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2-Mercapto-propylamine: radiopharmacology in mice, pharmacokinetic studies in mice and in rats, mutagenicity and differential distribution between tissues and EMT6 tumour in mice

Christian Paul Karl Schumacher [a,*], Yves Robbe [a], Marie-Thérèse Sicart [a], Guy Subra [a], René Delard [b], Jean-Bernard Dubois [b]

[a] *Laboratoire de Chimie Organique Pharmaceutique, Faculté de Pharmacie, 15 Avenue Charles Flahault, 34060 Montpellier Cedex, France*
[b] *Service de Radiothérapie, Centre Val d'Aurelle, rue de la Croix Verte, 34100 Montpellier, France*

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Abstract

Radiopharmacological studies conducted with 2-mercapto-propylamine (2MPA), a methylated derivative of cysteamine, indicated a good efficiency in whole body irradiated mice as observed over a period of 9 months. Its efficacy was also checked for supralethal irradiations of restricted body parts: in the brain and the rectum. The diffusion of ^{14}C-labelled 2MPA was assessed by an autoradiographic study and measurement of its distribution in the main organs in mice. 2MPA penetrated the blood brain barrier but concentrated preferentially in the liver, kidney and skin. Fixation on plasmatic proteins was much lower in rats than in mice but urinary and faecal eliminations were of the same order for the two species. An important biliary excretion of 2MPA or its metabolites in rats combined with their lack in the faeces underlies an entero-hepatic cycle. A differential diffusion of 2MPA between normal tissues in mice and EMT6 tumours was clearly revealed by autoradiographic observations. The ability of 2MPA to trap 2,2′-diphenyl 1-picryl hydrazyl, an organic free radical, was checked by in vitro studies. Its performance indicated that 2MPA acted at least as a free radical scavenger. Ames test demonstrated that 2MPA whatever the dose employed was not a mutagenic agent. Pharmacological and pharmacokinetical observations provided a better understanding of the activity of this drug.

Keywords: Aminothiol; Radioprotection; EMT6 tumor; 2-Mercapto-propylamine

1. Introduction

2-Mercapto-propylamine (2MPA) is a methylated derivative of cysteamine, half as toxic as the latter; its lethal dose of acute toxicity (LD_{50}) is 800 mg kg^{-1} in mice. It is easily synthesised from 1-amino-2-propanol; its dose reducing factor has a value of 1.85 [1]. 2MPA is soluble in water and is generated from carbon 5 methylated thiazolane rings in the organism [2]. Preliminary radiopharmacological studies indicated that 2MPA protected whole body irradiated mice.

The present studies are in keeping with the development of radioprotective drugs. The aim was to determine the exact radiopharmacological power of 2MPA and to obtain some fundamental pharmacokinetic results, in order to better understand the mechanism of action of this drug. 2MPA protects healthy mice from radiations but it was decided to check the differential diffusion between normal tissues and tumours, and Ames test was carried out to detect any possible mutagenicity of 2MPA.

2. Methods

2.1. Chemicals

Cysteamine was commercially purchased and WR 2721 was synthesised in our laboratory [3]. The synthesis of 2MPA has already been described [1]. ^{14}C labelled 2MPA (2-mercapto-1-propylamine, hydrochloride 2-^{14}C) was purchased from Isotopchim (France); specific activity 1968.4 MBq M^{-1}. All these drugs were used solubilised in physiological serum.

2.2. Animals

Tests of cephalic and pelvic irradiation were performed with female 10 PS C57 B2/6/JICO mice. Whole body irra-

* Corresponding author. Tel.: +33 04 67 54 80 00; fax: +33 04 67 54 80 39.

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diations were performed with female Swiss mice. For the study of the diffusion of 2MPA, female Swiss mice and female Wistar rats were used. All the animals originated from the IFFA CREDO (France) breeding. The mice were 9–10 weeks old and the rats weighed 250 g. After their irradiation until their death, the mice were housed in an air conditioned shelter with food and water at will.

3. Radiopharmacology

3.1. Irradiation of the cephalic region

Animals received an intraperitoneal injection of 2MPA at the $LD_{50}/4$ dose, volume of 0.1–0.2 ml, 15 min before their exposure. Control animals received an injection of the same volume of physiological serum. They were individually immobilised in an altuglass tube. Each tube was 2.5 cm diameter wide and had a hole at the bottom to allow sufficient aeration. 12 tubes were stuck together in 2 lines so that the heads of the mice were facing. The mice were kept in position by means of a pad of cotton wool, and the stopper of each tube had a notch for the tail which was fixed with self-adhesive tape. A single γ irradiation of 18 Gy (LD_{100} of irradiation at 30 days) was delivered simultaneously to the cephalic region of the 12 mice by means of a ^{60}Co irradiator. The source was 60 cm above a 2 mm thick altuglass lid covering the tubes. The collimator was placed 15 cm above the lid and its opening was set to limit a rectangular irradiation field which covered only the skull of the 12 mice. The radiation flow was 1.86 Gy min^{-1}.

3.2. Irradiation of the pelvis

15 min before irradiation the mice received the radioprotector at a dose of 1 mM kg^{-1} and an injection of Nesdonal at 40 mg kg^{-1} by the intraperitoneal route. The volume of the injected solution totalled 0.2 ml. Control animals were injected with physiological serum and Nesdonal. For each substance tested 16 mice were used. During the narcosis, the small intestine was pressed back by massage and kept away from the recto-sigmoid colon area by means of a sandow placed above the hip bones round the body, according to the protocol described by Ito et al. [4]. Then the animals were laid on their backs, in a circle, their legs and tail stuck with self-adhesive tape against an altuglass sheet. 8 mice were simultaneously irradiated. A single γ irradiation of 30 Gy (LD_{100} of irradiation at 120 days) was delivered by means of a ^{60}Co irradiator. The source was held 60 cm above the animals and the flow was 1.86 Gy min^{-1}. A 5 cm thick lead sheet having 8 holes of 20 mm diameter wide in the form of a circle acted as collimator. It was placed 15 cm above the animals, so that each irradiation field only covered the pelvic area of each mouse. Controls were made for both the irradiation protocols to ensure the homogeneity and the shape of the irradiation fields. The dosimetric films obtained by photodensitometry and the ionising chamber revealed that the doses were homogeneous by more or less 5% in each field.

4. Pharmacokinetics

4.1. Blood kinetics

4 rats and 4 mice were intraperitoneously injected with a solution of 2MPA at $LD_{50}/4$ in physiological serum containing 29.6 kBq/rat of ^{14}C labelled 2MPA for the rats and 2.96 kBq/mouse for the mice. The injected volume was 0.5 ml for the rats and 0.2 ml for the mice. Blood samples were taken at 15 min, 30 min, 1 h, 2 h, 3 h and 4 h after the injection. The rat and mice samples were treated the same; each sample received 1 ml of a mixture of toluene and isopropanol 1/1 to destroy the organic matter, then 3 h later 0.6 ml of a solution of 110 volumes of hydrogen peroxide. After discolouration the samples received 0.6 ml of a solution of N HCl for neutralisation and 10 ml of Instagel. The remaining fraction of radioactivity from the ^{14}C labelled 2MPA in the blood was determined by a scintillation counter.

4.2. Biliary kinetics

6 rats were anaesthetised with a 12% ethylurethane solution at a dose of 1.2 g kg^{-1} and their choledoch duct was catheterised. Bile was collected for 3 h after administration by the intraperitoneal route of the solution of 2MPA at $DL_{50}/4$ plus 29.6 kBq of ^{14}C labelled 2MPA. The ^{14}C radioactivity counting was made in the presence of 10 ml of Instagel for 100 mg of bile sample.

4.3. Urinary and faecal elimination

4 rats and 4 mice were intraperitoneally injected with a solution of 2MPA at $LD_{50}/4$ in physiological serum containing 29.6 kBq/rat of ^{14}C labelled 2MPA for the rats and 2.96 kBq/mouse for the mice. The injected volume was 0.5 ml for the rats and 0.2 ml for the mice. Urine and faeces were taken out of the metabolic cage 24 h after the administration of 2MPA. Urine samples were adjusted to 50 ml with water and faeces were crushed in liquid nitrogen then adjusted to 100 ml with water. Samples of 1 ml of this mixture were added to 10 ml of Instagel before determining the radioactivity with a scintillation counter.

4.4. Distribution of labelled 2MPA in the blood, liver, kidney, brain and lung

8 mice were used for this purpose. They were sacrificed by a prolonged anaesthesia with ether, 30 min after the administration of the solution of 2MPA at $DL_{50}/4$ containing 2.96 kBq of ^{14}C labelled 2MPA. Blood was taken by an intracardiac puncture. Organs were excised and crushed in a mortar with liquid nitrogen. Blood aliquots of 100 mg were treated

in the same way as in the blood kinetic study (see above); samples of 100 mg of each organ received 1 ml of toluene/isopropanol 1/1 mixture, 0.6 ml of hydrogen peroxide and 0.6 ml N HCl. After 30 min, 0.6 ml HCl and 10 ml Instagel were added to each sample before counting the ^{14}C radioactivity in a scintillation counter.

4.5. Organic radicals trapper

According to the protocol of Cier et al. [5], 75 μM of 2,2-diphenyl-1-picryl-hydrazyl (a free organic radical) and 75 μM of 2MPA were mixed at room temperature. The beginning of the addition of the second solution was taken as point 0 s for the kinetic study. The decrease of absorbance of the mixture was measured by spectrometry at 512 μm in relation to a solution of 75 μM of 2MPA. The half life of the organic radical was established by graphic extrapolation. Results expressed in seconds represent an arithmetical mean of three trials.

4.6. Autoradiographic study in mice bearing an EMT6 tumour

15 days before the test, mice were injected with EMT6 tumoral cells and antibiotics, colimycin and penicillin, by the intramuscular route in their backs. The day of the test, 10 mice were injected with the solution of 2MPA at $DL_{50}/4$ plus 2.96 kBq of ^{14}C labelled 2MPA by the intraperitoneal route. Mice were sacrified 30 min, 2 h and 24 h after the administration of 2MPA by immersion in liquid nitrogen. Sagittal cuts were made 1 week after the conservation of the animal at −30°C. Cuts were dried for 2 weeks before their deposit on Kodak Kodirex film for 12 weeks. The intensity of marking on the developed films indicated the distribution of the radiolabelled 2MPA and its metabolites.

4.7. Ames test

Ames test [6] determines the reversion frequency of mutation in two bacterial strains TA 98 and TA 100 (*Salmonella typhimurium*) with or without metabolic activators (rat liver extract S9 mix). These two strains exhibit mutations that impair their development on histidine deprived medium. The mutagenicity of substances in contact with the bacteria increases the frequency of reversion. 2MPA was distributed at 250, 500, 1000, 2500 and 5000 μg per dish of culture medium without histidine, in the presence or absence of rat liver extract microsomes (S9 mix), a source of enzymes that amplifies the eventual reverting power. A negative control (distilled water) and two positive controls (2-nitro-fluorene (NF) and 2-amino-anthracene (AA)) were also made. Plates were seeded with TA 98 or TA 100 strains (about 2×10^8 bacteria per dish). The mutagenicity was determined by counting the number of clones which appeared after 48 h at 37°C.

5. Results

5.1. Radiopharmacology

5.1.1. Long term survival

The survival of Swiss mice treated with 2 MPA at $LD_{50}/2$ was observed over a period of 9 months (Fig. 1). Batches of 10 mice were irradiated on their whole body for 15 min after their injection at 3, 5, 7, 9 and 11 Gy. For irradiation at 3 and 5 Gy all the mice survived for 7 months. 90% of the mice remained alive during the following 2 months. The lifespan increases (LI %) at 9 months were respectively 91.6 and 80.4%. For irradiation at 7 Gy the lifespan increase was 100% up to 6 months then lowered progressively; at 9 months it was 62%. All the mice irradiated at 9 Gy survived 3 months then a progressive mortality occurred between 3 and 8 months; at 9 months lifespan increase was 41.5%. At 11 Gy, 80% of the mice survived the supralethal dose of irradiation during the first month, during the following period of 5 months they died at a slow rate; the lifespan increase at 6 months was 75%. Then during the last period of observation the rate of mortality increased; at 9 months the lifespan increase was 20%.

5.2. Radioprotection of the brain

The survival of twelve C57 BL6 mice was checked for 120 days after their irradiation (Fig. 2). 100% of the mice treated

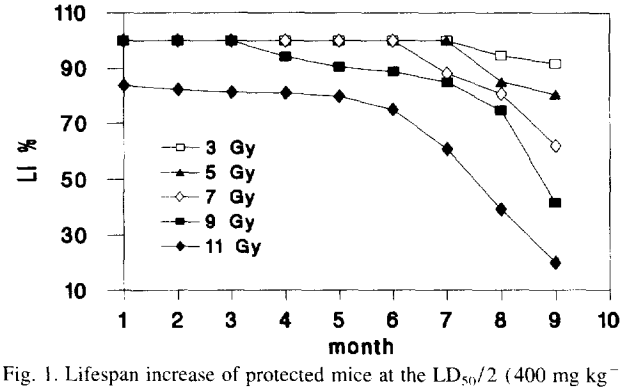

Fig. 1. Lifespan increase of protected mice at the $LD_{50}/2$ (400 mg kg^{-1}) as a function of the dose of irradiation, for whole body irradiated mice, observed over 9 months.

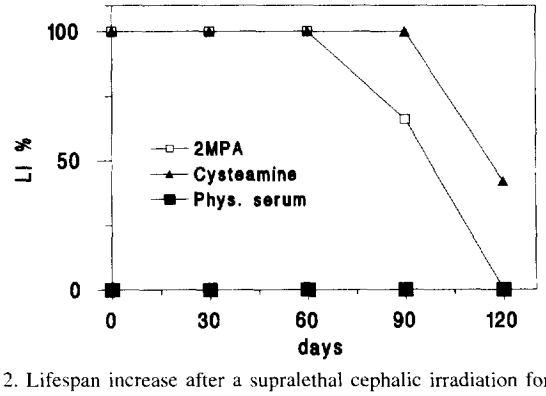

Fig. 2. Lifespan increase after a supralethal cephalic irradiation for mice protected with 2MPA or cysteamine at $DL_{50}/4$.

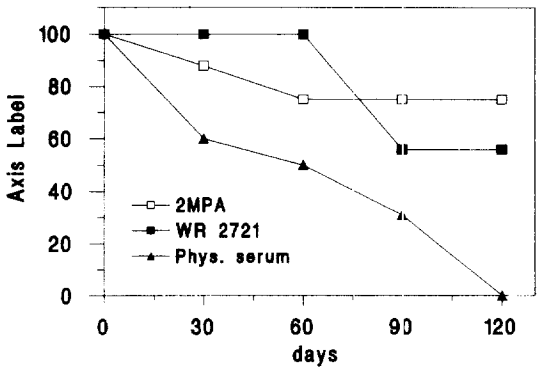

Fig. 3. Lifespan increase after a supralethal pelvic irradiation for mice protected with 2MPA or WR 2721 at a dose of 1 mM kg^{-1}.

with 2MPA at $DL_{50}/4$ survived until the end of the second month, and 66% of them remained alive at the end of the third month. At 120 days no mice had survived. The real survival mean time of the batch was 89 days. No control mice survived over the first month; for them the real survival mean time had a value of 11.5 days. The ratio of the value of the real survival mean time of treated mice and of control mice was 7.7. For comparison, the real survival mean time obtained with cysteamine at $DL_{50}/4$ was 156 and the ratio was 13.5.

5.3. Radioprotection of the rectum

Sixteen C57 BL6 mice were observed for 120 days after their irradiation (Fig. 3). Mice received 2MPA at a dose of 1 mM kg^{-1}. At this concentration 88% of the mice survived to the end of the first month, and 75% of them were alive at the end of the observation. The real survival mean time was 150 days. The value of this parameter for the control mice was 75 days; the ratio of both values was 2. The real survival time obtained with WR 2721 was 188 and the ratio was 2.5.

6. Pharmacokinetics

6.1. Blood kinetics of the ^{14}C labelled 2MPA in mice and rats (Fig. 4)

In rats, less than 0.2% of the injected 2MPA remained in the blood, whatever the time of the observation between 5 and 360 min. For mice 3.5% of the injected 2MPA was found in the blood 5 min after its intraperitoneal injection, then the quantity decreased slowly up to 2 h. 4 h after the injection, 0.85% of the ^{14}C labelled 2MPA remained in the mice blood.

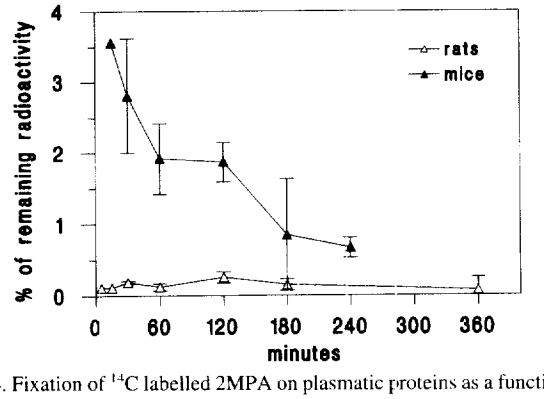

Fig. 4. Fixation of ^{14}C labelled 2MPA on plasmatic proteins as a function of time for mice and rats. Percent of the initial injection per tissue weight ∓ standard mean deviation.

6.2. Distribution of ^{14}C labelled 2MPA in the blood, liver, kidney, brain and lung (Table 1)

30 min after the injection, the maximal amount of 2MPA was found in the kidney and the liver, respectively 7.4 and 4% of radioactivity/tissue weight. To a lesser extent the brain and lung contained about 2.5% of radioactivity/tissue weight of the injected 2MPA.

6.3. Biliary kinetics of ^{14}C labelled 2MPA in rats (Fig. 5)

The radioactivity was determined on samples of 100 mg of bile. 11.3 ± 3% of the injected 2MPA were eliminated within 3 h in the bile. Maximal excretion occurred during the second hour after the injection with a rate of 5 1%.

6.4. Urinary and faecal elimination of ^{14}C labelled 2MPA in rats and mice (Table 2)

For the mice and rats, observed for 24 h, most of the 2MPA was excreted by the urinary route.

Table 1
Distribution of ^{14}C labelled 2MPA in the organs of mice 30 min after injection. Percent of the initial injection per tissue weight ∓ standard mean deviation

Tissues	Mean ∓ s.e.m.
Brain	2.34 ∓ 0.72
Liver	4.06 ∓ 1.11
Lung	2.75 ∓ 0.38
Kidney	7.39 ∓ 2.25
Blood	0.83 ∓ 0.42
Total	17.37 ∓ 4.88

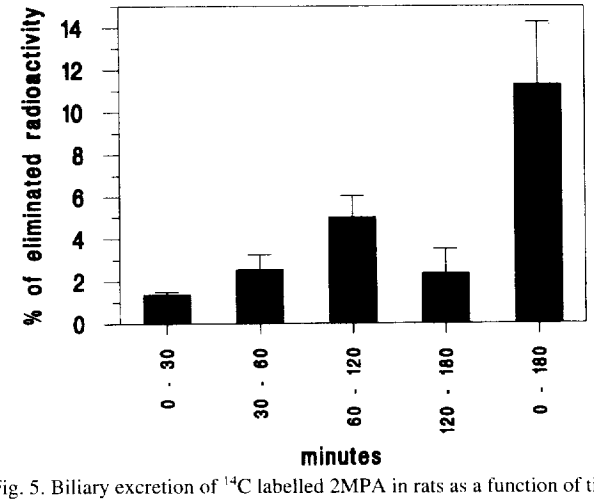

Fig. 5. Biliary excretion of ^{14}C labelled 2MPA in rats as a function of time. Percent of the initial injection per 100 mg of bile ∓ standard mean deviation.

Table 2
Urinary and faecal elimination of ^{14}C labelled 2MPA determined after 24 h for mice and rats. Percent of the initial injection ∓ standard mean deviation

	Urine	Faeces
Rats	49.55 ∓ 3.70	2.31 ∓ 1.88
Mice	47.89 ∓ 2.89	2.15 ∓ 1.22

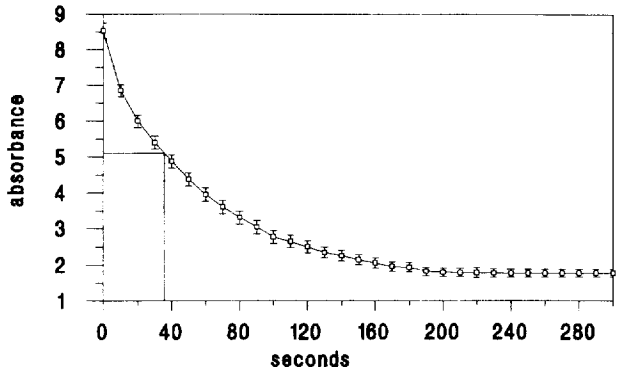

Fig. 6. Absorbance as a function of time of 75 μM diphenyl-picryl-hydrazyl in the presence of 75 μM 2MPA. Optical density ∓ standard mean deviation.

6.5. Autoradiographic study of mice with EMT6 tumour

30 min after the injection, the whole body of the mouse was intensively impregnated, including the central nervous system. Comparatively, only a feeble diffusion occurred in the tumour. 2 h after the injection the diffusion remained ubiquitous, but the skin, intestine, kidney, liver and brain were more intensively marked. The EMT6 tumour was almost free of marking. 24 h after the injection, the main part of the radioactivity was found in the skin; the tumours appeared with a feeble peripheral marking (results not shown).

6.6. Organic radical trapper (Fig. 6)

In the presence of 75 μM of 2MPA, the half life of 2,2-diphenyl-1-picryl-hydrazyl was 37.6 ∓ 8.2 s.

6.7. Ames test (Table 3)

Negative controls indicated the occurrence of spontaneous reversions. The positive controls indicated the response of the bacteria strains in the presence of highly mutagenic compounds. For the two strains TA 98 and TA 100, with or without metabolic activator, whatever the dose of 2MPA used, the number of developed clones did not differ from the values obtained for distilled water. In the presence of standard mutagenic agents the rate of reversion is amplified by a factor of 10 to 25.

7. Discussion

A dose dependent radioprotection offered by 2MPA for whole body irradiated mice at LD_{100} at 30 days has already been described [1]. Here, the radiopharmacology has been clarified by the determination of the lifespan increase over a long period of observation. Mice were irradiated on their whole body from 3 to 11 Gy, according to a protocol described in the preceeding paper [2]. When irradiated from 3 to 9 Gy, the protection brought by 2MPA at $DL_{50}/2$ (400 mg kg^{-1}) permitted the survival of all the mice for at least 3 months. Then, the beginning of the mortality in the different batches of mice and the rate of death occurred in proportion to the intensity of irradiation. The extent of radiolesion is a function of the dose of irradiation. Up to 9 Gy, 2MPA protected the mice from the early effects of radiation but could not prevent the spreading of all the radiolesions that caused the death of the mice long after their irradiation. For a supralethal irradiation of 11 Gy, only 80% of the mice survived after the first month. The remaining mice died rapidly with a high rate of death. At 11 Gy, the strength of the irradiation was above LD_{100} at 30 days and the mice died from acute radiolesions.

The in vitro test performed with a free organic radical demonstrated the rapid action of 2MPA and the autoradiographic study showed a rapid diffusion throughout the whole

Table 3
Number of TA 98 and TA 100 clones developed in the presence of distilled water (negative control), 2-nitrofluorene or 2-amino-anthracene (positive controls) and various doses of 2MPA. For each test in triplicate, with or without S9 mix

Substances (μg/dish)	Number of clones/dish											
	TA 98 − S9 mix			TA 98 + S9 mix			TA 100 − S9 mix			TA 100 + S9 mix		
Distilled water	34	35	37	31	33	32	168	130	116	156	136	169
2MPA 250	40	32	32	29	35	37	140	149	144	102	153	149
2MPA 500	24	39	31	28	28	31	135	125	164	137	128	144
2MPA 1000	34	27	37	41	35	40	114	120	136	131	124	132
2MPA 2500	27	33	31	35	33	25	90	103	113	93	109	120
2MPA 5000	31	33	30	42	28	37	105	119	123	109	109	134
NF 2.5	732	668										
AA 2.5				889	1015							
NF 1							572	740				
AA 1										1154	1350	

body. These two results accredited that 2MPA acted at least by trapping radio-induced free radicals. The extent of free radical formation depends, among many other factors, on the dose of irradiation [7]. Above 9 Gy, the amount of ionisation was so important that the generation of free radicals exceeded the capacity of the injected 2MPA to annihilate them. For the highest doses of radiations, in addition to the generation of free radicals, the ionisations might statistically directly affect the DNA structure to such an extent that 2MPA, even acting as a hydrogen donator or as a disulfide bond, was not able to restore the DNA damage.

The determination of the efficacy of 2MPA against irradiations of a selective body area continued the radiopharmacological study. When the irradiation concerned only the brain, drugs were administered at $DL_{50}/4$. For mice irradiated at LD_{100} at 30 days of irradiation, all of them survived 2 months, then all died within the two following months.

2MPA penetrated the blood brain barrier and diffused into the brain as seen in the autoradiographic picture and as determined in the study of the distribution in the organs. In this test, 2MPA was compared to cysteamine which exerted a very good radioprotection of the brain [8]. 2MPA was slightly less effective than cysteamine; the cell membrane transport systems that carry cysteamine [9] might be less able to carry 2MPA due to the steric space of its methyl group. It has been shown that the methylation of carbon 2 of cysteamine prevents its fast oxidation [10] so it is longer acting than cysteamine. The cell imperviousness to 2MPA might be compensated by its longer period of action. In addition, due to its electron-donator methyl group, 2MPA was susceptible to the easy loss of its thiol group for transulfuration reactions that might increase the pool of biological thiol compounds. In the rectal irradiation test, 80% of the mice survived over 3 months the irradiation at LD_{100} when protected by 2MPA at 1 mM kg^{-1}. In this test, the death of the mice appeared later and came from differed radiolesions such as sclerosis of the mucous membrane of the rectum and vascular thrombosis due to massive cell destruction [11]. The autoradiographic study indicated a great and early impregnation of ^{14}C labelled 2MPA in the intestinal tract. Compared to WR2721 at the same dosage, 2MPA was less effective. The dephosphorylation of WR2721 led to formation of cysteamine related compounds in the intracellular spaces [12]. The presence of the methylated group of 2MPA might impair the efficacy of the metabolic pathways which achieved the radioprotective effect of exogenous aminothiols.

A first set of pharmacokinetical observations demonstrated that the plasmatic circulation of ^{14}C labelled 2MPA was different in mice and rats. In the latter only a slight proportion of the intraperitoneally injected radioactive drug was measured as soon as 5 min after the administration. This observation combined with the earlier picture obtained from the autoradiographic study indicated that 2MPA rapidly reached the intracellular spaces. 2MPA penetrated the cells faster in rats than in mice. The autoradiographic pictures showed that 2MPA or its metabolites diffused throughout the whole body in mice and penetrated the blood brain barrier very quickly. More precisely, as seen by the measurements of its distribution in the body, 2MPA or its metabolites concentrated in the liver and kidney 30 min after its injection. These two organs were eminently implicated in the physiological process of excretion. Most of the injected ^{14}C labelled 2MPA or its metabolites were found in the urine of the rats and of the mice. They almost left the body by urinary excretion. Urine extracts submitted to chromatography showed multiple spots (result not shown). They corresponded to different metabolites issued from the degradation of 2MPA and to disulfur formation due to easy oxidation of the thiols with air; actually they are not identified. In rats 11.7% of the total injected ^{14}C labelled 2MPA or its metabolites were found in the biliary excretion during the three first hours after its injection and nearly nothing was detected in the faeces collected over 24 h. These two observations together indicated that 2MPA and its metabolites followed an entero-hepatic cycle. After 24 h, according to the results obtained on urine and faeces, 50% of the injected 2MPA or its metabolites remained in the body; the autoradiographic pictures confirmed the presence of ^{14}C in the animals after this period with a preferential distribution in the skin, liver and kidney. The autoradiographic study was undertaken with mice bearing EMT6 tumours; the pictures revealed a differential distribution of 2MPA between the normal tissues and tumours. 2MPA remained mainly in the peripheral cells of the tumours whatever the time of observation. This differential distribution could be useful in protecting normal tissues to a better extent than tumours in the case of radiotherapy treatment. Some thiols have been shown to be mutagenic and might add their effects to the troubles resulting from the ionisations. It has been shown that glutathione and cysteine were mutagenic in strains of *Salmonella tiphimurium* [13,14] because of the involvment of oxygen radicals formed through oxidation of cysteine. For 2MPA no dose dependant effects could be seen in TA 98 and TA 100 strains up to a dose of 5 mM per plate, even in the presence of liver post-mitochondrial supernatant fraction. 2MPA might be considered as non-mutagenic.

8. Conclusions

This study demonstrated a good efficiency of 2MPA as a radioprotective compound for acute supralethal irradiation of whole body and of limited body parts in mice. The pharmacokinetic observations indicated a rapid diffusion of 2MPA in the whole body, including the central nervous system and a long lasting impregnation. 2MPA concentrated preferentially in the liver, kidney and skin but did not penetrate EMT6 tumours cells as well as normal tissues. An exclusive urinary rejection and an important biliary excretion underlies an entero-hepatic cycle. Only small amounts of 2MPA were found fixed on plasmatic proteins, 2MPA rapidly reached the intracellular space and exerted its effects at least as an organic radical trapper. 2MPA has been shown to be non-mutagenic.

The acute toxicity of 2MPA may be lowered by the use of its prodrugs, for instance thiazolane rings methylated on carbon 5 [2]. A radioprotective compound that penetrates the blood brain barrier and diffuses differentially between normal tissues and tumours could be useful in radiotherapy.

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