmacological effect. These results are in agreement with previous studies in which co-administration of other cationic drugs was shown to increase the concentration of free lidocaine in serum and tissues (2,9).

Our *in vitro* results supported the displacement of lidocaine observed *in vivo* in the presence of clonidine, and suggested that the binding process may be partly responsible for this interaction. Estimation of percentage binding to proteins *in vitro* only in water-deprived tissues with addition of NaN_3 solution ensures that no metabolic reaction occurs in the examined tissues and reflects the actual binding capacity of the tissue proteins. Therefore it is almost certain that the results obtained with a lidocaine mixture (labeled + cold substance) reflect intact lidocaine without any metabolic products such as mono-ethylglycinexylidide (MEGX).

The pharmacokinetic interaction observed in the present study could have considerable implications for clinical practice, allowing determination of the appropriate dose of lidocaine in each individual case. A decrease in the effective analgesic dose of lidocaine could be achieved, avoiding any undesirable effects on the cardiovascular system. An ultrasound study of the heart has recently proved that there are no statistically significant pharmacodynamic alterations when clonidine is co-administered with lidocaine, in terms of heart function parameters (18).

Furthermore, studies comparing dental anesthesia for third molar surgery using a combination of lidocaine with clonidine, or lidocaine with epinephrine, have demonstrated no great differences of hemodynamic parameters between the two groups. However a significant reduction in heart

rate and systolic blood pressure was observed in the lidocaine plus clonidine group, and a significant increase in heart rate was evident in the lidocaine plus epinephrine group 10 min after third molar surgery (16,17). On the other hand, it has been shown that mepivacaine, a compound similar to lidocaine, might modulate the contractility of the heart and vascular system (25).

In conclusion, the fraction of free lidocaine is enhanced by co-administration of clonidine *in vivo* and *in vitro*, with a possible further increase of its pharmacodynamic (local anesthetic) effect. Thus enhancement of the free lidocaine fraction observed in the present study may permit the use of this drug combination for anesthesia of the maxillofacial area as an alternative to lidocaine and epinephrine, since it may potentiate the local anesthetic effect, thus altering patient compliance without any cardiovascular complications.

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