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The Influence of Diphenhydramine Administration on Lidocaine Protein Binding in Rat Serum and Tissues

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Abstract: Lidocaine is an amide type local anaesthetic and diphenhydramine is a first-generation antihistamine drug. The aim of the present study was to investigate the influence of diphenhydramine co-administration on the extent of lidocaine binding to rat serum and maxillofacial tissue proteins in order to determine a possible synergistic action that could enhance lidocaine's anaesthetic/analgesic activity. Twenty-eight Wistar rats divided in 4 groups (I, II, III, IV) received lidocaine in the masseter muscle. Groups II and IV received diphenhydramine per os 2 h before lidocaine administration. Groups I and II were sacrificed after 15 min and groups III and IV 30 min after lidocaine injection. Masseter and mandible samples were isolated and incubated in NaCl 0.9% solution while serum was obtained through blood centrifugation. Free lidocaine fraction in the tissues' incubation medium and the serum was obtained through ultrafiltration and determined by radioscopic method in a β-counter Lidocaine's free fraction levels (μg g^{-1}) were enhanced after 15 min under diphenhydramine co-administration in all samples from $(7.1319\pm1.4066)\times10^{-4}$ to $(12.1097\pm3.7528)\times10^{-4}$ in serum, from 0.9339±0.3077 to 2.6791±1.1648 in masseter ($p<0.01$) and from 0.3898±0.0879 to 0.6918±0.2743 in mandible ($p<0.05$). A statistically significant increase in free anaesthetic levels was also noticed after 30 min in serum from $(8.6227\pm0.6902)\times10^{-4}$ to $(13.9518\pm4.9849)\times10^{-4}$ ($p<0.05$). Lidocaine's increase could probably be attributed to mechanisms influencing its protein binding properties. Consequently, a possible synergistic action of the two drugs' combination is demonstrated which could enhance lidocaine's anaesthetic action, affecting depth and duration of anaesthesia.

Key words: Antihistamine, local anaesthetic, alpha-1-acid glycoprotein, displacement, pharmacokinetics interaction

INTRODUCTION

Lidocaine is an amide type local anaesthetic agent, widely used in dental practice. It is a cationic molecule with pKa value of 7.9, showing stronger binding affinity to alpha-1-acid glycoprotein than to albumin (Isohanni, 2009).

Diphenhydramine, a first-generation antihistamine drug, belongs to the ethanolamine class. In addition to the antihistamine activity, diphenhydramine produces sedation, possesses anticholinergic action and is effective as an antitussive and antivomiting agent. It also possesses a local anaesthetic effect . It is characterized as a basic molecule with pKa value of 9 and is mainly bound to alpha-1-acid glycoprotein. Its local anaesthetic properties can be useful in cases of dental or emergency patients claiming to have allergy to the commonly used local anaesthetic drugs (Gallo and Ellis, 1987; Malamed, 1973; Pavlidakey *et al*., 2009; Pollack and Swindle, 1989; Uckan *et al*., 1998). Diphenhydramine is shown to be effective for dermal surface anaesthesia (Green *et al*., 1994; Ernst *et al*., 1994), as well as when applied topically to rabbit corneas (Suffridge *et al*., 2009). However, diphenhydramine is reported to possess less effective anaesthetic action than lidocaine and moreover to cause adverse reactions since it may be irritating on injection and post-injection phase or result in skin necrosis (Dire and Hogan, 1993; Willett *et al*., 2008).

Diphenhydramine may serve as an alternative not only for patients with a history of allergy but also in the management of large lacerations in order to avoid an amide anaesthetic overdose (Ernst *et al*., 1993). Consequently, these two drugs' synergistic action may reduce the required amide anaesthetic dosage, thus decreasing the possibility of lidocaine's overdose and the side effects concerning the central nervous and cardiovascular system.

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The aim of the present study was to investigate the influence of diphenhydramine co-administration on free lidocaine fraction in rat serum, masseter muscle and mandibular bone, in order to determine a possible synergistic action that could enhance lidocaine's anaesthetic/analgesic activity, since only the free drug is pharmacologically active.

MATERIALS AND METHODS

Twenty-eight male Wistar rats with a bodyweight of 250±10 g, aged almost 6 weeks, divided into 4 groups (I, II, III and IV, n = 7) were used. All groups received lidocaine (Xylocaine inj. 2% 50 mL Astra Zeneca) in a single dose of 4 mg kg^{-1} in the masseter muscle. The injection mixture consisted of labeled lidocaine (Carbonyl-^{14}C -lidocaine hydrochloride, with specific activity 50 m Ci/mmol)and cold substance (2.85 μg mL^{-1} cold substance +7 μL labeled lidocaine). Groups II and IV received diphenhydramine per os by a gastroesophagal catheter (Benadryl syr. 12.5 mg/5 mL Pfizer) in a single dose of 1.25 mg kg^{-1} 2 h before lidocaine administration. The animals were sacrificed 15 (groups I and II) and 30 (groups III and IV) minutes after lidocaine administration. The groups used in study design are shown in Table 1.

Blood was collected and centrifuged in order to obtain serum. Masseter muscle and mandibular bone were isolated, removed, weighted and incubated in 3 mL of NaCl 0.9% for 12 h at 8°C. The medium used for tissues' incubation and the serum were subjected to ultrafiltration in order to obtain the free drug fraction according to previous studies (Kotsiou *et al*., 2006; Tesseromatis *et al*., 1987, 2007; Tigka *et al*., 2009, 2011). Free lidocaine levels were estimated by radioscopic method in a β-scintillation Packard counter, using 200 μL of ultrafiltrate added to 15 mL scintillation solution (Tesseromatis *et al*., 1987, 2007; Tigka *et al*., 2009, 2011).

The animals received care according to the Guide for the Care and Use of Experimental animals (NRC, 1985). Every effort was made to minimize animal suffering and to use the minimum number of animals required in order to reach safe conclusions.

Statistical analysis: Statistical analysis was performed via t-test. Differences were considered significant at the level of $p<0.05$. SPSS t-test was used to conduct the analysis.

RESULTS AND DISCUSSION

As shown in Table 2, the levels of free lidocaine fraction in serum and the examined maxillofacial tissues were increased when diphenhydramine was co-administered.

Free lidocaine fraction (μg g^{-1}) in serum was significantly increased from $(7.1319\pm1.4066)\times10^{-4}$ estimated 15 min after initial lidocaine administration to $(12.1097\pm3.7528)\times10^{-4}$ at the same time point under the influence of diphenhydramine ($p<0.01$). Free lidocaine's fraction $[(8.6227\pm0.6902)\times10^{-4}]$ estimated 30 min after its initial administration was also statistically significantly ($p<0.05$) increased to the value of $(13.9518\pm4.9849)\times10^{-4}$ under diphenhydramine co-administration. A significant enhancement of free lidocaine levels under diphenhydramine treatment was also noticed in masseter [from 0.9339±0.3077 to 2.6791±1.1648 ($p<0.01$)] and in mandible [from 0.3898±0.0879 to 0.6918±0.2743 ($p<0.05$)] 15 min after the anaesthetic administration compared to the free anaesthetic concentration at the same time point in the absence of antihistamine treatment. Masseter and mandible lidocaine levels at 30 min were increased in the presence of diphenhydramine but the observed difference was not statistically significant.

Drugs bind reversibly to plasma and tissue proteins, forming complexes which are pharmacologically inactive and represent a drug's storage form. Only free-unbound drug is able to exert pharmacologic action. A drug's displacement from its binding sites on proteins may result in increased drug's diffusion to action sites and consequently increased pharmacologic effect.

Table 1: Groups used in study design

Group I	Intramuscular (IM) lidocaine administration and animal sacrifice 15 min later
Group II	Per os diphenhydramine administration 2 h before IM lidocaine administration. Animal sacrifice 15 min after lidocaine administration
Group III	IM lidocaine administration and animal sacrifice 30 min later
Group IV	PO diphenhydramine administration 2 h before IM lidocaine administration. Animal sacrifice 30 min after lidocaine administration

Table 2: Free fraction of lidocaine

	Serum μg/g^{+}	Masseter μg/g^{++}	Mandible μg/g^{++}
Group I Lid 15 min	$(7.1319\pm1.4066)\times10^{-4}$	0.9339±0.3077	0.3898±0.0879
Group II Lid+diph 15 min	$(12.1097\pm3.7528)\times10^{-4}$	2.6791±1.1648	0.6918±0.2743
Group III Lid 30 min	$(8.6227\pm0.6902)\times10^{-4}$	1.0032±0.7842	0.3803±0.0636
Group IV Lid+diph 30 min	$(13.9518\pm4.9849)\times10^{-4}$	1.3606±0.346	0.4374±0.116

Lid = lidocaine, diph= diphenhydramine, $^{+}$μg/total body weight, $^{++}$ μg/weight of tissue, Serum I/II $p<0.01$ and III/IV $p<0.05$, Masseter I/II $p<0.01$, Mandible I/II $p<0.05$

Many drugs and endogenous substances can influence another drug's protein binding or displace other drug molecules, resulting in altered free drug's concentration and in a clinical impact on its pharmacologic outcome (Hu *et al*., 1993; Juarez-Olguin *et al*., 2002; Saranteas *et al*., 2002, 2003, 2004; Tsivou *et al*., 2005).

According to the results of the present study, an interaction exists between the two administered substances which enhance free lidocaine levels in serum and the examined tissues. As both drugs are cationic and are also both mainly bound to alpha-1-acid glycoprotein, a possible pharmacokinetics interaction which influences lidocaine's protein binding, takes place. Lidocaine is displaced from its serum and tissues' protein binding sites and its free fraction, which is the pharmacologically active, is increased under the administration of diphenhydramine.

An additional analgesic effect can therefore be observed in clinical practice under the co-administration of the two drugs. Moreover the increase of active lidocaine's concentration, apart from pain relief, can further protect tissues, since it exerts antinociceptive action (Muth-Selbach *et al*., 2009).

The present results are in agreement with previous studies, in which the free fraction of lidocaine was increased under the co-administration of other cationic drugs (Tigka *et al*., 2009, 2011; Tesseromatis *et al*., 2007). Adminstration of clonidine, a cationic drug with pKa = 8.25 and stronger binding affinity to alpha-1-acid glycoprotein, interferes with the pharmacokinetics of lidocaine, resulting in an increase of lidocaine free fraction in serum and tissues (heart, masseter muscle, mandible bone), probably through the anaesthetic drug's displacement from its protein binding sites (Tigka *et al*., 2009, 2011). An *in vitro* experimental model also indicates that propranolol administration can displace lidocaine from liver proteins, therefore increasing its free fraction excreted by the liver (Tesseromatis *et al*., 2007).

The interaction of lidocaine with other drugs is also shown by Saranteas *et al*. (2003), who suggest that paracetamol or propranolol administration increases lidocaine concentration in serum. They also suggest that propranolol changes lidocaine's protein binding in the tissues examined.

It would be wise to have in mind that the free fraction's increase of a displaced drug may be followed by redistribution or altered elimination process which could influence the displacement's clinical outcome. Other interaction procedures may also be implicated, affecting the final clinical result of lidocaine's and diphenhydramine's co-administration. Similar study is referred by Rahman *et al*. (2001) demonstrating a competition for the same binding site on albumin taking place when dexamethasone phosphate and testosterone phenyl propionate are concurrently administered. It may be noticed that when warfarin is co-administered with the above substances, site I is blocked and an increase of the free drug's concentration occurs. When site I is not blocked by warfarin, the displaced from its high affinity binding site II drug is rebound to its low affinity site I, showing a lower free concentration than that obtained in the presence of warfarin. Similarly, a site-to-site displacement occurs during concurrent administration of captopril and ciprofloxacin with captopril's free fraction increase depending on the presence of the site II specific probe diazepam (Mahbubul Alam *et al*., 2004).

However, according to the results of the present study, diphenhydramine administration causes an increase in lidocaine's free levels, despite the presence of any processes which possibly take place.

Summarizing, it is demonstrated that apart from sedation caused by co-administered diphenhydramine, the two drugs' combination can lead to a synergistic action, affecting depth and duration of anaesthesia. Moreover, diphenhydramine as a co-administered drug can obtain a decrease in lidocaine's dosage and consequently in the side effects caused by its overdose.

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