Int J Legal Med (2000) 114 : 29–35

ORIGINAL ARTICLE

E. A. De Letter · P. De Paepe · K. M. Clauwaert
F. M. Belpaire · W. E. Lambert · J. F. Van Bocxlaer
M. H. A. Piette

Is vitreous humour useful for the interpretation of 3,4-methylenedioxymethamphetamine (MDMA) blood levels?

Experimental approach with rabbits

Received: 15 September 1999 / Accepted: 7 January 2000

Abstract As drug instability and redistribution are factors known to affect the interpretation of post-mortem blood levels, we questioned whether post-mortem vitreous humour concentrations could be useful as predictors for the MDMA load at the time of death. In a first series of in vivo experiments using rabbits, 3,4-methylenedioxymethamphetamine (MDMA) concentrations in plasma, blood and vitreous humour were studied as a function of time after intravenous (iv) administration of MDMA. Equilibration between the vascular compartment and vitreous humour was attained about 1 h after iv MDMA administration. In a second series of experiments, the post-mortem stability of MDMA in vitreous humour in relation to ambient temperature was investigated. Post-mortem MDMA concentrations in vitreous humour were closer to the ante-mortem blood levels when compared to cardiac blood samples. These preliminary investigations in the rabbit model indicate that measurements of vitreous humour concentrations could also be of interest for predicting the blood concentration at the time of death in humans.

Key words 3,4-Methylenedioxymethamphetamine · MDMA · Vitreous humour · Pharmacokinetics · Post-mortem stability · Rabbits

E. A. De Letter · M. H. A. Piette (✉)
Department of Forensic Medicine, Ghent University,
J. Kluyskensstraat 29, 9000 Ghent, Belgium
e-mail: Michel.Piette@rug.ac.be;
Tel.: +32-9-2649291; Fax: +32-9-2649495

P. De Paepe · F. M. Belpaire
Heymans Institute of Pharmacology, Ghent University,
De Pintelaan 185, 9000 Ghent, Belgium

K. M. Clauwaert · W. E. Lambert · J. F. Van Bocxlaer
Department of Toxicology, Ghent University,
Harelbekestraat 72, 9000 Ghent, Belgium

Introduction

As post-mortem drug levels in blood do not necessarily reflect the concentration at the time of death, the question whether a detected level played an important role in the mechanism of death remains a complex problem in the forensic practice. In particular, post-mortem instability and redistribution of drugs are important thanato-chemical factors [1]. This toxicological nightmare is an established fact for various drugs e.g. cocaine [2] and many therapeutic drugs such as barbiturates [1] and digitalis [3, 4]. To a certain extent, the influence of these post-mortem phenomena can be avoided by sampling blood as soon as possible after death from an isolated peripheral vein such as the femoral vein [5]. However, bearing in mind this general recommendation, a single blood sample is often insufficient to draw appropriate conclusions. Another sample (tissue or fluid) should not only be used as an analytical control for the blood level determined, but could also provide information on the pharmacokinetic phase and as a result the time of drug intake. Vitreous humour is one of these supplementary samples and is an interesting medium because the vitreous fluid is less influenced by autolytic processes and is convenient (e.g. simple to sample and not affected by hemolysis). Formerly, vitreous humour determinations have been performed in order to detect various drugs, in particular ethanol [6] and also morphine [7], cocaine [8] and amitriptyline [9] for example.

To our knowledge, the post-mortem drug distribution in humans has barely been explored for amphetamines and analogues, or for MDMA in particular, with the exception of a few case reports [10, 11, 12].

To investigate whether vitreous humour concentrations could be more helpful than blood for determining post-mortem MDMA levels in humans, preliminary experiments in rabbits were carried out. Rabbits were chosen as the animal model because they have a vitreous volume of about 1.4 ml [13] which is much larger than in rats and the chemical characteristics of vitreous humour in rabbits are comparable to those in humans [14]. As pharmacokinetic

data for MDMA in the rabbit are lacking, we first investigated this after intravenous (iv) administration. We then studied whether determination of MDMA in vitreous humour is possible and whether there was a correlation in vivo between the vitreous humour levels and the plasma or blood concentrations. Finally, to explore whether the post-mortem vitreous concentrations could be useful to estimate the blood MDMA levels at the time of death, the influence of the post-mortem interval and the ambient temperature was examined.

Materials and methods

The study protocol was approved by the Ethics Committee for animals of the Medical School, Ghent University (request number ECP 98/1 and ECP 99/9). 3,4-Methylenedioxymethamphetamine hydrochloride was provided by Sigma-Aldrich (Belgium).

Animals and procedures

Female white New Zealand rabbits (weight 2050–4500 g) were purchased from Iffa Credo, Belgium. The animals were fasted overnight before the experiment but were allowed free access to water.

In vivo experiments

The broad study design is shown in Fig. 1. A polyethylene catheter (P.E. 50) filled with heparin solution (100 IU/ml) was implanted (xylocaine 1%) into the main central artery of the right ear in 18 rabbits under local anaesthesia.

In one group of six rabbits (t = 120 min), arterial blood samples (2 ml) were taken at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120 min after drug administration for the assay of MDMA and MDA (3,4-methylenedioxyamphetamine) concentrations in plasma. Additional samples (1 ml at 10, 20, 60, 120 min) were taken for whole blood determinations. Pentobarbital anaesthesia was induced according to Prince (30–35 mg/kg body weight [15]).

In all cases, sampled blood was replaced by the same amount of saline. Blood samples were centrifuged at 3500 rpm for 10 min and plasma and whole blood samples were frozen at –30 °C until analysis. Both eyes were immediately frozen after enucleation by immersion in liquid nitrogen for 1–2 min. The eyes were preserved at –30 °C until the vitreous bodies were dissected as described by Abel and Boyle [16].

Finally, in three rabbits the urinary excretion of MDMA and MDA was followed for 96 h after MDMA iv administration (1 mg/kg). Urine was collected in a metabolism cage and the volume measured. These samples were also frozen until analysis.

Post-mortem experiment

The broad study design is shown in Fig. 1. Sample preservation and dissection of the eyes were carried out as previously described.

Protein binding

Protein binding of MDMA was determined by ultrafiltration using the Amicon centrifuge micro-partition system and YMT membrane discs (Grace, Amicon Division, Beverly, Mass.). Blank rabbit plasma samples were spiked with 400 ng/ml MDMA, 1 ml-plasma samples were transferred to the micro-partition system and centrifuged at 1500 rpm for 30 min resulting in a volume between 100 and 200 µl. The MDMA concentration was measured in a 50 µl aliquot of the ultrafiltrate with HPLC. Adsorption of MDMA to the filter was negligible.

Drug assay

Concentrations of MDMA and MDA were determined using a fully validated HPLC (high pressure liquid chromatography) procedure with fluorescence detection (λ_{ex} 288, λ_{em} 324 nm). The samples (250 µl) were liquid/liquid extracted with hexane : ethylacetate (70 : 30 v/v) at pH 9.5 using MDEA (3,4-methylenedioxyethylamphetamine) as the internal standard. Chromatographic separation was achieved using Hypersil BDS C18 columns (3 µm, 100 × 2.1 mm, Alltech, Deerfield, Il.) isocratically eluted at 0.2 ml/min with a mixture of water/methanol/acetonitrile containing 0.1 M ammonium acetate. The method proved linear from 2 to 1000 ng/ml (2 ng/ml being the quantitation limit both for MDMA and MDA, between-day reproducibility < 25%). With each batch of samples, a calibration curve prepared in the corresponding blank matrix (except for the vitreous humour which was substituted with water for reasons of practical unavailability) and quality control samples (7 and 500 ng/ml) were analysed. Accuracy was between 97–102% (n = 7) and total precision (CV, coefficient of variation) was lower than 13% (n = 7).

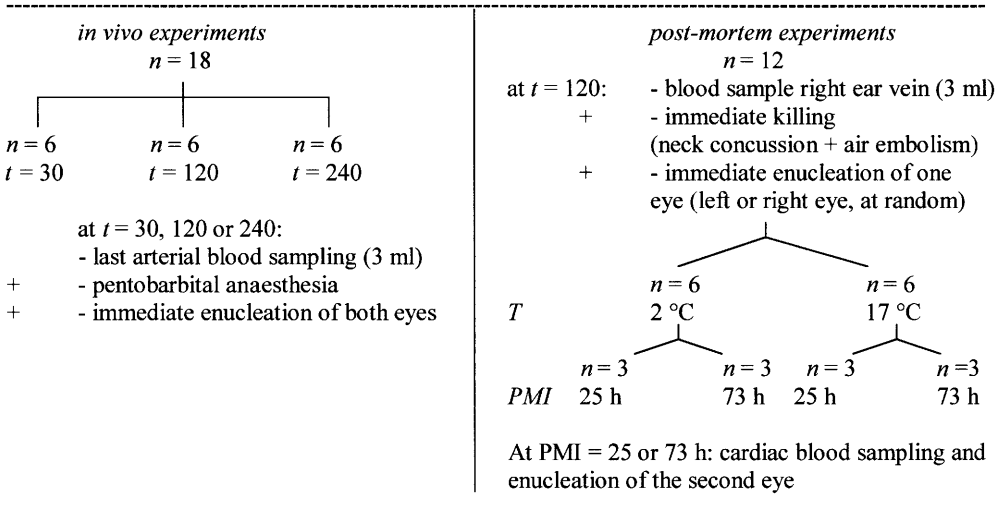

Fig. 1 Study design in rabbits receiving 1 mg/kg MDMA slowly infused via the left ear vein [t time expressed in minutes, T temperature in °C, *PMI* post-mortem interval (h)]

Analysis of data

The results are expressed as means (± SD).

The plasma concentration-time profiles of MDMA after iv administration were individually analysed using a pharmacokinetic computer programme (WinNonlin version 1.5 – Scientific Consulting, Inc.) and were best characterized on the basis of the Akaike Information Criterion [17] by a 2-compartment model using the equation, $C = Ae^{-\alpha t} + Be^{-\beta \tau}$, where C is the plasma concentration at time t, α and β are hybrid rate constants and A and B are the coefficients of the exponential terms. Calculation of the pharmacokinetic parameters (half-life, volume of distribution at steady state, volume of distribution of the central compartment, clearance, area under the curve (AUC) and mean residence time) was done according to Gibaldi and Perrier [18].

Statistical processing of the data was performed using non-parametric tests. The Wilcoxon Rank test was used for analysis of interindividual differences in concentrations between the left and right eyes. The Friedman test for repeated measurements was used to compare the ratios of blood to plasma MDMA concentrations (10, 20, 60 and 120 min after infusion). The ratios between the vitreous humour MDMA concentration and the corresponding plasma or blood level sampled 30, 120 and 240 min after administration, were compared using the non-paired Kruskal-Wallis test and when appropriate, this was followed by the Mann-Whitney U-Test. The correlation between vitreous humour and plasma or blood levels was investigated with the Spearman correlation test. For all tests, *P* values less than 0.05 were considered to be statistically significant.

Results

In vivo experiments

Figure 2 shows the time course of the mean plasma concentrations of MDMA following a 1 mg/kg iv dose ($n = 6$). The data from each animal were well fitted according to a two compartment model. The corresponding pharmacokinetic parameters are summarized in Table 1. The mean blood/plasma ratios calculated at 10, 20, 60 and 120 min after infusion, were 1.2 ± 0.1, 1.2 ± 0.2, 1.3 ± 0.5 and 1.3 ± 0.2, respectively. Statistical analysis did not reveal significant differences.

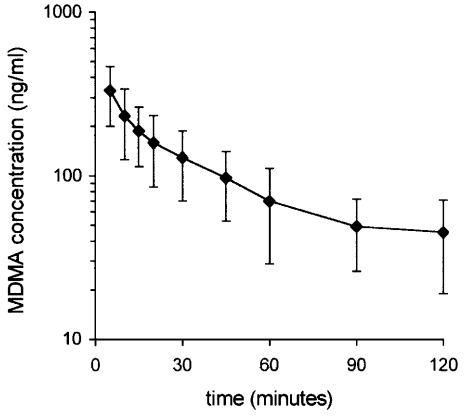

Fig. 2 Plasma concentrations of MDMA as a function of time in rabbits after an iv dose of 1 mg/kg MDMA ($n = 6$). Results are expressed as means ± SD

Table 1 Pharmacokinetic parameters of MDMA in rabbits after an intravenous dose of 1 mg/kg (results expressed as means ± SD)

AUC (area under the curve; μg.min/l)	16,937 ± 7,849
Alpha half-life[a] (min)	5.0 ± 1.8
Beta half-life[b] (min)	63.5 ± 34.2
Systemic clearance (1/kg per h)	4.1 ± 1.4
Volume of distribution of the central compartment (1/kg)	1.9 ± 0.8
Volume of distribution at steady state (l/kg)	4.9 ± 2.6
Mean residence time (min)	78.1 ± 46.7

[a] Half-time in the distribution phase
[b] Half-time in the elimination phase

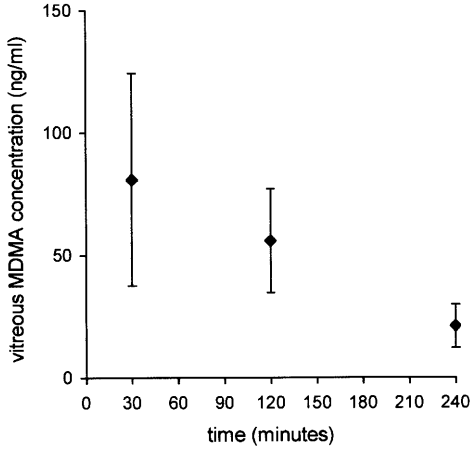

Fig. 3 Mean vitreous humour MDMA concentrations as a function of time in rabbits after an iv dose of 1 mg/kg MDMA ($n = 6$ for each time point). Results are expressed as means ± SD

MDMA concentrations were measured in the vitreous fluid 30, 120 and 240 min after administration ($n = 6$ for each time point, see Fig. 3). As there were no statistical differences in MDMA concentrations between the left and the right eyes ($n = 18$), the mean values for both eyes were used.

The ratios of the vitreous MDMA concentrations ($MDMA_{vitreous}$) to the plasma ($MDMA_{plasma}$) (see Fig. 4 A) or whole blood ($MDMA_{blood}$) concentrations 30 min after administration (see Fig. 4 B) were less than one and significantly different from the values obtained at 120 and 240 min when both ratios were higher than one, but no significant difference was observed between both values (ratios at $t = 120$ min 1.4 ± 0.3 and 1.1 ± 0.3, at $t = 240$ min 1.6 ± 0.2 and 1.1 ± 0.4, respectively).

Figure 5 shows the scatterplot of the $MDMA_{vitreous}$ versus $MDMA_{plasma}$ (Fig. 5 A) levels or $MDMA_{blood}$ (Fig. 5 B) in the elimination phase. The Spearman (r_s) correlation coefficients for the $MDMA_{vitreous}$ and $MDMA_{plasma}$ or $MDMA_{blood}$ in the elimination period (at $t = 120$ and 240 min) were 0.98 and 0.95, respectively. These correlations were significant at the 0.01 level.

MDA plasma and blood concentrations were low, ranging between 2 ng/ml and 6 ng/ml. The MDA levels in the vitreous humour were below the limit of quantitation (2 ng/ml).

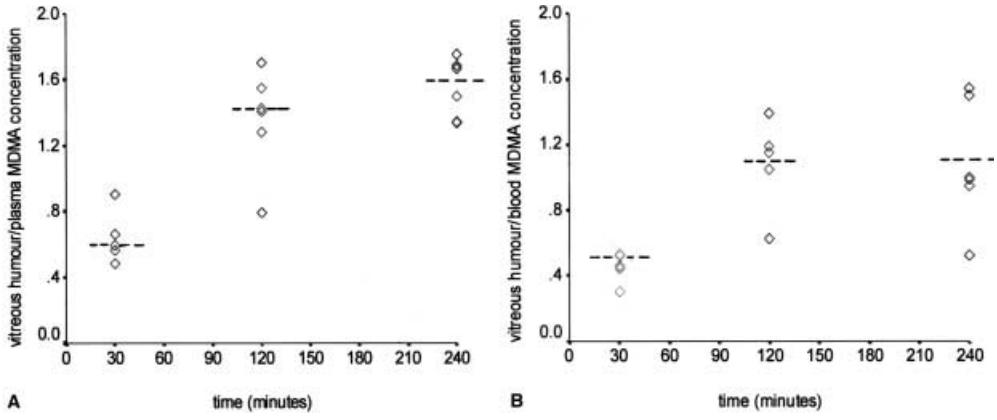

Fig. 4 Individual and mean ratios of the vitreous humour MDMA concentrations to plasma (**A**) or blood (**B**) concentrations as a function of time in rabbits after an iv dose of 1 mg/kg MDMA (n = 18)

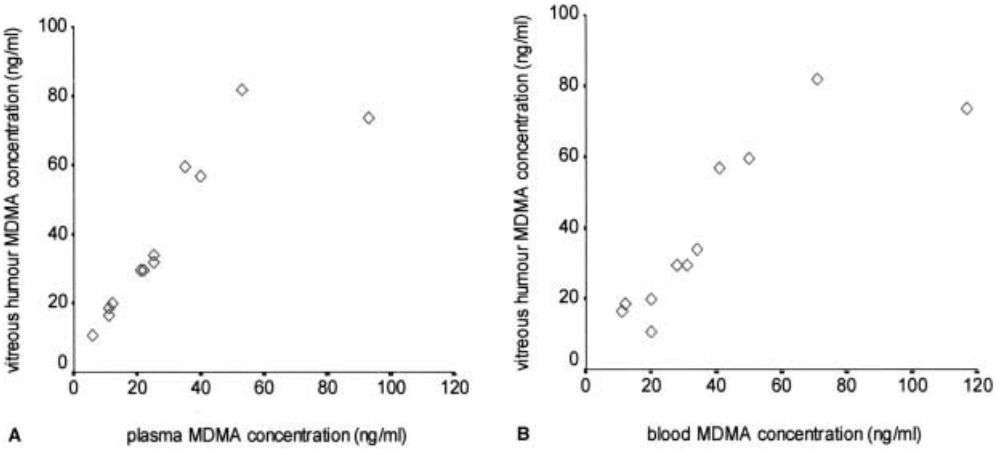

Fig. 5 Scatter plot of vitreous humour versus plasma (**A**) or blood (**B**) MDMA concentrations 120 and 240 min after an iv dose of 1 mg/kg MDMA (n = 12)

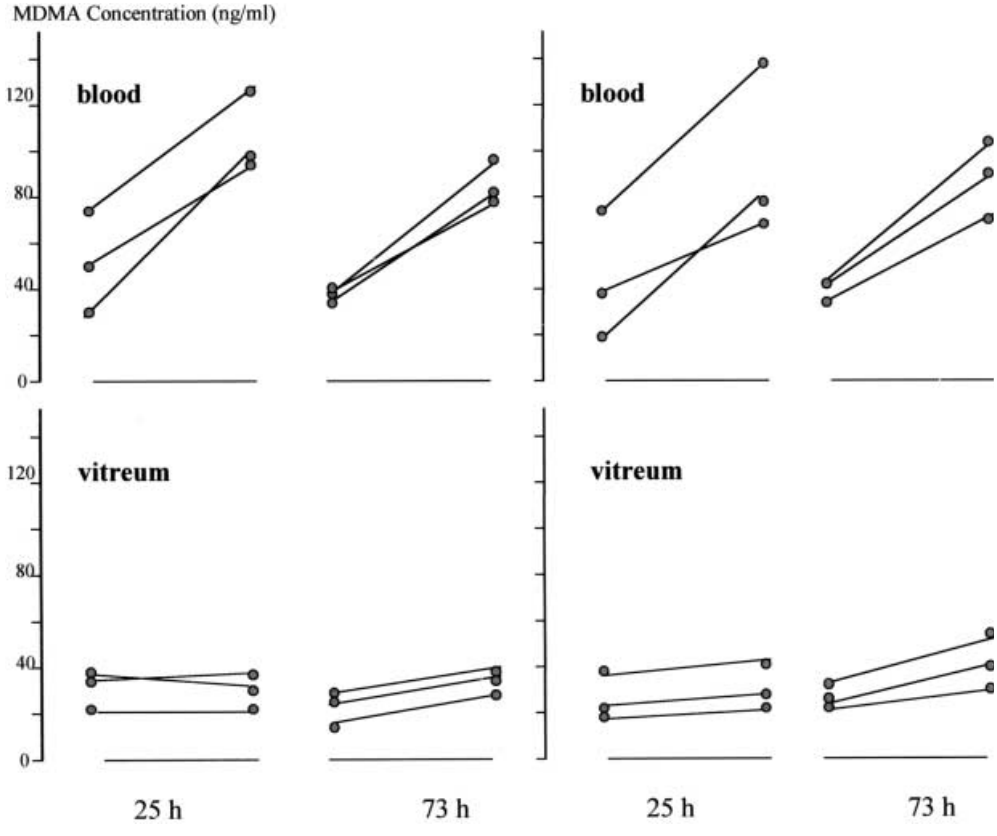

Fig. 6 Individual blood and vitreous MDMA concentrations (ng/ml) in rabbits 120 min after an iv dose of 1 mg/kg MDMA (ante-mortem values) and preserved at 2 °C *(left panel)* and 17 °C *(right panel)*, either 25 h or 73 h post mortem

The percentages of the administered MDMA dose recovered in urine of three rabbits were 12.9, 3.4 and 4.1%, respectively. MDA levels were below the limit of quantitation. The mean unbound fraction of MDMA in plasma was 63% ± 3 (n = 6) at a concentration of 400 ng/ml.

Post-mortem experiment (see Fig. 6)

The mean plasma, blood levels and the vitreous humour concentrations of one eye 120 min after MDMA infusion (just prior to killing of the animals) were 32 ± 17, 42 ± 16, and 27 ± 7 ng/ml, respectively (n = 12).

Blood MDMA concentrations were clearly increased post mortem compared to the values obtained ante mortem, whereas the vitreous MDMA concentrations did not change substantially post mortem. An overall slight increase in vitreous MDMA levels was observed, somewhat more pronounced at 17 °C 73 h post mortem and vitreous MDMA levels tended to be more stable at 2 °C.

Discussion

In this study, we examined whether determination of vitreous MDMA levels can be useful to predict the MDMA blood concentrations at the time of death with the help of a rabbit experiment.

In order to interpret the post-mortem concentrations of MDMA in the rabbit, we first investigated the pharmacokinetics of MDMA after an intravenous dose of 1 mg/kg. The plasma concentration versus time curves of MDMA were fitted according to a 2-compartment model. MDMA has a high volume of distribution (5 l/kg), a high systemic clearance (4.1 l/kg per h) and a relatively short half-life (1 h). Our results were fairly consistent with previously reported pharmacokinetic parameters of MDMA after intravenous (iv) and subcutaneous (sc) administration in rats [19].

The mean blood/plasma ratios in the distribution as well as the elimination phase indicate a certain accumulation of MDMA in red blood cells.

In our experiments, only 6% of the MDMA dose was found unchanged in urine and MDMA is probably eliminated by biotransformation or by excretion via the bile. In addition, MDA concentrations in rabbit plasma were very low (below 6 ng/ml) and were below the limit of quantitation in urine. In rats and mice, MDA was identified in plasma as an important metabolite [20] whereas in rat urine, either R(–) MDA and S(+) MDA were rarely found [19]. In humans, MDA was originally assumed to be the major metabolite [21], but it was demonstrated that conjugated HMMA (4-hydroxy-3-methoxy-methamphetamine) and HHMA (3,4-dihydroxy-methamphetamine) were the main urinary metabolites of MDMA [22, 23, 24, 25].

The mean binding of MDMA in rabbit plasma (37%) is similar to that in dogs [26].

In our experiments, after a single intravenous dose, equilibration between plasma and vitreous humour was obtained between 30 and 120 min, as the $MDMA_{vitreous}$/$MDMA_{plasma}$ ratios did not differ significantly between 120 and 240 min post administration. Physiologically, the blood-retina barrier can be compared with the blood-brain barrier and Chu et al. [27] demonstrated that an equilibration between plasma and brain concentrations of MDMA was obtained within 30 min after sc administration of MDMA in rats. The $MDMA_{vitreous}$ / $MDMA_{plasma}$ ratios at 120 (1.4 ± 0.3) and 240 min (1.6 ± 0.2) and the corresponding ratios of $MDMA_{vitreous}$ to $MDMA_{blood}$ (1.1 ± 0.3 and 1.1 ± 0.4, respectively) indicate a slight accumulation of MDMA in the vitreous compartment. The accumulation is in fact even more important as the plasma protein binding of MDMA is ± 30%. A possible explanation could be binding to the vitreous humour, but in vitreous fluid protein concentration is only 1–3% of the total serum protein concentration [14]. For some drugs with an important level of plasma protein binding, such as fleroxacin [28], vitreous to plasma concentration ratios lower than 1 were seen. However, higher vitreous humour levels compared to serum concentrations were noticed in rabbits for fluconazole [29]. For ethanol too, a higher vitreous humour level compared to blood concentration at steady state was observed [6] which was explained by the smaller dry matter content of vitreous humour [30].

To our knowledge, there are only two case reports of MDMA determination in human vitreous humour; both revealing a vitreous humour/blood concentration ratio below 1. Crifasi and Long [31] published a traffic accident fatality attributed to the use of MDMA. The vitreous-blood ratio was 0.5 and very little MDA was detected. These authors concluded that their results support an acute event (i.e. that death occurred before distribution was complete). In the second case report, (an acute poisoning, probably accidental, due to combined intake of MDMA, cocaine and heroin) [32], the ratio of total vitreous-blood MDMA levels was calculated as 0.66; an analogous explanation for their results could be assumed.

Since ambient temperature and post-mortem interval are important thanato-chemical factors, four different conditions, 2 °C or 17 °C and 25 or 73 h post mortem, respectively, were investigated in the second part of our study. We demonstrated that MDMA concentrations in cardiac blood increased post mortem, whereas vitreous MDMA levels were much more stable and thus more representative of the ante-mortem blood levels. Relatively small differences were noticed between the peri- and post-mortem vitreous values obtained at ambient temperatures of either 2 °C or 17 °C. The elevation of the vitreous MDMA concentration 73 h after death in rabbits preserved at 17 °C could partially be explained by the dehydration which occurred and, theoretically a low level of post-mortem redistribution. On the other hand, the MDMA concentration increase in our rabbit heart blood samples, taken 73 h post mortem in particular, point to a post-mortem redistribution. Moriya and Hashimoto [33] demonstrated post-mortem diffusion of methamphetamine from lung tissue in rabbits. In humans, post-mortem cardiac blood levels of amphetamine [5] and methamphetamine [34, 35] were

found to be higher than femoral blood concentrations and in one of the cases reported by Rohrig and Prouty, the MDMA concentration in heart blood was reported to be 5 times higher than in femoral blood [11]. However, recently, in two human fatal cases associated with amphetamine intake [36], a post-mortem increase in amphetamine concentrations of 50–60% in femoral blood was noticed. Further thanato-chemical experiments are needed to explore the mechanism of these increases. Instability of MDMA itself is not very likely as in vitro stability studies of MDMA and MDA in aqueous solutions and dog plasma demonstrated that these products are fairly stable [26]. Human blood samples containing amphetamine and methamphetamine stored in preservation products are sufficiently stable up to 5 years [37]. Nagata and colleagues [38] investigated the stability of amphetamine and methamphetamine in post-mortem rabbit tissues and concluded that these products are sufficiently stable.

In summary, after intravenous administration, MDMA can easily be identified in the vitreous humour of rabbits and an equilibration between the vitreous humour and the vascular compartment was established after about 1 h. In addition, our results confirm that heart blood samples cannot be used for post-mortem toxicological analysis. In fact rabbit vitreous MDMA was more stable than post-mortem cardiac blood levels.

Vitreous sampling for MDMA determination seems to be a good autopsy practice if blood is lacking (e.g. as a result of severe blood loss or putrefaction). Moreover, after equilibration, vitreous humour could be a suitable control sample in cases of erratic blood values due to either sampling site bias or analytical errors. In fact vitreous humour MDMA levels could be more representative than blood MDMA concentrations when there is a prolonged post-mortem interval. Further thanato-chemical investigations of routine autopsy cases should be performed to confirm these preliminary observations in rabbits.

Acknowledgements The authors gratefully thank Prof. M. Bogaert for advice in the development of this study and critically reading of the manuscript. Our gratitude also goes to Prof. A. P. De Leenheer for overall support (equipment, chemicals and accommodation). We would like to thank as well Mr. G. Van Maele for statistical recommendations, Mrs Thérèse De Vuyst for assistance in the English grammar and Mr R. Declercq for skilled technical assistance.

References

1. Pounder DJ, Jones GR (1990) Post-mortem drug redistribution – a toxicological nightmare. Forensic Sci Int 45: 253–263
2. Hearn WL, Keran EE, Wei H, Hime G (1991) Site-dependent postmortem changes in blood cocaine concentrations. J Forensic Sci 36: 673–684
3. Donnelly B, Balkon J, Bidanset JH, Belmonte A, Barletta M, Manning T (1991) Comparative kinetics of serum and vitreous humor digoxin concentrations in a guinea pig model. Part I: intravenous administration of digoxin. J Anal Toxicol 15: 60–62
4. Balkon J, Donnelly B (1992) Comparative kinetics of digoxin in serum and vitreous humor in a guinea pig model. Part II: Single oral dose administration. J Anal Toxicol 16: 155–157
5. Prouty RW, Anderson WH (1990) The forensic implications of site and temporal influences on postmortem blood-drug concentrations. J Forensic Sci 35: 243–270
6. Chao TC, Lo DST (1993) Relationship between postmortem blood and vitreous humor ethanol levels. Am J Forensic Med Pathol 14: 303–308
7. Bermejo AM, Ramos I, Fernández P, López-Rivadulla M, Cruz A, Chiarotti M, Fucci N, Marsilli R (1992) Morphine determination by gas chromatography/mass spectroscopy in human vitreous humor and comparison with radioimmunoassay. J Anal Toxicol 16: 372–374
8. McKinney PE, Phillips S, Gomez HF, Brent J, MacIntyre M, Watson WA (1995) Vitreous humor cocaine and metabolite concentrations: do postmortem specimens reflect blood levels at the time of death? J Forensic Sci 40: 102–107
9. Hilberg T, Ripel Å, Smith AJ, Slørdal L, Mørland J, Bjørneboe A (1998) Postmortem amitriptyline pharmacokinetics in pigs after oral and intravenous routes of administration. J Forensic Sci 43: 380–387
10. Dowling GP, McDonough ET, Bost RO (1987) 'Eve' and 'Ecstasy'. A report of five deaths associated with the use of MDEA and MDMA. JAMA 257: 1615–1617
11. Rohrig TP, Prouty RW (1992) Tissue distribution of methylenedioxy-methamphetamine. J Anal Toxicol 16: 52–53
12. Fineschi V, Masti A (1996) Fatal poisoning by MDMA (ecstasy) and MDEA: a case report. Int J Legal Med 108: 272–275
13. Gardner SK (1987). Ocular drug penetration and pharmacokinetic principles. In: Lamberts DW, Potter DE (eds) Clinical ophthalmic pharmacology. Little Brown and Company, Boston Toronto, pp 1–52
14. Berman ER (ed) (1991) Vitreous. In: Biochemistry of the eye. Plenum Press, New York London, pp 291–307
15. Prince JH (ed) (1964) The rabbit in eye research. Charles C Thomas, Springfield, Ill., p 618
16. Abel R Jr, Boyle GL (1976) Dissecting ocular tissue for intraocular drug studies. Invest Ophthalmol 15: 216–219
17. Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automat Control 19: 716–723
18. Gibaldi M, Perrier D (eds) (1982) Pharmacokinetics. Marcel Dekker, New York
19. Fitzgerald RL, Blanke RV, Poklis A (1990) Stereoselective pharmacokinetics of 3,4-methylenedioxymethamphetamine in the rat. Chirality 2: 241–248
20. Fitzgerald RL, Blanke RV, Rosecrans JA, Glennon RA (1989) Stereochemistry of the metabolism of MDMA to MDA. Life Sci 45: 295–301
21. Verebey K, Alrazi J, Jaffe JH (1988) The complications of "Ecstasy" (MDMA). JAMA 259: 1649–1650
22. Boer D de, Tan LP, Gorter P, van de Wal RMA, Kettenes-van den Bosch JJ, Bruijn EA de, Maes RAA (1997) Gas chromatographic/mass spectrometric assay for profiling the enantiomers of 3,4-methylenedioxymethamphetamine and its chiral metabolites using positive chemical ionization ion trap mass spectrometry. J Mass Spectrom 32: 1236–1246
23. Lanz M, Brenneisen R, Thormann W (1997) Enantioselective determination of 3,4-methylenedioxymethamphetamine and two of its metabolites in human urine by cyclodextrin-modified capillary zone electrophoresis. Electrophoresis 18: 1035–1043
24. Lim HK, Foltz RL (1989) Identification of metabolites of 3,4-(methylenedioxy)methamphetamine in human urine. Chem Res Toxicol 2: 142–143
25. Helmlin HJ, Bracher K, Bourquin D, Vonlanthen D, Brenneisen R, Styk J (1996) Analysis of 3,4-methylenedioxymethamphetamine (MDMA) and its metabolites in plasma and urine by HPLC-DAD and GC-MS. J Anal Toxicol 20: 432–440
26. Garrett ER, Seyda K, Marroum P (1991) High performance liquid chromatographic assays of the illicit designer drug "Ecstasy," a modified amphetamine, with applications to stability, partitioning and plasma protein binding. Acta Pharm Nord 3: 9–14

27. Chu T, Kumagai Y, DiStefano EW, Cho AK (1996) Disposition of methylenedioxymethamphetamine and three metabolites in the brains of different rat strains and their possible roles in acute serotonin depletion. Biochem Pharmacol 51: 789–796
28. Miller MH, Madu A, Samathanam G, Rush D, Madu CN, Mathisson K, Mayers M (1992) Fleroxacin pharmacokinetics in aqueous and vitreous humors determined by using complete concentration-time data from individual rabbits. Antimicrob Agents Chemother 36: 32–38
29. O'Day DM, Foulds G, Williams TE, Robinson RD, Allen RH, Head WS (1990) Ocular uptake of fluconazole following oral administration. Arch Ophthalmol 108: 1006–1008
30. Felby S, Olsen J (1969) Comparative studies of postmortem ethyl alcohol in vitreous humor, blood and muscle. J Forensic Sci 14: 93–101
31. Crifasi JC, Long C (1996) Traffic fatality related to the use of methylenedioxymethamphetamine. J Forensic Sci 41: 1082–1084
32. Moore KA, Mozayani A, Fierro MF, Poklis A (1996) Distribution of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) stereoisomers in a fatal poisoning. Forensic Sci Int 83: 111–119
33. Moriya F, Hashimoto Y (1999) Redistribution of basic drugs into cardiac blood from surrounding tissues during early-stages post mortem. J Forensic Sci 44: 10–16
34. Logan BK, Weiss EL, Harruff RC (1996) Case report: distribution of methamphetamine in a massive fatal ingestion. J Forensic Sci 41: 322–323
35. Miyazaki T, Kojima T, Yashiki M, Wakamoto H, Iwasaki Y, Taniguchi T (1993) Site dependence of methamphetamine concentrations in blood samples collected from cadavers of people who had been methamphetamine abusers. Am J Forensic Med Pathol 14: 121–124
36. Hilberg T, Rodge S, Mørland J (1999) Postmortem drug redistribution – human cases related to results in experimental animals. J Forensic Sci 44: 3–9
37. Giorgi SN, Meeker JE (1995) A 5-year stability study of common illicit drugs in blood. J Anal Toxicol 19: 392–398
38. Nagata T, Kimura K, Hara K, Kudo K (1990) Methamphetamine and amphetamine concentrations in postmortem rabbit tissues. Forensic Sci Int 48: 39–47