Isotopic Effect Study of Propofol Deuteration on the Metabolism, Activity, and Toxicity of the Anesthetic

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The use of isotopic substitution to delay the oxidative metabolism of the anesthetic propofol **1** was studied. The aromatic hydrogens of propofol **1** were replaced by deuterium to produce the mono- and trideuterated derivatives **4** and **5**. In vitro metabolic studies on human hepatic microsomes showed no isotopic effect in the para hydroxylation of propofol, and **1**, **4**, and **5** display similar hypnotic activity and toxicity in mice.

Introduction

Propofol **1** (2,6-diisopropylphenol) is a short-acting hypnotic agent used for inducing and maintaining anesthesia. It is intravenously administered either by repeated bolus injections or by continuous injection.[1] The exact neurochemical mechanism of action of propofol remains unclear. However, it is well-known that the general neurochemical mechanism of anesthetics undergoes interactions between the anesthetic and the GABA receptor.[2]

The propofol is quickly and widely distributed into the body, intensively metabolized, and eliminated. Thus, 88% of the initial dose is found in the urine within 5 days and 2% in the feces.[3] The major metabolic pathway of propofol is its glucuronidation consuming 50−60% of the total dose. The second metabolic pathway is the para hydroxylation of propofol producing compound **2** (Scheme 1). The final metabolic pathways are the 1- and 4-glucuronidation or the 4-sulfation of the metabolite **2**.[4] All these metabolic pathways reduce or even suppress the propofol activity.

The oxidative metabolism of propofol at low concentrations involves cytochrome CYP2C9 (at least 50%) and other isoforms such as CYP2A6, 2C8, 2C18, 2C19, and 1A2. The role of the latter group of cytochromes is amplified with increasing concentrations of propofol and decreasing concentrations of CYP2C9. For this reason, the metabolism of propofol has low interindividual variability and low interactions with other drugs.[5]

Drugs labeled with stable isotopes can be used as ideal internal standards in quantitative studies, and stable isotopes having no isotopic effect such as ^{13}C or ^{15}N are then preferred. However, the deuterium isotope is often employed. Indeed, it may induce some modifications of the chemical and physicochemical properties of the labeled drugs (polarity, molar volume, electron donation, van der Waals forces, dipolar moment, and lipophilicity) and therefore may contribute to the modification of metabolism kinetics and biological properties such as biodistribution and affinity for the receptors.

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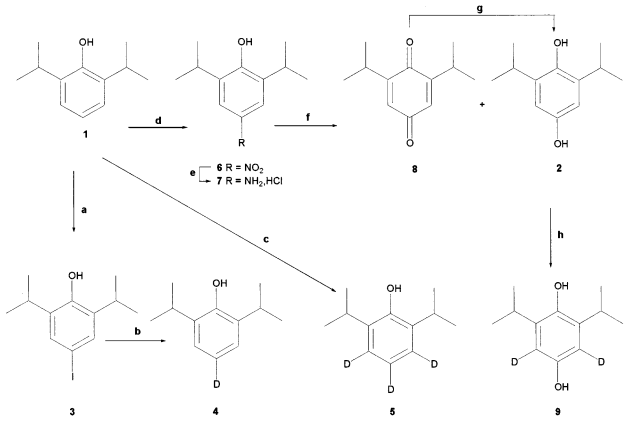
Scheme 1[a]



[a] Reagents: (a) ICl/AcOH; (b) D_2/Pd; (c) DCl/D_2O; (d) HNO_3/AcOH; (e) Sn/HCl; (f) H_2SO_4/$NaNO_2$; (g) $Na_2S_2O_4$/NaOH; (h) DCl/D_2O.

Various biological consequences of deuterium labeling can be observed. Thus, lower metabolism kinetics has been established for the N-deethylation of deuterated lidocaine[6] and for the debenzylation of deuterated 1-benzyl-4-cyano-4-phenylpiperidine[7] while the para hydroxylation of the phenytoin[8] (5,5-diphenylhydantoin) is not modified by deuteration of the metabolism site.

Concerning the in vivo biological properties, the deuterated amphetamines (phenyl-2-aminopropane) have a lower locomotor activity[9] than the protio compound, the deuterated 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) has the same cytotoxic activity[10] as the protio compound, whereas the inhibitory activity on the gastric secretion of *N*,*N*-dimethyl-*N*′-[2-(diisopropylamino)ethyl]-*N*′-(4,6-dimethyl-2-pyridyl)urea is increased by deuterium substitution.[11] Accordingly, we set out to investigate the influence of deuterium labeling on the metabolism and the pharmacological activity and toxicity of propofol.

Chemistry

Monodeuterated propofol **4** was synthesized by a catalyzed (Pd/C) exchange reaction under D_2 atmosphere from 4-iodo-2,6-diisopropylphenol **3**, which was obtained as previously described[12] by direct iodination

Table 1. K_m and V_m on Human Hepatic Microsomes, HD_{50} (Hypnotic Dose), LD_{50} (Lethality Dose), and TI (Therapeutic Index) on Mice, Effects of a 24 mg/kg (2 × HD_{50}) Dose on Mice, LRR (Loss of Righting Reflex), RW (Recovery to Walking), Loss of Sensibility (LS), and Loss of Painful Sensibility (LPS)

	V_{max}, nmol mg^{-1} min^{-1}	K_m, μM	HD_{50}, mg kg^{-1}	LD_{50}, mg kg^{-1}	TI	LRR, min	RW, min	LS, min	LPS, min
1	1.6 ± 0.2^a	12.3 ± 3.8^a	15	32	2.1	6.8 ± 1.6^b	8.8 ± 3.2^b	4.4 ± 0.7^b	3.1 ± 0.6^b
4	1.6 ± 0.3^a	16.7 ± 4.1^a	13	39	3	5.2 ± 1.4^b	6.4 ± 1.9^b	3.3 ± 1.0^b	2.1 ± 0.7^b
5	1.7 ± 0.3^a	16.4 ± 2.8^a	13	35	2.7	5.4 ± 1.8^b	7.3 ± 3.0^b	3.8 ± 1.3^b	2.3 ± 0.9^b

[a] The results are expressed as means and standard deviations of four determinations from four separate microsomal experiments. The differences in the K_m and V_m among **1**, **4**, and **5** are not statistically significant (Student's *t* test). [b] The results are expressed as means and standard deviations of 10 determinations.

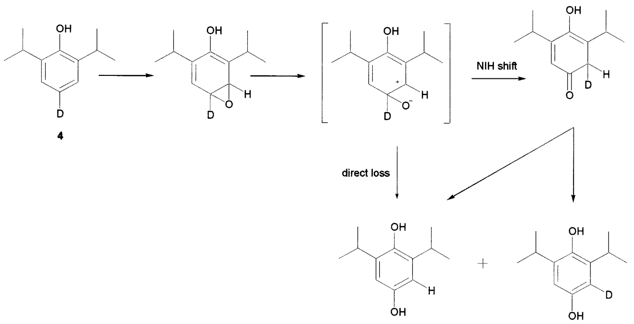
of propofol **1** (Scheme 1). The trideuterated propofol **5** was synthesized by an exchange reaction in DCl/D_2O at 140 °C under pressure. The synthesis of 4-hydroxy-2,6-diisopropylphenol **2** was undertaken by a Sandmeyer reaction from 4-amino-2,6-diisopropylphenol **7**, which was prepared as previously described[12] by nitration of propofol **1** and reduction of 2,6-diisopropyl-4-nitrophenol **6**. The Sandmeyer reaction leads to a mixture of 4-hydroxy-2,6-diisopropylphenol **2** and 2,6-diisopropyl-1,4-quinone **8**, which was reduced to 4-hydroxy-2,6-diisopropylphenol **2**. 3,5-Dideuterium-4-hydroxy-2,6-diisopropylphenol **9** was synthesized by an exchange reaction in DCl/D_2O at 140 °C under pressure. The monodeuterated propofol **4**, the trideuterated propofol **5**, and 3,5-dideuterium-4-hydroxy-2,6-diisopropylphenol **9** were obtained at low isotopic dilutions (3%, 6%, and 3%, respectively). The compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry, and microanalysis data.

In Vitro Experiments and GC−MS Analysis

Gas chromatography−mass spectrometry (GC−MS) has previously been used[13−16] for propofol kinetic studies in plasma[16] or whole blood[13] and for metabolic studies on human hepatic microsomes.[14] The propofol metabolism was followed by measuring the propofol decrease[13] or followed indirectly[14] by measuring 2,6-diisopropyl-1,4-quinone **8**, produced from 4-hydroxy-2,6-diisopropylphenol **2** in alkaline conditions. The GC−MS method selected in the present work includes a derivatization by silylation[16] in order to improve the stability and peak shapes of compounds in the course of gas chromatography.

The metabolism of propofol **1**, propofol-d_1 **4**, and propofol-d_3 **5** was followed using the appearance of the related hydroxylated metabolites. A quadratic regression analysis was carried out between 10 and 10 000 ng/mL 4-hydroxy-2,6-diisopropylphenol **2** or 3,5-dideuterium-4-hydroxy-2,6-diisopropylphenol **9**, and equations of the mean plots ($n = 4$) were $y = (2.71 \times 10^{-8})x^2 + (1.73 \times 10^{-4})x + 1.47 \times 10^{-2}$ ($r = 0.999$) for the 4-hydroxy-2,6-diisopropylphenol **2** and $y = (6.15 \times 10^{-9})x^2 + (2.55 \times 10^{-4})x + 3.44 \times 10^{-2}$ ($r = 0.999$) for the 3,5-dideuterium-4-hydroxy-2,6-diisopropylphenol **9**.

The incubation of propofol **1** with human microsomes and NADPH produced only one metabolite, 4-hydroxy-2,6-diisopropylphenol **2**. Indeed, the occurrence of the metabolite 2,6-diisopropyl-1,4-quinone **8**, systematically followed using the characteristic ion at m/z 149, has never been observed. The incubation of propofol-d_1 **4** produced a single metabolite, 4-hydroxy-2,6-diisopro-



4 **2** **10**

Figure 1. Arene oxidative pathway in microsomal oxidation of aromatic substrate.

tion (following the characteristic ion at m/z 339), owing to a NIH-shift mechanism[17] (Figure 1), was not found. The incubation of propofol-d_3 **5** likewise produced only one metabolite, 3,5-dideuterium-4-hydroxy-2,6-diisopropylphenol **9**.

Propofol **1** and its deuterated derivatives **4** and **5** displayed similar kinetics of metabolism. The Michaelis−Menten plots gave mean values of $V_m = 1.6 \pm 0.3$ nmol mg^{-1} min^{-1} and $K_m = 15.1 \pm 3.6$ μM (Table 1).

In Vivo Experiments

The hypnotic activity (HD_{50}) and the toxicity (LD_{50}) of propofol **1** and of the two deuterated compounds propofol-d_1 **4** and propofol-d_3 **5** were compared on mice. The speed of induction, the sleeping time, and the recovery time were noted and joined to a quantitative assessment of the analgesia for a 24 mg/kg dose (about 2 × HD_{50}) (Table 1). Concerning the hypnotic activity, propofol-d_1 **4** and propofol-d_3 **5** presented slightly lower HD_{50} compared to those obtained for the standard propofol **1**. Concerning the toxicity, the deuterated compounds and mainly propofol-d_1 **4** were found to be less toxic than propofol ($LD_{50propofol} < LD_{50propofol-d3} < LD_{50propofol-d1}$). Further experiments performed in mice at a 24 mg/kg dose showed that the induction times, the sleeping times, and the recovery times of the three compounds were similar and that the quality of the analgesia was preserved. Times of loss of sensibility and times of loss of painful sensibility were equivalent.

Discussion

The in vitro experiments of the three compounds give mean values of V_m and K_m ($V_m = 1.6 \pm 0.3$ nmol mg^{-1} min^{-1} and $K_m = 15.1 \pm 3.6$ μM), which are similar and in good agreement with those obtained for propofol **1** by Guitton et al.[5] The deuterium substitution of the

isotopic effect in the aromatic hydroxylation of the propofol indicates that the cleavage of the C−H bond is not the rate-limiting step.

Concerning the metabolism of the propofol-d_1 **4**, for any deuterated metabolite detected, the mechanism of hydroxylation of the propofol avoids using the NIH shift pathway (Figure 1), which has previously been described for the aromatic hydroxylation of different molecules such as phenytoin,[18] warfarin,[19] and oxprenolol.[20]

Slight differences between the three analogues of propofol are observed in the in vivo experiments. The deuterated compounds (more particularly propofol-d_1 **4**) seem to have lower HD_{50}, higher LD_{50}, and as a result higher therapeutic index. However, these differences are not statistically significant and it may be partly related to the low sensibility of the pharmacological tests.

Concerning the propofol anesthetic activity (HD_{50}), our results are in agreement with those obtained by Anderson et al.[21] We also point out that lower toxicity has already been observed with deuterated compounds compared to their protio analogues (i.e, amphetamines[9] and 2,6-di-*tert*-butyl-4-methylphenol[22]), which is interesting for a such a category of short-acting anesthetics requiring repeated injections.

Conclusion

This work demonstrates that the deuteration procedure, commonly used in quantitative studies with internal standards or for the identification of metabolic pathways, does not delay the metabolism kinetics of propofol. The cleavage of the C−H bond is not the rate-limiting step in the mechanism of para hydroxylation of propofol, and this mechanism avoids using the NIH shift pathway. Additionally, in vivo experiments show that the deuteration does not abolish anesthetic properties of propofol.

Supporting Information Available: General experimental procedure for preparation, physical and spectral characterization (^{1}H and ^{13}C NMR, mass spectrometry, IR spectroscopy, and elemental analysis) of the synthesized compounds, and general experimental procedures for in vitro biological studies on human hepatic microsomes and in vivo biological studies in mice. This material is available free of charge via the Internet at http://pubs.acs.org.

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