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THE DISPOSITION AND METABOLISM OF METHYL ACRYLATE IN MALE WISTAR ALBINO RATS

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Abstract. The prestent study was designed to investigate methyl-[2,3-^{14}C]acrylate (MA) distribution, excretion, and metabolism. Data presented here show that the radioactivity derived from MA is rapidly absorbed after i.p. and p.o. administration and distributed into all major tissues of rats.

The highest concentration of MA-derived radioactivity was detected mainly in the liver and kidneys at 1 (i.p.) or 2 (p.o.) hours after dosing. There were only slight differences observed in the dynamics of tissue distribution and excretion in relation to the route of administration.

The major route of MA excretion was CO_2 exhalation (approximately 54% of the administered dose in 48 h) followed by urinary excretion. Two metabolites were identified in the urine, namely, N-acetyl-S-(2-methylcarboxyethyl)cysteine and N-acetyl-S-(2-carboxyethyl)cysteine, and ratio between those was about 1:1.

INTRODUCTION

Acrylic acid esters are used mainly as monomers in the production of acrylic resins and fibres, in the preparation of emulsion polymers for latex paints and varnishes, and have numerous applications in the paper and textile industries. The main components of acrylic resin are acrylic acid esters, the simplest being methyl acrylate.

The volatility and acute toxicity of acrylic acid esters decrease as the chain length of the alcohol component increases (1, 11). Acrylates are irritating to the skin and mucous membranes, and their irritative potential decreases according to the increasing chain length.

Methyl acrylate is a volatile substance and if inhaled can produce toxic effects of varying severity. This compound is highly irratative to mucous membranes, and at high concentrations or repeated exposures induces degenerative changes in the liver and kidneys (1,7,11,16). Despite its widespread use and interesting biological properties, relatively few studies of the distribution, excretion and metabolism of methyl acrylate have been reported.

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It was reported that methyl acrylate is hydrolyzed by several rat tissue carboxylesterases; dose-dependent decrease of the non-protein thiol levels in these tissues was observed. The inhibition of these carboxylesterases potentiated the toxic effects of methyl acrylate (10,15). In the study with methyl[2,3-^{14}C] acrylate in guinea pigs and rats some 36 to 52% of the given dose was excreted in the expired air as $^{14}CO_2$, and about 25–49% in urine within 72 h (13,14). Delbressine et al. (3) reported only some 7% of a given dose of methyl acrylate to have been excreted in urine of rats as S-(2-carboxyethyl)mercapturic acid or its methyl ester. The kinetics of the distribution and excretion of methyl acrylate has not been adequately explained and it is still not known what metabolites are present in urine of animals exposed to this compound.

The present study aimed at investigating the toxicokinetics of methyl [2,3–^{14}C]acrylate including blood and tissue distribution, excretion and metabolism after single administration of the compound to rats.

MATERIALS AND METHODS

Methyl-[2,3–^{14}C]acrylate (specific activity 48 MBq/mmol), chromatographically pure, labelled in the vinyl moiety was purchased from the Institute of Nuclear Research, Świerk, Poland. Unlabelled MA (BDH) was freshly distilled. All the other chemicals (BDH) were of analytical grade. Tissues, erythrocytes and plasma were digested according to Mahin and Lofberg (9).

All radioactivity measurements were carried out using Rack Beta 1209 (LKB) Liquid Scintillation Counter with DMP option and Tritosol (6) as scintillation mixture. The correction of counting was made by the external standard method.

Synthesis of Metabolite Standards

(1) N-Acetyl-S-(2-methylcarboxyethyl) cysteine: N-Acetyl-L-cysteine (80 mmol; 13.06 g), methyl acrylate (80 mmol; 8.01 g), triethylamine (400 mmol; 56 ml), and absolute ethanol (400 ml) were refluxed for 18 h and cooled, and the volatiles were removed by rotary evaporation. The oily residue was dissolved in 200 ml of water, extracted with 400 ml of ethyl acetate, dried over anhydrous sodium sulfate, and the filtrate was evaporated to a viscous oil with a rotary evaporator. A sample of this material was analysed by gas chromatograph – mass spectrometry system (GC-MS).

(2) N-Acetyl-S-(2-carboxyethyl)cysteine: N-Acetyl-L-cysteine (80 mmol; 13.06 g), 3-chloropropionic acid (80 mmol; 8.68 g), triethylamine (400 mmol; 56 ml), and absolute ethanol (400 ml) were refluxed for 18 h and cooled, and the volatiles were removed on a rotary evaporator. The oily residue was dissolved in 200 ml of water, acidified with hydrochloric acid, and extracted with ethyl acetate, and the aqueous phase was discarded. The ethyl acetate solution was dried over sodium sulfate and filtered, and the filtrate was evaporated to a viscous oil with a rotary evaporator. A sample of this material was converted to dimethyl ester with diazomethane, and analysed by GC-MS. The fragmentation pattern of both the above synthetic standards was consistent with the assigned structure.

Chromatographic System

An LKB Series 2105 HPLC with Pharmacia-LKB Ultrapac ODS 120 TSK reversed-phase C18 column (5 μm particle size 0.46 × 25 cm) was connected to a Hewlett-Packard 1050 UV-Vis detector (215 nm was used). The mobile phase used in HPLC was a gradient mixture:

A: 0.25 M Ammonium formate, pH 3.4

B: Acetonitrile (HPLC-grade)

Program: 0 – 6 min. 0%B; 6 – 30 min. 0 – 20%B with a flow rate of 0.5 ml/min. 0.5 ml fractions were collected for radioactivity measurement.

Identification of metabolites was carried out using a Hewlett-Packard 5890A Gas Chromatograph with 5970 Mass Selective Detector, HP-5970 C ChemStation. A column HP-5 was used. The temperatures were as follows: injector 180°C, oven: initial temp. 150°C rate 5°C/min, final temp. 180°C. Helium was used as a carrier gas, at the flow rate of 0.45 ml/min (about 25 cm/sec).

Experimental

The experiments were performed on 72 male Wistar rats (approximate body weight 250 g ± 10%) divided into 2 groups. The animals were put individually in glass metabolism cages (Simax, Czechoslovakia) and acclimatized for 48 hours. Subsequently, both groups of animals were administered 100 mg/kg of MA-^{14}C (about 3.7 MBq/kg) intraperitoneally (Group I) and intragastrically (Group II). The MA-^{14}C solution, with a specific activity of 870kBq/ml was prepared directly before administration by adding an appropriate amount of the medium (MA without an isotope marker) and dissolving it in 0.1% Tween 80. Immediately after the administration, the rats were placed in individual metabolism cages which enabled the collection of separate samples of urine and faeces. The expired air was pulled out of cages with the use of a water pump through 3 washers, fixed one after another and containing 25 ml of isopropanol (I) and 25 ml of ethanolamine (II and III). The washers were exchanged every hour durng the first 12 h of experiment and then every 12 hours.

Blood samples were collected from the tail veins using calibrated, heparinized capillaries at periods of 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, 12, 24 and 48 hours following administration of the compounds; 30 μm of blood was collected each time. After centrifugation (haematocrite centrifuge, Unipan) the amount of haematocrite was checked; the capillary was cut at the division line. Plasma and blood cells were put separately into scintillation vials. In order to count ^{14}C content in the whole blood, the value of 7 ml of blood/100 g body weight was adopted (4).

Rats were decapitated under light-ether narcosis at time intervals given in Tables 2 and 3. The liver, kidney, lung, brain, spleen, heart, stomach, a fragment of sciatic nerve and a sample of fat from the abdomen were examined.

Urine was acidified with 10 μl glacial acetic acid/ml urine and centrifuged at low speed to remove particulate matter. The supernatant was further purified through chromatography using Florisil (100 – 120 mesh) glass column (5 × 2 cm) and distilled water as a solvent. The eluate was condensed by rotary evaporation and then analysed by HPLC. The fractions containing ^{14}C were separately evaporated till complete dryness, dissolved in a small amount of methanol and then analysed by

GC-MS. Part of the analysed fractions were converted to methyl derivatives with diazomethane.

Results

The excretion of ^{14}C in the expired air, urine and faeces within the first 72 h is presented in Table 1. The expired air proved to be the main route of excretion

Table 1. Total radioactivity excreted (% of a dose) following oral or intraperitoneal administration of methyl [2,3−^{14}C]acrylate to rats (values represent mean for 6 rats ± S.E.M.)

Route of administration	Expired air (% of a dose)			Urine (% of a dose)			Faeces (% of a dose)	Total excretion (% of a dose)
	Time intervals (in hours)							
	0−24	24−48	48−72	0−24	24−48	48−72	0−72	
i.p.	51.8±4.6	2.4±0.3	trace	38.7±0.8	1.3±0.3	0	1.5±2.1	96.70
p.o.	38.6±6.5	trace	trace	49.3±7.4	1.9±1.2	0	1.5±0.5	91.30

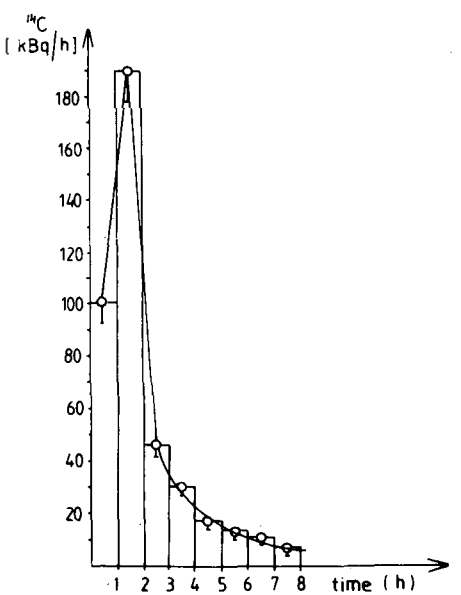

Fig. 1. The rate of ^{14}C excretion in the expired air after a single interperitoneal administration of methyl-[2,3-^{14}C]acrylate (100 mg/kg) to rats. Each point represents the mean of six animals ±SD.

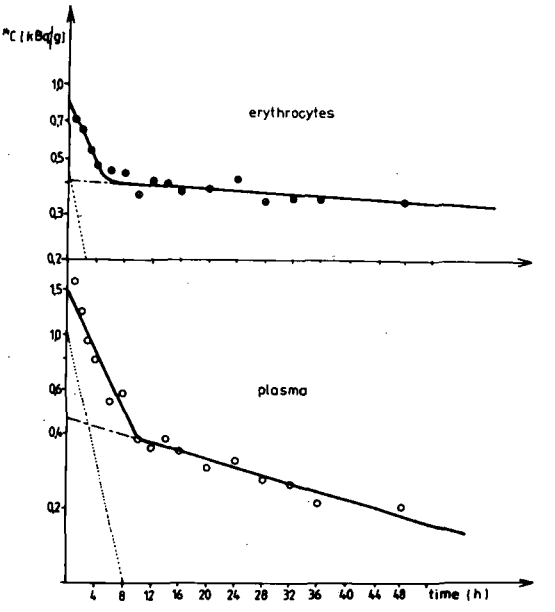

Fig. 2. Decay of radioactivity in blood following a single interaperitoneal administration of methyl[2,3-^{14}C]acrylate (100 mg/kg) to rats.

of ^{14}C. More than 40–50% of the given dose of ^{14}C was eliminated in this way during 24 h and the maximum excretion took place within the second h after the administration of MA (Fig. 1), irrespective of the route of absorption. The absence of ^{14}C radioactvity in the washers containing isopropanol excluded the presence of unchanged MA. Radioactivity was found only in the ethanolamine trapping solution, which indicates the presence of $^{14}CO_2$. Apart from the expired air, ^{14}C was also excreted in a considerable amount (40–50%) in urine, mainly during the first 24 h (Table 1). Only trace amounts of ^{14}C were excreted in faeces.

It was found that 72 h after oral and i.p. administration the total amount of the axcreted ^{14}C reached 91% and 97%, respectively.

The decay of radioactivity in blood plasma and erythorcytes after i.p. administration of MA is shown in Fig 2. The loss of ^{14}C in the plasma and erythrocytes was biphasic. The half-lives ($T_{1/2}$) of ^{14}C loss, counted by means of a graphic technique, for the fast phase were found to be the same for plasma and erythrocytes (about 2 h). They were, however, different for the slow phase (36 and 172 h, respectively).

Table 2. Distribution of radioactivity in tissues of rats following a single i.p. administration of methyl [2,3-^{14}C]acrylate (all results are presented as a mean of six rats; all SEM values $\leqslant \pm$ 10%)

Tissues	Time after administration (in hours)							
	1		8		24		48	
	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue
Liver	3.62	2.81	1.28	0.94	0.85	0.84	0.46	0.47
Kidneys	3.55	0.55	1.00	0.16	0.55	0.10	0.35	0.05
Spleen	1.38	0.05	0.66	0.02	0.68	0.03	0.24	0.01
Lung	2.70	0.30	0.88	0.10	0.61	0.06	0.50	0.06
Brain	1.72	0.32	0.47	0.07	0.32	0.06	0.20	0.03
Fat	0.64	–	0.26	–	0.29	–	0.16	–
Sciatic nerve	1.42	–	0.30	–	0.23	–	0.16	–
Plasma*	1.64	1.81	0.58	0.64	0.32	0.35	0.22	0.24
Erythrocytes*	0.72	0.53	0.33	0.32	0.42	0.31	0.36	0.27

*The results are presented in kBq/ml.

The tissue distribution of radioactivity after i.p. administration of MA (Table 2) showed the highest initial specific radioactivity in the liver, kidneys and the lungs, 1 h after dosage. The radioactivity declined rapidly with time in all tissues, particurly between 1 and 8 h, and then more slowly, especially in erythrocytes and the spleen. At 48 h after dosage the total radioactivity present in the examined tissues amounted only to about 1% the administered dose.

Table 3 displays the distributation of ^{14}C-radioactivity in the tissues after oral administration. The maximum ^{14}C concentration in most of the examined ttissues was observed 2 h after MA-^{14}C administration. Kidneys, liver, plasma and erythrocytes were tissues wih the highest initial radioactivity at this point of time. As in the case of i.p. administration, the radioactivity declined rapidly with time in all tissues between 2 and 8 h, and then more slowly, especially in the spleen and the lung.

Table 3. Distribution of radioactivity in tissues of rats following a single p.o. administration of methyl [2,3-^{14}C]acrylate (all results are presented as a mean of six rats; all SEM valueas $\leqslant \pm$ 10%)

Tissues	Time after administration (in hours)							
	2		8		24		48	
	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue
Liver	2.14	2.10	1.23	1.00	0.77	0.65	0.54	0.43
Kidneys	2.71	0.48	0.68	0.11	0.47	0.07	0.38	0.06
Spleen	0.55	0.02	0.42	0.01	0.36	0.01	0.41	0.01
Lung	0,44	0.06	0.38	0.05	0.33	0.04	0.30	0.03
Brain	0.29	0.06	0.15	0.03	0.11	0.02	0.12	0.02
Fat	0.08	–	0.10	–	0.11	–	0.10	–
Sciatic nerve	0.36	–	0.27	–	0.20	–	0.15	–
Plasma*	2.63	2.91	1.23	1.36	0.71	0.79	0.40	0.44
Erythrocytes*	1.32	0.97	0.64	0.47	0.37	0.27	0.21	0.46

*The results are presented in kBq/ml

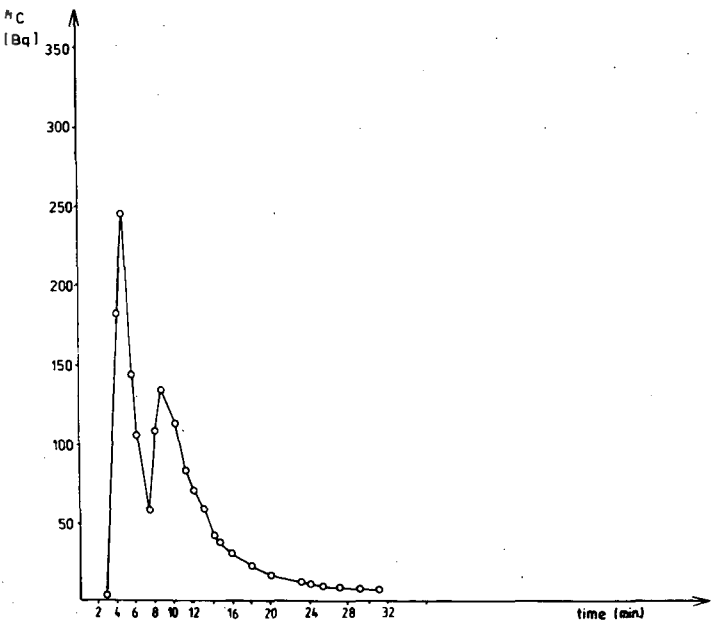

Fig. 3. Representative HPLC chromatogram of cumulative 24-hr urine from a rat treated with an i.p. dose of 100 mg/kg methyl [2,3-^{14}C]acrylate to rats.

The major portion of the dose was metabolised to CO_2; however two radioactive peaks were present in the chromatograms of cumulative 24 – h urine of both i.p. and orally treated rats (Fig. 3). The first peak having an average R_T of 4.3. min had the same HPLC R_T as synthetic N-acetyl-S-(2-methylcarboxyethyl)cysteine. GC-MS examination of the sample and the standard after derivatisation by treatment with

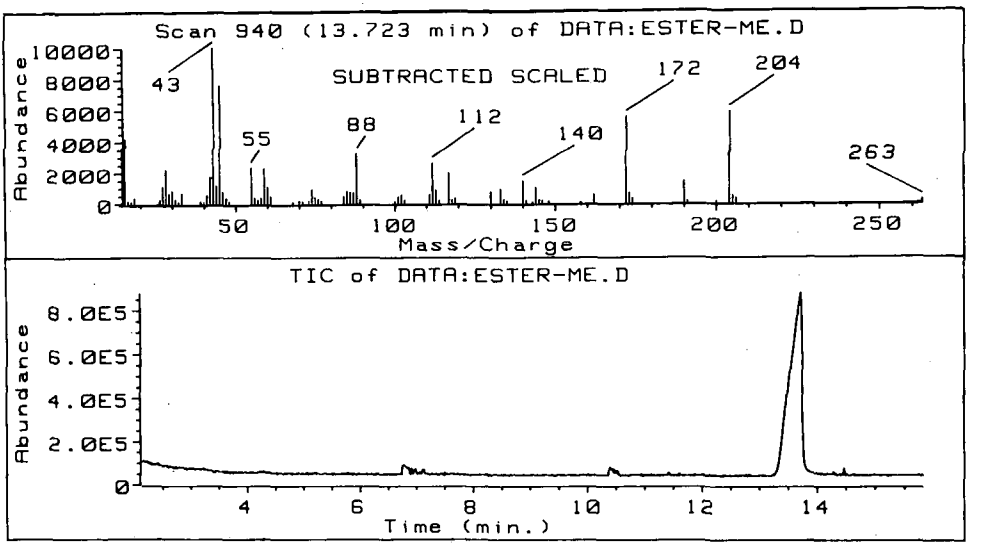

Fig. 4. Representative GC-chromatogram and mass spectrum of the methylated synthetic standard (N-acetyl-S-(2-methylcarboxyethyl)cysteine methyl ester).

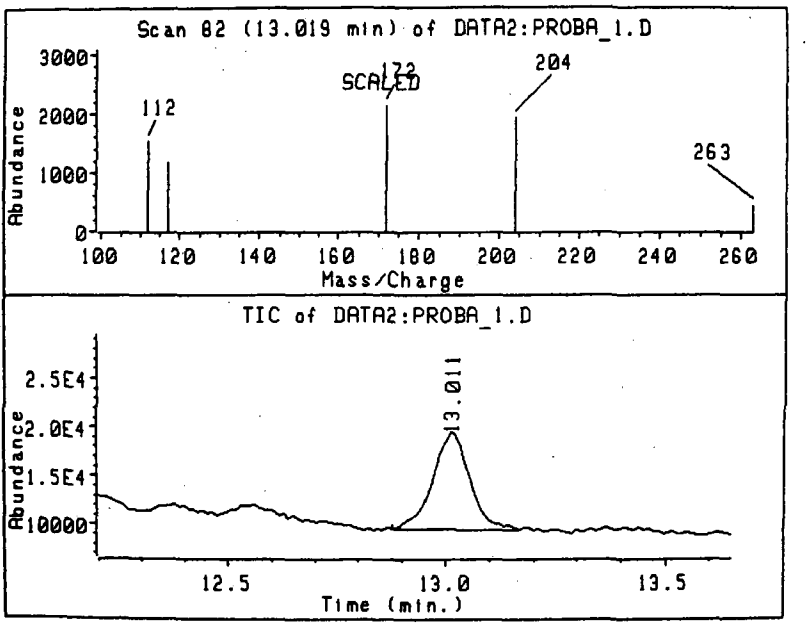

Fig. 5. Represantative GC-chromatogram and mass spectrum of the methylated samples from peaks I and II, by Selected Ion Monitoring.

diazomethane revealed that these compunds were the same (Figs 4 and 5). The second HPLC peak, with an average R_T of 8.8 min was identified as N-acetyl-S-(2-carboxyethyl)cysteine by comparing HPLC retention times as well as by GC-MS analysis after derivatisation with diazomethane (Fig. 5). The ratio between the two excreted metabolites, dicarboxylic acid and monomethyl ester, calculated from the mean ratio between peak I and peak II (Fig. 3) was about 1:1.

DISCUSSION

MA was rapidly absorbed from the peritoneal cavity and less rapidly from the gastrointestinal tract, and quickly and completely metabolised after administration. The experiments on the distribution of ^{14}C in tissues revealed the highest initial concentration of ^{14}C in the liver, kidneys, lung and blood, followed a rapid decrease which confirms the metabolic and excretion processes in those tissues. The observed small differences in the rate of tissue distribution derived probably from prolonged absorption of MA from the gastrointestinal tract as compared to i.p. route of administration.

Previous investigations of acrylate metabolism have established that the acrylic acid esters can be hydrolysed to free acid or react with sulfhydryls to from thioethers. Miller et al. (10) demonstrated that methyl, ethyl, and butyl acrylates were hydrolysed to acrylic acid in the liver, kidneys and lung homogenates *in vitro*. This hydrolysis was inhibited by tri-o-tolyl phosphate (TOTP) of non-specific carboxylesterases, with a subsequent decrease in non-protein sulfhydryls (15) N-acetyl-S-(2carboxyethyl)cysteine and N-acetyl-S-(2-methylcarboxyethyl) cysteine were identified in the urine of rats treated with MA at the amount of about 7% of the dose. The ratio between the excreted dicarboxylic acid and monomethyl ester was 20:1, whereas the administration of TOTP affected this ratio which was then 1:2. Similar mercapturic acid derivatives as the main metabolites were also detected in the urine of rats which were given ethyl and butyl acrylates (2,8,12).

The results of the present study revealed that the metabolism likewise that of ethyl and butyl acrylates was a two-way process. The major portion of MA dose in rats was hydrolysed by carboxylesterases to acrylic acid and methanol, whereas the minor one was conjugated with endogenous glutathione (GSH) and subsequently excreted as mercapturic acid in urine (Table 1, Fig. 5). After hydrolysis of MA to acrylic acid the double bond can be hydrated to form 3-hydroxypropionic acid which can then be oxidized to malonic acid and further in normal metabolic pathways to form $^{14}CO_2$ (5). The two mercapturic acid identified in urine are the products of MA-GSH conjugation. It is likely that the conjugation occurs between GSH and the intact MA molecule rather than the acrylic acid as the acrylic acid has been shown to be much less reactive with GSH *in vitro* than its esters (10).

To conclude, the results of this study have indicated that MA belongs to the fast-circulating compounds readily absorbed from the gastrointestinal tract and peritoneal cavity and rapidly and completely metabolised in the system. Thus, it is rather unlikely in the case of chronic exposure that MA is accumulated in the body.

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