TECHNICAL NOTE

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An Experimental Methodology for the Study of Postmortem Changes in Toxic Concentrations of Drugs, Using Secobarbital As an Example

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ABSTRACT: Postmortem changes in alcohol and drug concentrations are well known today. The authors used an HPLC assay of barbiturates in postmortem tissue, and developed a rat model in order to evidence postmortem changes in toxics concentrations. Postmortem changes in secobarbital concentrations were evidenced using a rat-secobarbital model. This work emphasizes the difficulty of postmortem toxicology, as concentrations found at the time of autopsy may be different from concentrations at the time of death.

KEYWORDS: toxicology, secobarbital, postmortem changes, autopsy, HPLC

It could be suggested that drug concentration in biological fluids and tissues, after forensic autopsy, remains roughly the same, whatever the delay between death and collection of samples. Yet postmortem changes in toxic concentrations are well known today, for alcohol [*1–12*] as well as barbiturates [*13*], chlorpromazine [*14*], benzodiazepines [*15*], amitriptyline [*16*], digoxin [*17–19*], morphine [*20*], carboxyhaemoglobin and methaemoglobin [*21*], and in fact various drugs or toxics [*22–24*]. The aim of this work was to develop a rat experimental model for studying such postmortem changes.

Experimental Work

Sodium secobarbital was chosen for its fast action, the stability of the molecule in postmortem tissues [*13*] and the persistently high frequency of its use in suicide attempts.

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The extraction and quantification of sodium secobarbital in postmortem tissues were achieved by an HPLC method [25]. The mobile phase used was diammonium phosphate (50 mmol/L at pH 8.1) and methanol (1:1). Other technical characteristics were as follow: reversed-phase column (Waters C18 Novapak 3.9 mm × 15 cm; 4 μm), gard-column (Waters, C18 Bondapak, 7 μm); ambient temperature; flow-rate 0.8 mL/mn; UV 240 nm. Extraction was achieved with a 0.5 mol/L phosphate buffer (pH 5.5 = 1 mL) mixed with hexane-ethylic ether-isopropanol (49:49:2 = 12 mL). Then a Na3PO4 solution (0.01 mmol/L, pH = 11.7, 5 mL) and HClO4 solution (0.02 mol/L, pH = 2, 5 mL) were used. At the end the organic layer was evaporated under stream nitrogen at 45°C and residue was dissolved in methanol (100 μL) and injected into HPLC.

This method allowed the separation and analysis of all barbiturate derivates commercialized in France. The purification obtained was quite satisfactory, even in putrefied tissues and fluids.

The extraction efficiency is low (50%), but an internal standard is used (Aprobarbital) which can be easily separated from sodium secobarbital and from other barbiturates. The absence of interference was noted both with endogenous compounds and other barbiturates. Linearity, within-run precision, day-to-day precision and instrumental detection limits were quite satisfactory [26].

Animal Experiment

Twenty wistar rats received a high oral dose of sodium secobarbital in methanol solution (250 mg/kg) via a stomach tube and were sacrificed by cervical dislocation two hours after intoxication. One group of five rats was autopsied immediately after death on day 0, another on day 2, another on day 4, and another on day 7. Dead animals were left at room temperature (20°C) between death and collection of the samples. The extraction procedure and HPLC analysis were carried out on heart and kidney tissues, to extend the former work [26].

Results

As previously reported in blood, liver and spleen, the average of five analyses shows an increase of concentrations with postmortem delay in heart and kidney (Table 1).

Discussion

The concentration increases are quite stunning, but data must be studied by statistical analysis. Table 2 shows the comparison of observed means by analysis of variance. These data prove that postmortem increase of secobarbital in rat is a real issue for liver, spleen, and blood ($P < 0.001$). In the case of heart and kidney, the variations of concentrations are not statistically significant. Furthermore variations in blood, liver and spleen are compatible with a first order kinetics [26] and various constants can be defined and calculated: first-order rate constant, time needed for the toxic concentration to double with regard to the concentration measured at Day zero.

TABLE 1—*Average of five analyses in kidney and heart.*

		Day 0	Day 2	Day 4	Day 7	Day 7/Day 0
Kidney	Average (μg/g)	68.86	32.04	124.44	125.02	1.81
	S.D.	32.00	17.46	117.93	35.49	...
Heart	Average (μg/g)	23.12	46.92	61.36	84.34	3.65
	S.D.	13.82	54.26	37.20	50.68	...

TABLE 2—*Comparison of observed averages by analysis of variance.*

	F(c-1, N-c)	*P*
Blood[b]	F (3, 14)[a] = 26.73	$P < 0.001$
Liver[b]	F (3, 16) = 17.59	$P < 0.001$
Spleen[b]	F (3, 16) = 19.10	$P < 0.001$
Kidney	F (3, 16) = 2.56	NS
Heart	F (3, 16) = 1.39	NS

[a] Blood impossible to collect at day 4 and day 7 on 2 animals, due to putrefaction.

[b] Statistical data non previously published.

The equation is:

$$C(t) = Co.e^{kt}$$

Co: concentration at the time of death
K (day $-$ 1): first order rate constant
a (day) time needed for the concentration to double
a and *k* are connected by the equation:

$$a = (\text{Log } 2)/k$$

It was noted that the speed constants were about the same on the three tissues studied, which would suggest almost similar slopes.

At least it was observed an important kidney concentration decrease on the second postmortem day which implies a real difficulty of modelization.

Levine [*13*] pointed out the postmortem stability of five commonly prescribed barbiturates and Thiopental in blood and liver at room temperature and at 4°C. Greater than 75% of the drugs were detected at the end of the two- to three-month period. These changes were not considered significant. Therefore barbiturates appear to be stable in blood and liver under the conditions of these experiments, which enhanced several hypotheses that are put forward to explain postmortem rediffusion of the drug: cellular lysis; impairment of physiological barriers and protein bindings; rediffusion from interstitial liquids; dehydration and concentration of drug in blood; postmortem metabolism. Parker [*27*] demonstrated postmortem diffusion of secobarbital from the stomach of rats dosed with high levels after death. This diffusion was believed to depend on the delay in performing the autopsy. Postmortem dissolution, expanding gases of putrefaction, and body position were considered to contribute to barbiturate diffusion into adjacent tissues.

Conclusion

Taking the example of sodium secobarbital, we developed a rat experimental model in order to study the postmortem changes of toxics concentrations.

Our data prove that a statistical analysis of the data is necessary to discuss the results.

Furthermore the existence of postmortem kinetics in the rat blood, liver and spleen was demonstrated. There is a great variation of secobarbital concentrations in rat heart and also in rat kidney, with an important decrease on the second postmortem day, but these data are not statistically significant.

This draft illustrates a difficult problem in postmortem toxicological assessment of secobarbital, that is, the levels found at the time of forensic autopsy do not necessarily correspond to the levels found at the time of death [*22*].

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