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ORIGINAL ARTICLE

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Post-mortem redistribution of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") in the rabbit

Part II: post-mortem infusion in trachea or stomach

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Abstract Drug concentrations in autopsy samples can also be influenced by post-mortem gastric diffusion when the stomach contains a substantial amount of the drug or by diffusion from the trachea when agonal aspiration or post-mortem regurgitation of vomit occurs. This was studied in a rabbit animal model in which MDMA solutions were infused post mortem either in the trachea or in the stomach. At 24, 48 or 72 h post mortem, samples including cardiac blood, vitreous humour, urine, bile, gastric content and several tissues were taken for toxicological analysis. After *post-mortem tracheal infusion*, MDMA can easily diffuse not only into the lungs but also in great quantities into the cardiac blood and, to a lesser extent, into the cardiac muscle. MDMA was also found in the closely adjacent diaphragm and in the upper abdominal organs, including the liver and the stomach. Following *post-mortem infusion into the stomach*, considerable MDMA levels were found in cardiac blood and muscle, both lungs, diaphragm and liver tissue when the solution was concentrated nearby the lower oesophageal sphincter. However, when the MDMA solution was present deeper in the stomach, MDMA levels were high in the spleen and the liver and relatively low in cardiac blood and muscle. In both experiments, MDA levels in most tissues were low or below the limit of quantitation, but were substantial in cardiac blood and muscle, lungs and diaphragm, indicating that MDMA can be metabolised to MDA after death. These results in the rabbit model indicate that the diffusion of MDMA out of the stomach content, or due to aspirated vomit and gastro-oesophageal reflux can lead to considerable post-mortem redistribution and thus should be taken into account in current forensic practice in order to draw the right conclusions when a peripheral blood sample is not available.

Keywords 3,4-Methylenedioxymethamphetamine (MDMA) · 3,4-Methylenedioxyamphetamine (MDA) · Post-mortem tracheal and gastric infusion · Post-mortem redistribution · Rabbits

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Introduction

Post-mortem drug levels can be difficult to interpret due to interfering thanato-chemical processes such as drug instability and post-mortem redistribution [1]. These processes can result from diffusion of the substance out of adjacent organs. They can also be due to diffusion from high concentrations present in the gastric content and/or from vomit aspiration in the airways or even from post-mortem regurgitation [2, 3].

Post-mortem absorption of ethanol, paracetamol and propoxyphene from simulated vomit aspiration was found to result in an increase in post-mortem cardiac blood concentrations in five human bodies [4].

Diffusion of ethanol from the stomach cavity after death has been investigated extensively for more than 50 years [5]. As this post-mortem diffusion results in an elevation of the blood alcohol level in cardiac blood [6] and even in aortic blood [7], peripheral blood sampling such as from the femoral (or external iliac) vein is recommended because it is obviously less liable to post-mortem changes. Post-mortem diffusion from gastric residues into blood and surrounding tissues has also been studied for a few other drugs including zopiclone [8], benzodiazepines [9], paracetamol [9] and amitriptyline [10, 11].

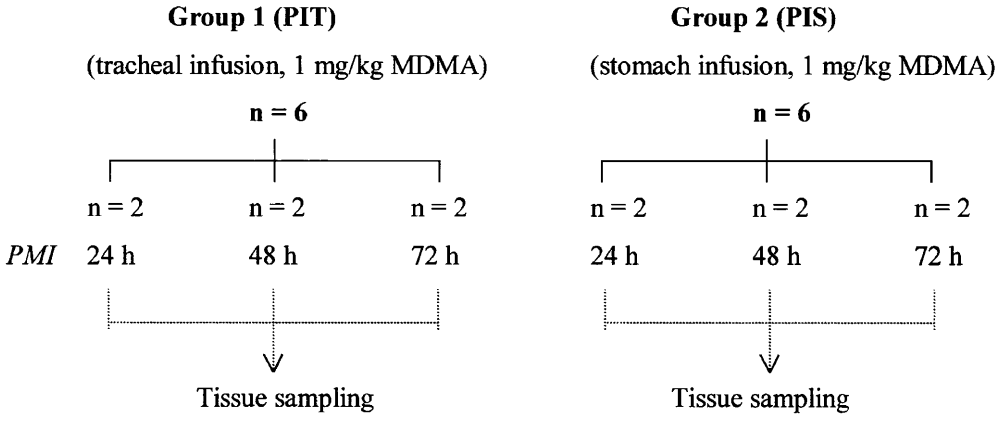


Fig. 1 Scheme of the study design of post-mortem infusion in rabbits receiving 1 mg/kg in the trachea (*Group 1, PIT, left panel*) or in the stomach (*Group 2, PIS, right panel*) (*PMI* post-mortem interval expressed in hours)

The amphetamine derivative, 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") is stable in blood and plasma in vitro [12]. In *part one*, post-mortem redistribution due to diffusion of MDMA out of several organ tissues was evaluated after simulation of a complete distribution of the substance prior to death [13]. In this study, post-mortem diffusion of MDMA from a "reservoir" in the stomach or from agonal vomit aspiration was explored using a rabbit animal model. This can be compared with the condition when somebody dies shortly after MDMA ingestion (e.g. due to cardiac arrhythmia) and therefore an incomplete absorption occurred, or when substantial regurgitation or vomit aspiration takes place in the peri-mortem period. When blood and tissue concentrations (studied up to 72 h after death) in both experimental settings change substantially, this should be taken into account in the interpretation of the toxicological results in humans.

Materials and methods

Provision of MDMA and rabbits as well as handling of the animals prior to the onset of the experiments and preservation of the samples took place as previously described [13].

Animals and procedures

The rabbits (weight 1,900–2,420 g) were killed using a CO_2/O_2 gas chamber (70/30%). Thereafter, two groups of rabbits were created: group 1 (n=6) was used for post-mortem infusion into the trachea (PIT) and group 2 (n=6) for post-mortem infusion into the stomach (PIS). Randomisation of the animals occurred and infusion of the MDMA solution (diluted in saline; 1 mg/kg) took place within the first hour post mortem.

The study design is presented in Fig. 1. After preparation of either the trachea or the oesophagus, ligation towards the laryngeal/pharyngeal region took place. For group 1 (PIT), a highly concentrated MDMA solution was used in order to reduce the volume of fluid to be infused (<0.5 ml). The solution was injected into the trachea using a 1-ml syringe and 26G needle. In group 2 (PIS), a polyethylene catheter (inner diameter of 2.5 mm) was inserted into the oesophagus up to the lower oesophageal sphincter. After infusion of MDMA, flushing of the catheter with saline took place. All rabbits were left in a supine position at ambient temperature (15°C). Dissection was carried out in a strict manner so as to avoid contamination of the samples and sampling took place in the same sequence for all rabbits. The samples taken in group 1 (PIT) were: cardiac blood and muscle, left and right lungs, left and right diaphragm, liver, stomach wall and stomach content, spleen, left and right iliopsoas muscle, abdominal adipose tissue, left and right kidneys, urine, cerebrum, cerebellum, brainstem, and both eyes. In the second group (PIS), furthermore, lower vena cava blood, duodenal wall and content, distal small bowel and content, and left and right abdominal muscle wall were sampled. Although the rabbits were fasted overnight, the stomach was not empty due to coprophagia. All organs were taken in toto for toxicological analysis. The eyes were handled as previously described [14].

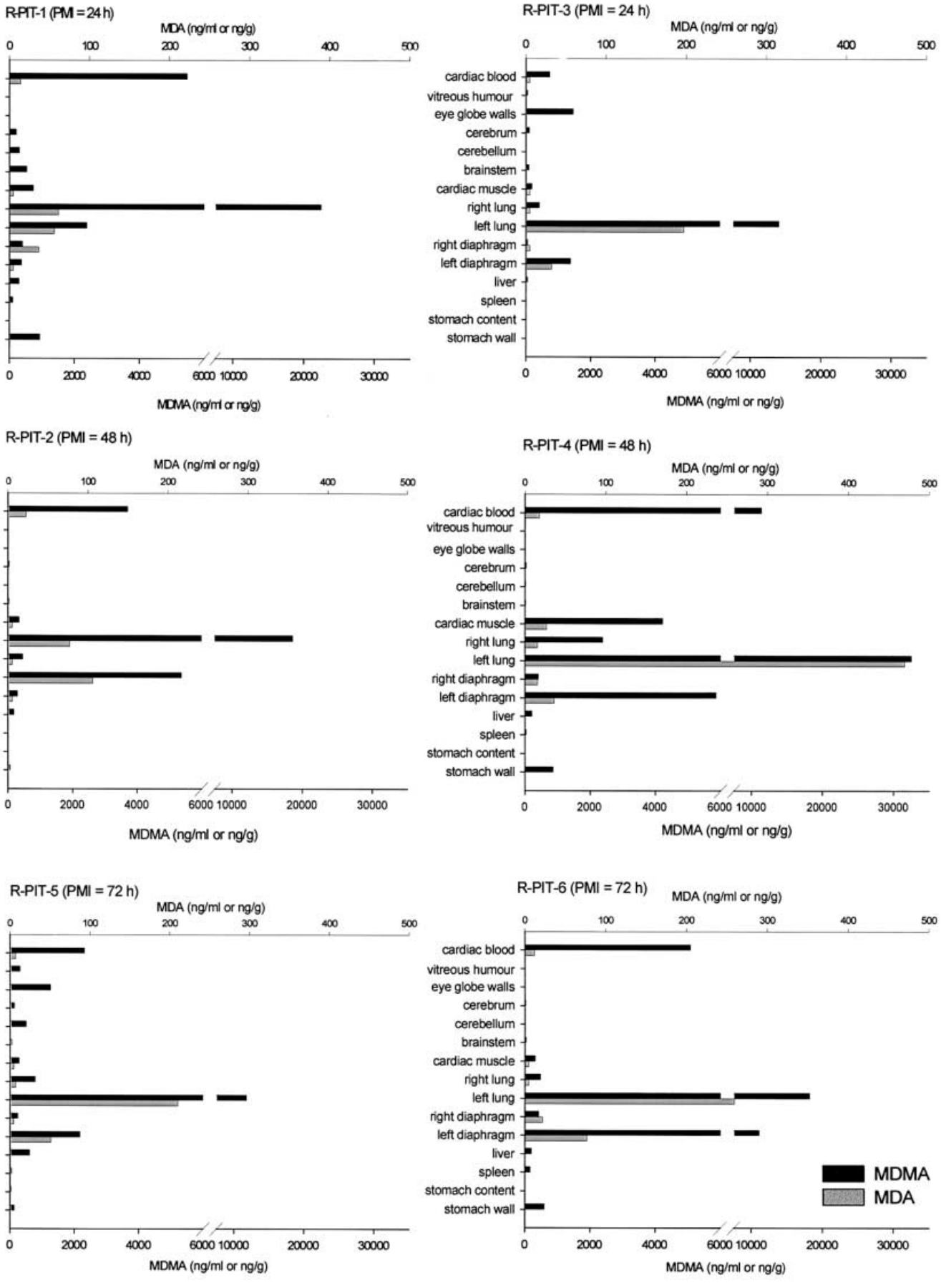
Analytical methods

MDMA and MDA concentrations in the tissues were assayed by HPLC with fluorescence detection as described earlier [13].

Results

Figure 2 shows the individual concentrations of MDMA and MDA in group 1 (PIT). The data indicate that the extent of post-mortem diffusion depends mainly on whether the MDMA solution flowed into the left (R-PIT-3,-4,-5,-6) or into the right (R-PIT-1,-2) bronchus. MDMA concentrations were substantial in the organs most directly adjacent to the lung containing the highest MDMA levels, such as the corresponding hemi-diaphragm. In most rabbits, very high MDMA levels were found in the cardiac blood. When the MDMA solution was concentrated in the left lung, MDMA was quantifiable in the stomach wall beginning 24 h after administration, and even in the stomach content and kidneys after 48 and 72 h. This is visually represented in Fig. 3, where the post-mortem redistribution is shown in 2 rabbits in which the highest MDMA amounts were found in the left bronchus at 24 and 48 h post mortem. In most tissues, the MDA levels were below the limit of quantitation (LOQ <10 ng/g), except in those having very high MDMA concentrations: cardiac blood and muscle, both lungs and diaphragm. However, the MDA concentrations were relatively low (<500 ng/g; see Fig. 2).

Fig. 2 Individual MDMA and MDA concentrations in rabbits ▶ (n=6) after post-mortem infusion of 1 mg/kg MDMA in the trachea (*PIT*), 24, 48 and 72 h after administration


R-PIT-1 (PMI = 24 h)
R-PIT-3 (PMI = 24 h)
R-PIT-2 (PMI = 48 h)
R-PIT-4 (PMI = 48 h)
R-PIT-5 (PMI = 72 h)
R-PIT-6 (PMI = 72 h)
MDA (ng/ml or ng/g)
MDMA (ng/ml or ng/g)
0
100
200
300
400
500
2000
4000
6000
10000
20000
30000
cardiac blood
vitreous humour
eye globe walls
cerebrum
cerebellum
brainstem
cardiac muscle
right lung
left lung
right diaphragm
left diaphragm
liver
spleen
stomach content
stomach wall
MDMA
MDA

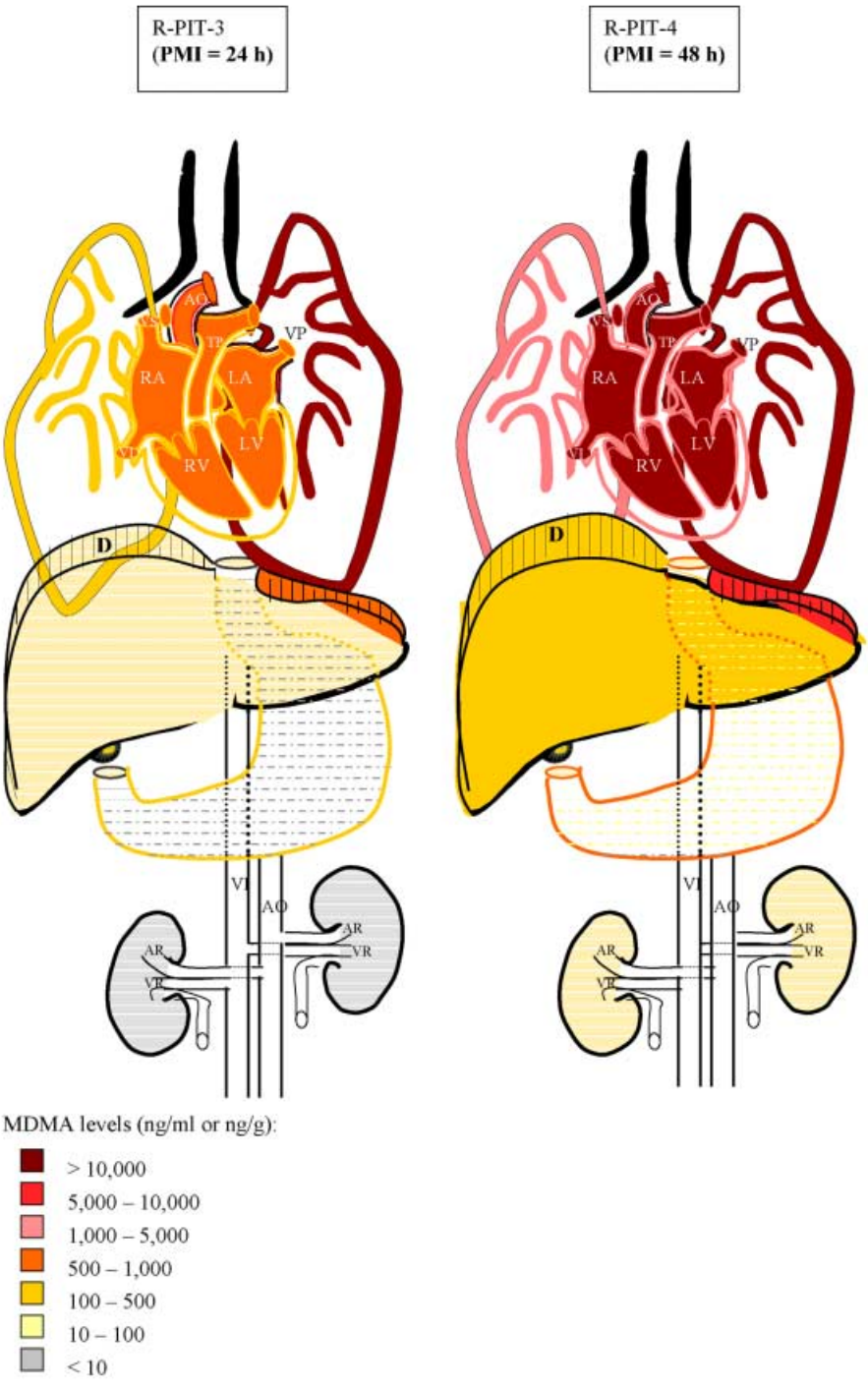
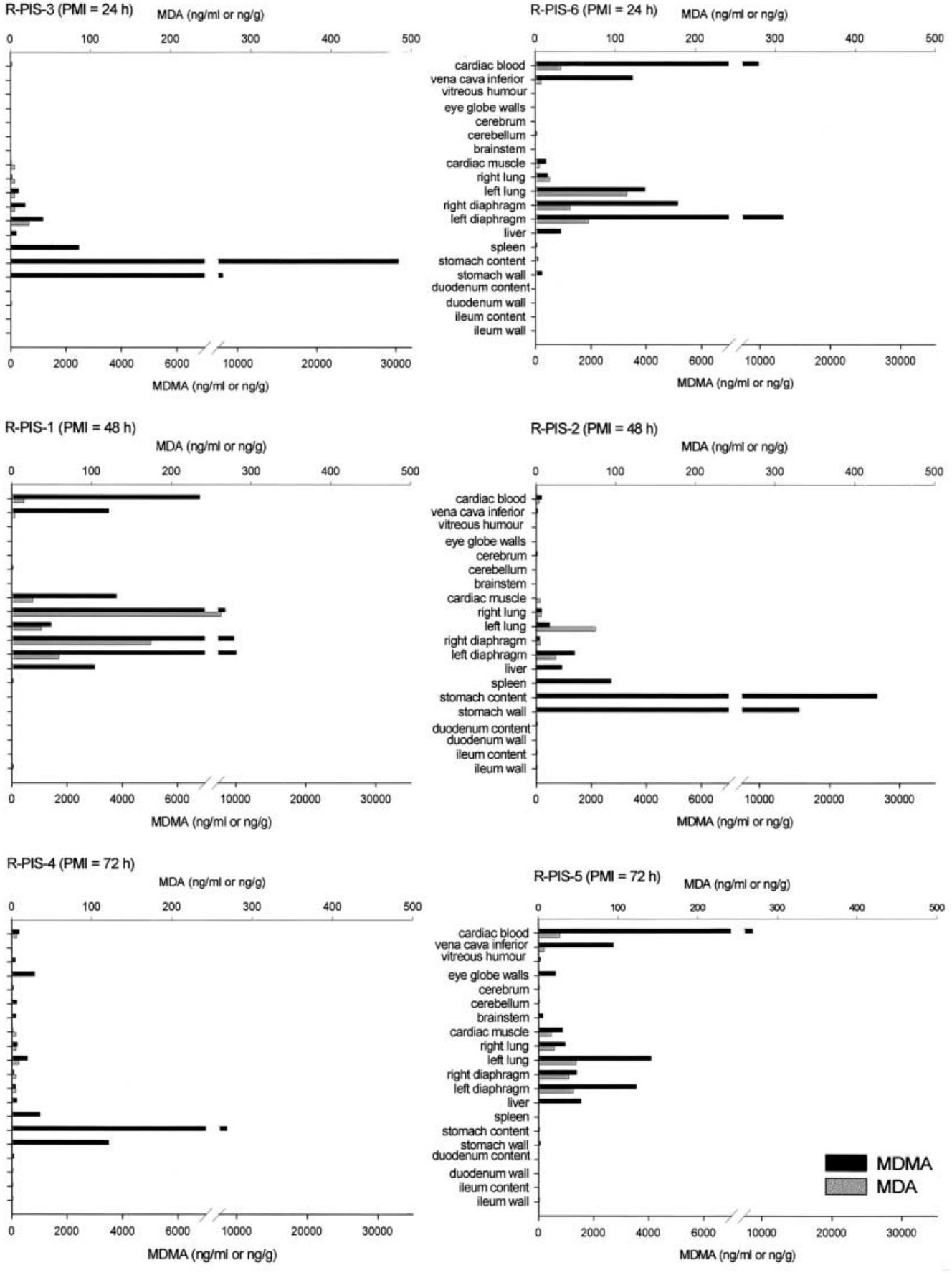


Fig. 3 Thoracic and abdominal post-mortem diffusion after tracheal instillation of 1 mg/kg MDMA in rabbits (*n*=2), in which spreading of the solution occurred predominantly in the left bronchus, 24 and 48 h after administration. MDMA levels (ng/ml or ng/g) *(RA* right atrium, *RV* right ventricle, *LA* left atrium, *LV* left ventricle, *TP* truncus pulmonalis, *VP* venae pulmonales, *AO* aorta, *VI* inferior vena cava, *VS* superior vena cava, *AR* arteria renalis, *VR* vena renalis, *D* diaphragm)

Figure 4 presents the individual MDMA and MDA levels in the rabbits of group 2 (PIS). Two patterns of post-mortem redistribution can be distinguished: an "intra-gastric" and a "supra-diaphragmatic" pattern. In the intra-gastric pattern (*n*=3; R-PIS-2,-3,-4), high MDMA levels were found in the left diaphragm and lung, as well as in the spleen. In addition, substantial MDMA concentrations were present in the liver. When the MDMA solution was concentrated just above the lower oesophageal sphincter (supra-diaphragmatic pattern; *n*=3; R-PIS-1,-5,-6), high MDMA levels were found in both hemi-diaphragms, the liver, the cardiac blood and muscle, and both lungs. These findings are visually documented in Fig. 5. The two different patterns, i.e. intra-gastric (Fig. 5a) and supra-diaphragmatic (Fig. 5b), are presented 72 h after infusion.

Fig. 4 Individual MDMA and MDA concentrations in rabbits ▶ (*n*=6) after post-mortem infusion of 1 mg/kg MDMA in the stomach (*PIS*), 24, 48 and 72 h after administration


R-PIS-3 (PMI = 24 h)
R-PIS-6 (PMI = 24 h)
R-PIS-1 (PMI = 48 h)
R-PIS-2 (PMI = 48 h)
R-PIS-4 (PMI = 72 h)
R-PIS-5 (PMI = 72 h)
MDA (ng/ml or ng/g)
MDMA (ng/ml or ng/g)
0
100
200
300
400
500
2000
4000
6000
10000
20000
30000
cardiac blood
vena cava inferior
vitreous humour
eye globe walls
cerebrum
cerebellum
brainstem
cardiac muscle
right lung
left lung
right diaphragm
left diaphragm
liver
spleen
stomach content
stomach wall
duodenum content
duodenum wall
ileum content
ileum wall
MDMA
MDA

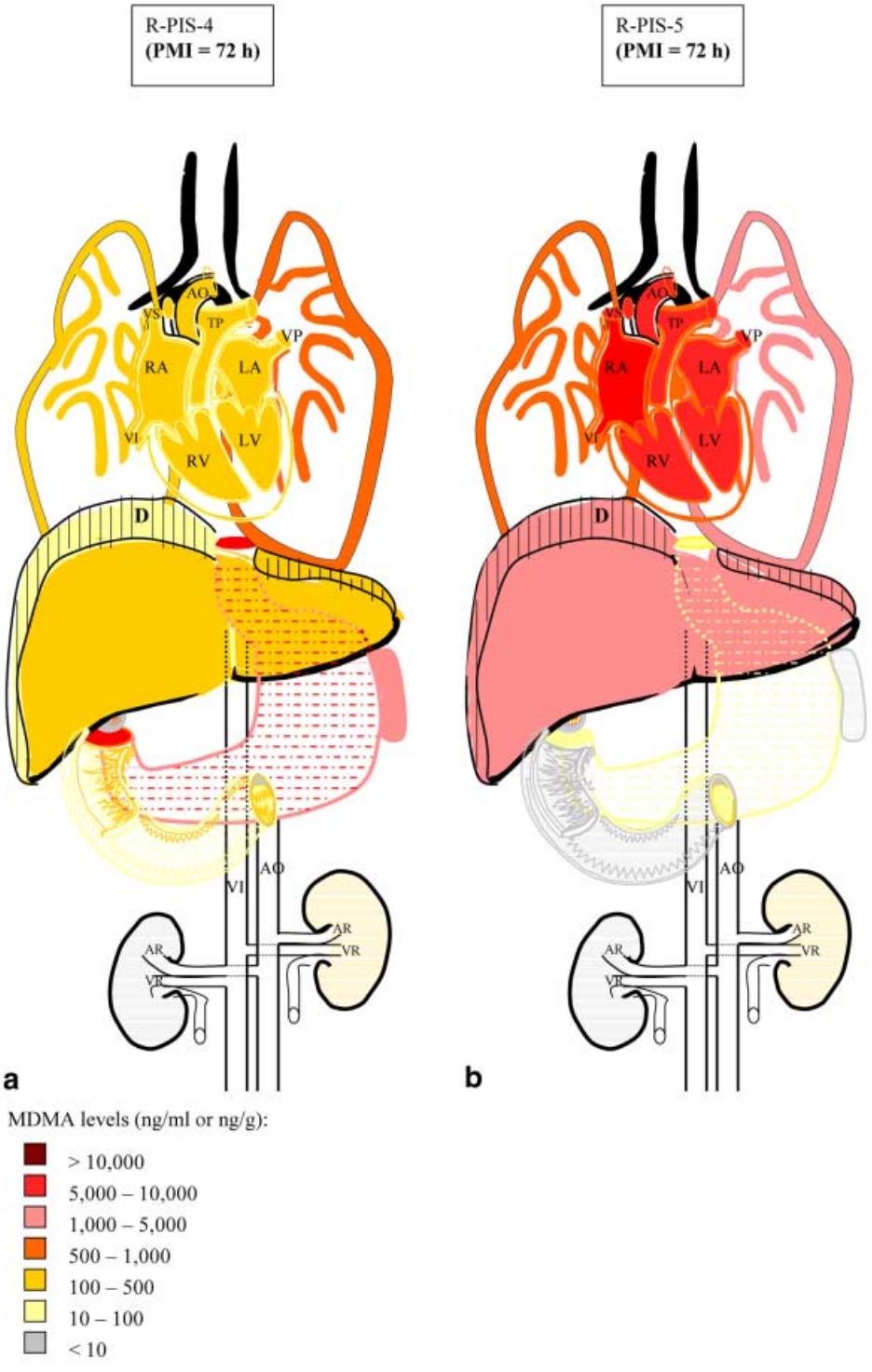


Fig. 5 Thoracic and abdominal post-mortem diffusion after gastric instillation of 1 mg/kg MDMA in rabbits (n=2), showing the difference between the intra-gastric **a** and supra-diaphragmatic pattern **b** 72 h after instillation. MDMA levels (ng/ml or ng/g) *(RA* right atrium, *RV* right ventricle, *LA* left atrium, *LV* left ventricle, *TP* truncus pulmonalis, *VP* venae pulmonales, *D* diaphragm, *AO* aorta, *VI* inferior vena cava, *VS* superior vena cava, *AR* arteria renalis, *VR* vena renalis, *D* diaphragm)

For all rabbits, an inter-individual variation was observed. As a result, no clear relationship between the concentrations and the post-mortem interval can be postulated.

In all rabbits, the MDMA levels were either very low or below LOQ (<10 ng/g) in the brain, eye globe walls and vitreous humour, small bowel wall and content, kidneys, iliopsoas muscle, abdominal adipose tissue, muscle of the abdominal wall, and urine (max 500 ng/g). However, in two rabbits of each group (R-PIT-3 and R-PIT-5, 24 h and 72 h after infusion, and R-PIS-4 and R-PIS-5, both rabbits 72 h after infusion) MDMA concentrations were non-negligible in the eye globe walls and vitreous humour. In addition, the levels in the eye globe walls were obviously higher than in the vitreous humour (max 2,000 and 360 ng/g, respectively).

In the rabbits of the intra-gastric pattern, MDA was barely quantifiable and was also very low in the supra-diaphragmatic pattern (<300 ng/g; see Fig. 4).

Discussion

MDMA tissue levels after *post-mortem tracheal instillation* depend on the dispersion of the solution into either the left or the right bronchus. In both cases, however, the MDMA concentrations were high in the cardiac blood and to a lesser extent also in the cardiac muscle. In addition, our data show that MDMA can easily diffuse out of the trachea into the thoracic and upper abdominal organs, and the amounts diffused increased erratically with the post-mortem interval. In the lower abdominal tissues, such as the kidneys, the iliopsoas muscle and adipose tissue, the MDMA levels were either very low or below the quantitation limit.

After *post-mortem instillation in the stomach*, two different diffusion patterns were observed depending on whether the MDMA solution was concentrated intra-gastrically or supra-diaphragmatically. The supra-diaphragmatic situation is comparable to gastro-oesophageal reflux, which involves substantial diffusion into cardiac blood and muscle, both lungs and liver. When the MDMA solution was concentrated more deeply in the stomach, post-mortem redistribution did affect the thoracic organs to a minor extent, and the intra-gastric solution diffused mainly into the closely adjacent spleen. Our results indicate that peri- or post-mortem gastro-oesophageal reflux is obviously more responsible for the redistribution of MDMA than a high MDMA concentration in the stomach itself.

In four rabbits, non-negligible MDMA levels were found in vitreous humour and eye globe walls. These levels were clearly higher than in the corresponding brain, which indicates that another mechanism other than pure diffusion from the brain should be assumed. One possible explanation is that there was direct or indirect reflux into the naso-pharynx with diffusion of MDMA into the sinuses, the skull base and the orbitae. Such diffusion has formerly been established for ethanol: in a human model, diffusion from an ethanol solution in the mouth and pharynx into the skull and also into the vitreous humour was observed, although at relatively longer post-mortem intervals (more than 60 or 72 h) [15].

In all the experiments in rabbits performed, we observed that the MDMA concentrations in the iliopsoas muscle were not subject to post-mortem diffusion, and thus remained stable after death. Therefore, iliopsoas muscle can be an interesting specimen when the usual samples for drug assay are lacking. However, muscle sampling is not recommended for some other substances (such as temazepam, prothiaden, paracetamol and amitriptyline) [16, 17]. These studies did not include concentrations in iliopsoas muscle, however.

In both post-mortem instillation experiments, the MDA levels were only quantifiable when very high local MDMA concentrations were found, which proves that MDMA can be metabolised post mortem into MDA. The MDA concentrations were lower when the MDMA solution was concentrated intra-gastrically instead of supra-diaphragmatically. This is in accordance with a previous study in which we hypothesised that the lungs play a role in the metabolism of MDMA to MDA [13].

Conclusions

In this experiment, we used *tracheal* instillation of MDMA to demonstrate that agonal vomit aspiration can lead to substantial post-mortem redistribution, mainly into cardiac blood and muscle, and into both lungs. To a less pronounced extent, MDMA also diffused to the liver tissue and the lower abdominal organs. Using infusion into the *stomach*, we proved that peri- or post-mortem gastro-oesophageal reflux gives rise to significant post-mortem diffusion of MDMA. When the MDMA reservoir is concentrated in the stomach itself, the thoracic organs are not substantially affected by redistribution up to 72 h post mortem. These rabbit experimental results could be extrapolated to humans as agonal aspiration in the lungs or post-mortem regurgitation frequently occurs in medico-legal practice. Our results demonstrate once more that peripheral sampling should be recommended in current practice. However, when this is not possible, the MDMA and MDA levels should be interpreted with great caution, especially regarding toxic or lethal levels. Finally, as in all experiments performed at present, the iliopsoas muscle concentrations remain stable post mortem, this specimen can be useful in current forensic practice when an appropriate blood sample is lacking.

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