Laboratory Investigation

Cigarette smoke increases bupivacaine metabolism in rats

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This study was designed to document possible changes in bupivacaine kinetics in rats after exposure to cigarette smoke. Rats (n = 15) were exposed to cigarette smoke (Borgwaldt type Hamburg II) for ten minutes per day during four days (C) or eight days (B); controls (A) were used simultaneously without exposure to cigarette smoke. After bupivacaine 20 mg · kg^{-1} ip at day 4 (C) or day 8 (B), blood was sampled (0.5 ml of blood collected by puncture at the retro-orbital sinus 0.25, 0.5, 1, 2, 4, 6 and 8 hours after administration) and bupivacaine and its main metabolite i.e., desbutylbupivacaine (PPX) were determined by gas liquid chromatography. The sensitivity of the method was 15 ng · ml^{-1} and the reproductibility was <6%. Serum bupivacaine concentrations were plotted against time and the pharmacokinetic variables were determined assuming a two compartment open model: Cmax, Tmax were derived directly from individual data. The β phase elimination half-lives ($T_{1/2}\beta$), the area under the serum concentration curve (AUC_0^∞), the total plasma clearance (Cl) and the total volume of distribution (Vd) were calculated. These variables were assessed according to non-linear fitting method. Cigarette smoking exposure did not change the pharmacokinetic variables of bupivacaine. However, the pharmacokinetic parameters of PPX, Cmax (0.175 ± 0.007 μg · ml^{-1}, 0.119 ± 0.014 μg · ml^{-1} and 0.312 ± 0.023 μg · ml^{-1}, for groups A, B and C, respectively), AUC (0.170 ± 0.006 μg · ml^{-1} · hr^{-1}, 0.104 ± 0.013 μg · ml^{-1} · hr^{-1} and 0.433 ± 0.017 μg · ml^{-1} · hr^{-1} for groups A, B and C, respectively) and the ratio AUC PPX/ AUC bupivacaine (0.306 ± 0.042, 0.153 ± 0.021 and 0.660 ± 0.054 for groups A, B and C, respectively) were higher (P < 0.0001) for group C. These results indicate an enzymatic induction after only short exposure to cigarette smoking and justify further studies to document possible variations of the metabolism of bupivacaine induced by exposure to cigarette smoke in humans.

Le but de ce travail était de rechercher l'influence éventuelle du tabac sur la cinétique de la bupivacaïne chez le rat. Les animaux (n = 15) ont été exposés à la fumée de cigarette durant dix minutes par jour, soit pendant quatre jours (C), soit pendant huit jours (B), à l'aide d'une machine à fumer (Borgwaldt type Hamburg II); les témoins (A, non exposés à la fumée de cigarette) ont été utilisés simultanément et ont reçu une dose unique de 20 mg · kg^{-1} ip de bupivacaïne, ainsi que les animaux des groupes C au quatrième jour d'intoxication et ceux du groupe B au huitième jour. Les prélèvements de sang ont été faits au sinus rétro-orbitaire à 0,25, 0,5, 1, 2, 4, 6 et 8 heures après l'administration et la bupivacaïne, ainsi que son principal métabolite, la desbutylbupivacaïne (PPX), ont été déterminés par chromatographie en phase gazeuse. La sensibilité de la méthode était de 15 ng · ml^{-1} et la reproductibilité était de <6%. Les paramètres pharmacocinétiques ont été déterminés selon un modèle ouvert a deux compartiments: Cmax et Tmax ont été déterminés par inspection. La β demi-vie d'élimination ($T_{1/2}\beta$), l'aire sous la courbe des concentrations (AUC_0^∞, la clairance plasmatique totale (Cl) et le volume de distribution (Vd) ont été calculés selon une méthode d'ajustement non linéaire. L'exposition des animaux à la fumée de cigarette n'a pas modifié les paramètres pharmacocinétiques de la bupivacaïne. A l'inverse, en ce qui concerne le PPX, le Cmax (0,175 ± 0,007 μg · ml^{-1}, 0,119 ± 0,014 μg · ml^{-1} et 0,312 ± 0,023 μg · ml^{-1}, respectivement pour les groupes A, B et C), l'AUC (0,170 ± 0,006 μg · ml^{-1} · hr^{-1}, 0,104 ± 0,013 μg · ml^{-1} · hr^{-1} et 0,433 ± 0,017 μg · ml^{-1} · hr^{-1} respectivement pour les groupes A, B et C) et le rapport AUC PPX/ AUC bupivacaïne (0,306 ± 0,042, 0,153 ± 0,021 et 0,660 ± 0,054

Key words
ANESTHETICS, LOCAL: bupivacaine;
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respectivement pour les groupes A, B et C) ont été plus élèvé (P < 0,0001) après exposition au tabac durant quatre jours (C) mais pas durant huit jours (B). Ces résultats seraient donc en faveur d'une induction enzymatique après exposition durant quatre jours et justifient d'autres études pour préciser les modifications du métabolisme de la bupivacaïne après exposition a la fumée de tabac chez l'homme.

Many environmental, physiopathological or pharmacological factors which may influence the kinetic behaviour of one of the most commonly used local anaesthetics, bupivacaine, are under investigation in rodents in our laboratory. There are numerous studies demonstrating the influence of smoking on the kinetics of many drugs.[1] Cigarette smoke has been shown to cause induction of drug metabolizing enzymes[2] and thus may modify the metabolism of drugs metabolized by the liver such as local anaesthetics. However, the influence of cigarette smoking on local anaesthetic kinetics has not yet been studied.

The aim of the present work was to document possible changes in the pharmacokinetic behaviour of bupivacaine and its main metabolite, desbutylbupivacaine (PPX), induced by cigarette smoke inhalation in rats.

Methods

Animals

Adult male Wistar AF IOPS rats ($n = 15$, mean weight $= 215 \pm 2.5$ g) were housed one per cage for a minimum of two weeks before use under controlled, relative humidity (50–55%), temperature 25 ± 1°C and synchronization by light-dark cycle (06.00–18.00 hr, 18.00–0.600 hr) with free access to food and water, between January and February 1993.

Apparatus for cigarette smoking

A smoking apparatus was used for exposing the rats to cigarette smoke (Borgwaldt, Type Hamburg II, graciously supplied by SEITA). This apparatus consisted of a smoking head to which 30 cigarettes can be attached, a smoking channel, a smoking chamber slide piece, an inhalation chamber and ten animal holders for exposing the rats to smoke. The animal holders were modified to receive adult rats (the original holders being too small for holding adult rats). Briefly, the sequence of exposure consisted in lighting individually the cigarettes attached to the smoking head and then the smoking head was turned. The smoke from the lighted cigarettes was mixed with air at a ratio of 1/7 and passed to the inhalation chamber. Thirty cigarettes were lit. The animals were exposed to smoke (smoke:air, 1:7) for ten minutes. The duration of inhalation time from the smoking head and the frequency were 28 sec and $1 \cdot min^{-1}$, respectively. Five animals were exposed to the smoke simultaneously. The cigarettes used were standard cigarettes (Gitanes®, without filter, nicotine content: 1.46 mg, tar content: 14.7 mg).

Protocol

Two durations of cigarette smoke exposure were tested: four and eight days. Three groups of five rats were used for this experiment; during eight days (group B) or during four days (group C), rats were exposed as previously described to cigarette smoke; five rats (group A = controls) were placed under the same experimental conditions into the animal holders without exposure to cigarette smoke to apply the same stress conditions.

On day 4 and day 8 (at 09.00 hr, 15 min after the last exposure to smoke), animals of group B and C received bupivacaine (0.5%) 20 $mg \cdot kg^{-1}$ *ip*. Controls were also injected with bupivacaine (0.5%) 20 $mg \cdot kg^{-1}$ *ip*. Blood (0.5 ml) was collected from each animal by puncture at the retro-orbital sinus 0.25, 0.5, 1, 2, 4, 6 and 8 hr after administration. After each sample, 0.5 ml saline was injected *ip* in order to correct for blood loss. Blood was centrifuged and plasma was immediately frozen at −20°C until assayed.

Determination of bupivacaine and pharmacokinetic variables

Total bupivacaine and its main metabolite, desbutylbupivacaine (PPX), serum concentrations were determined by a specific gas liquid chromatographic method according to the modified technique of Bjork *et al.*[3] The sensitivity of the method was 15 $ng \cdot ml^{-1}$ and the reproductibility was <6%. Concerning the interday reproductibility, a CV of 0.68 and 1.83 for bupivacaine and PPX at a level of 1 $\mu g \cdot ml^{-1}$, respectively, was found.

Serum bupivacaine concentrations were plotted against time and pharmacokinetic variables were determined assuming a two compartment open model as previously described.[4] The Cmax and the Tmax were derived directly from individual data. The β phase elimination half-lives ($T_{1/2}\beta$) and the area under the serum concentration curve (AUC_0^{∞}) were calculated. Total plasma clearance (Cl) and total volume of distribution (Vd) were calculated according to the following equations: $Cl = F \times dose/AUC$ where F is equal to 1 and $Vd = Cl/Kel$.

These variables were assessed according to previously reported non-linear fitting methods (Bruguerolle, 1992) using a personal computer programme. All data were summarized (means ± SEM) and comparisons were done using ANOVA. Pairwise comparisons by Dunett t test were performed when a difference was detected after ANOVA (Statview II® program).

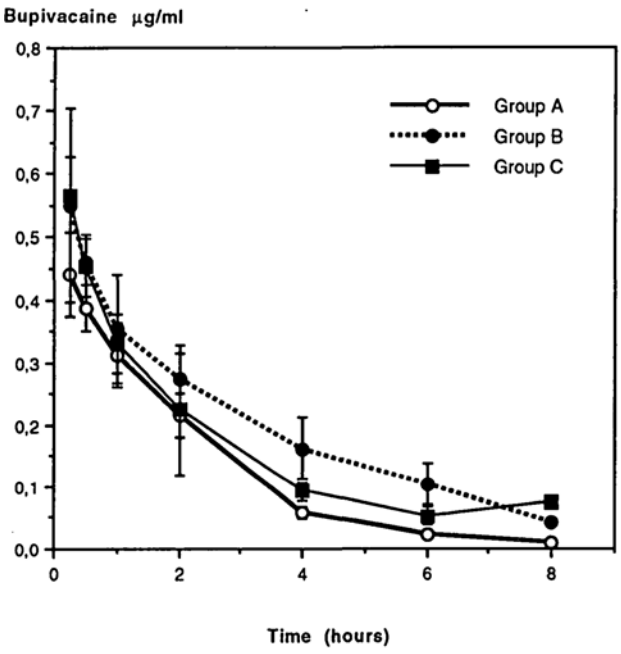

FIGURE 1 Plasma bupivacaine concentrations versus time with or without cigarette smoke inhalation.

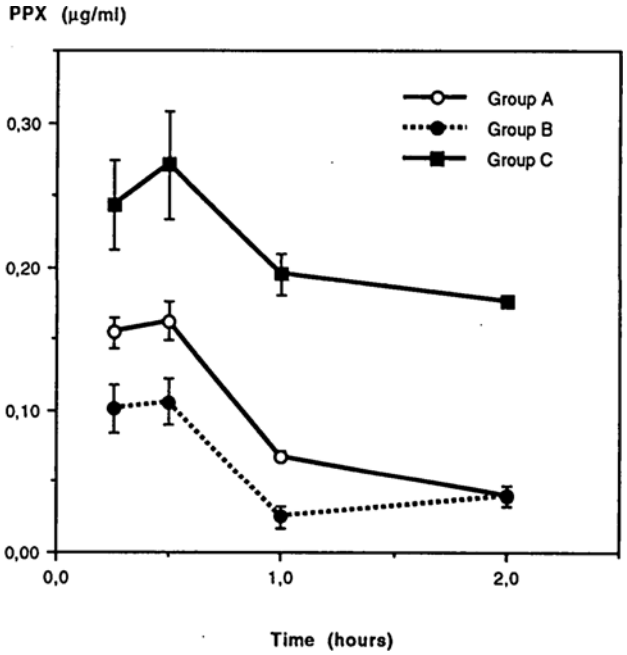

FIGURE 2 Plasma PPX concentrations versus time with or without cigarette smoke inhalation.

Results

Mean ± sem bupivacaine and PPX plasma concentrations in the three groups of rats are shown in Figures 1 and 2, respectively. The pharmacokinetic variables and statistical evaluations in each of these groups are listed in Tables I and II. Cigarette smoking exposure did not change the pharmacokinetic behaviour of bupivacaine. The PPX was detected in plasma only during the first two hours. But, the pharmacokinetic variables of PPX, Cmax (0.175 ± 0.007 $\mu g \cdot ml^{-1}$, 0.119 ± 0.014 $\mu g \cdot ml^{-1}$ and 0.312 ± 0.023 $\mu g \cdot ml^{-1}$, for groups A, B and C, respectively), AUC (0.170 ± 0.006 $\mu g \cdot ml^{-1} \cdot hr^{-1}$, 0.104 ± 0.013 $\mu g \cdot ml^{-1} \cdot hr^{-1}$ and 0.433 ± 0.017 $\mu g \cdot ml^{-1} \cdot hr^{-1}$ for groups A, B and C, respectively) and the ratio AUC PPX/ AUC bupivacaine (0.306 ± 0.042, 0.153 ± 0.021 and 0.660 ± 0.054 for groups A, B and C, respectively) were higher ($P < 0.0001$) for the group C (Dunett t test, Table II).

Discussion

The present work demonstrated that bupivacaine pharmacokinetic behaviour is not modified by exposure to cigarette smoke for four or eight days. On the contrary, our data indicated increasing metabolism of the drug during cigarette smoke exposure: PPX plasma concentrations were increased at day 4 of exposure but not at day 8. This may indicate hepatic microsomal enzyme induction during the first days of exposure.

Few studies have been devoted to the influence of cigarette smoking on the kinetics of local anaesthetics. Variations of protein binding of lidocaine have been studied[5,6] but the data are conflicting. For some authors, cigarette smoking decreased lidocaine protein binding due to induced variations of alpha 1 glycoprotein acid and albumin, while for some others, protein binding and plasma proteins are not modified.[5]

The effects of cigarette smoke on rats have been studied for drugs such as indomethacin, nicorandil, theophylline ...[7-9]; nicotine and other components of cigarette smoke have been shown to cause induction of drug metabolizing enzymes.[2] Animal studies support the view that nicotine is an enzyme inducer. The metabolism of meprobamate, benzo(a)pyrene, ethylmorphine, norcodeine, aniline ... were reported to be accelerated subsequent to nicotine administration by various route and dosages.[1]

Gomita *et al.*[7,8] and Yoshida *et al.*[9] reported, in animal studies, that plasma concentrations of some drugs such as indomethacin, nicorandil, theophylline ... were decreased by exposure to cigarette smoke. These effects were attributed to nicotine in the cigarette and/or other constituents of tobacco smoke. Concerning indomethacin,[9] it was suggested that acute exposure to cigarette smoke caused a decrease in indomethacin plasma concentrations due to delayed absorption from the gastrointestinal tract (since plasma concentrations were not influenced by the cigarette smoke when administered intravenously or rectally). The cigarette smoke may not have any influence on the metabolism of indomethacin.

TABLE I Mean ± sem bupivacaine pharmacokinetic variables in plasma; AUC: area under concentration curve ($\mu g \cdot ml^{-1} \cdot hr^{-1}$), Cmax: maximal concentration in serum ($\mu g \cdot ml^{-1}$), Tmax: time to reach the Cmax (hr), $T_{1/2}\beta$: β elimination half-life (hr), Cl: total plasma clearance ($ml \cdot hr^{-1}$) and Vd: total volume of distribution (ml)

	AUC ($\mu g \cdot ml^{-1} \cdot hr^{-1}$)	*Cmax* ($\mu g \cdot ml^{-1}$)	*Tmax* (hr)	$T_{1/2}\beta$ (hr)	*Cl* ($ml \cdot hr^{-1}$)	*Vd* (ml)
Group A (Controls)	0.981 ± 0.142	0.471 ± 0.042	0.300 ± 0.05	1.565 ± 0.105	5.854 ± 0.230	10.665 ± 0.46
Group B (8 days)	1.556 ± 0.245	0.623 ± 0.092	0.312 ± 0.06	2.233 ± 0.212	4.670 ± 1.786	12.987 ± 3.614
Group C (4 days)	1.267 ± 0.192	0.567 ± 0.059	0.250 ± 0.00	1.710 ± 0.249	6.085 ± 1.378	8.634 ± 0.984
ANOVA						
F	2.803	1.246	0.459	3.507	0.379	1.111
P	0.108	0.329	0.645	0.07	0.694	0.367

TABLE II Mean ± sem PPX pharmacokinetic variables in plasma; AUC: area under concentration curve ($\mu g \cdot ml^{-1} \cdot hr^{-1}$), Cmax: maximal concentration in serum ($\mu g \cdot ml^{-1}$), Tmax: time to reach the Cmax (hr). Statistical comparison was done by ANOVA

	Cmax ($\mu g \cdot ml^{-1}$)	*Tmax* (hr)	AUC_0^2 ($\mu g \cdot ml^{-1} \cdot hr^{-1}$)	*Ratio* AUC_0^2 *PPX/* AUC_0^2 *bupivacaine*
Group A (Controls)	0.175 ± 0.007	0.400 ± 0.061	0.170 ± 0.006	0.306 ± 0.042
Group B (8 days)	0.119 ± 0.014	0.375 ± 0.072	0.104 ± 0.013	0.153 ± 0.021
Group C (4 days)	0.312 ± 0.023	0.562 ± 0.157	0.433 ± 0.017	0.660 ± 0.054
ANOVA				
F	57.828	0.977	199.7	35.45
P	0.0001	0.410	0.0001	0.0001
Dunett t test	$P < 0.05$		$P < 0.05$	$P < 0.05$
	A vs B		A vs B	A vs C
	B vs C		B vs C	B vs C
	A vs C		A vs C	

The possible enzymatic induction documented after a short exposure to cigarette smoking must be discussed taking into account results from Raunio *et al.*[10] who reported a similar increase (i.e., induction) in liver ornithine decarboxylase and arylhydrocarbonhydroxylase activities in rats after cigarette smoking inhalation for the three first days of exposure and a decrease (i.e., an inhibition) after ten days. Also, Ali *et al.*[11] reported that chronic (28 days) administration of tobacco resulted in a marked increase in the rate of N-demethylation of amidopyrine, morphine and pethidine but the N-demethylation of these drugs was not affected by tobacco treatment for two or seven days.

In conclusion, our data indicate increasing metabolism of bupivacaine during cigarette smoke exposure: PPX plasma concentrations being increased after four days of exposure. This may indicate a possible hepatic microsomal enzyme induction during the first days of exposure. Future investigations will document possible changes in bupivacaine metabolism after different durations of exposure to cigarette smoke.

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