Biochemical Pharmacology, 1965, Vol. 14, pp. 1891–1893. Pergamon Press Ltd., Printed in Great Britain.

SHORT COMMUNICATIONS

Observations on the use and action of monoacetin in fluoroacetate poisoning

(*Received* 21 *July* 1965; *accepted* 25 *August* 1965)

IT IS generally believed that monoacetin, $CH_3CO \cdot O \cdot CH_2 \cdot CH(OH) \cdot CH_2OH$, must be given within 10 min of the administration of fluoroacetate to be effective as an antidote, and that its action is to stop the lethal synthesis of fluoroacetate to fluorocitrate (F. cit.), probably by intracellular liberation of acetate.[1, 2] In connection with attempts to find an antidote against fluorocitrate *in vivo*, we have studied further the effects in rats of monoacetin given by the intracerebral route (Valzelli technique) on intracerebral fluorocitrate. We have recently proved that fluorocitrate is at least 200 times more potent in causing convulsions than fluoroacetate.[3] The study has revealed some new points about monoacetin, and also about fluoroacetate toxicity. In particular, monoacetin can prevent the entry of the minute amount of F.cit. into the brain required to cause convulsions; even so it does not prevent in rats a fatal termination.

Methods and chemicals. Monoacetin was the technical glycerol monoacetate, BDH. When examined by thin layer chromatography, 3 compounds were present; the interpretation was that there was present a main compound of monoacetin, some diacetin and a trace of acetic acid. Fluoroacetate and fluorocitrate were described in a recent note.[3] Intracerebral injection was by Valzelli's technique.[4]

RESULTS

We proved first that monoacetin given by the intracerebral route in 0·01 ml was not toxic (Table 1, Exp. 1–4). For 1·4 g rat brain (the average) 0·004 ml is a dose of $\sim$ 3·8 g/kg. In experiment 5, 15 μg F. cit. (crude) was injected together with 0·004 ml of monoacetin. The average time to convulsions was approximately the same, as has been found recently.[3] Hence monoacetin in the brain, even at this high level, had no effect. We conclude that in brain tissue, monoacetin is not an antidote to fluorocitrate.

To determine whether monoacetin administered intraperitoneally could alter the effects of intracranial fluorocitrate involved finding the permissible dose of monoacetin for our rats. Experiments 6 and 7 (Table 1) confirm the facts quoted by Chenoweth *et al.*[1] and of Li, Sah and Anderson[5] (1941) for doses of 5–6 ml/kg in rat. Our experiments underline the general point that dosage with monoacetin must be kept below these amounts to avoid fatal results. We have used 0·2–0·3 ml injected intraperitoneally. In Exp. 8, it was found that monoacetin given immediately cannot alter the effect of intracerebral fluorocitrate. The effects on rat brain differed therefore from the cardiac effects reported for monkeys[1] by Chenoweth *et al.* (see below). They endorse the failure of monoacetin to act as antidote to fluorocitrate.

The effect of monoacetin on rats dosed with fluoroacetate (Exp. 9) shows that an intraperitoneal dose of 10 mg/kg fluoroacetate killed rats in an average time of 65 min. In another experiment with 5 mg/kg all rats died, but $\frac{1}{5}$ survived for over 7 hr. Using a dose of 10 mg/kg, monoacetin (0.3 ml) was given by the intraperitoneal route 15 and 20 min after the fluoroacetate. Experiments 10 and 11 show that even when given as late as 15 or 20 min after the dose of fluoroacetate, monoacetin alters the symptomatology and the time to death is much prolonged. The rats become flaccid and show little tendency to convulse. This does not appear to be due to a delay in absorption, because the injection i.p. of a neutral oil (Exp. 12), had no effect on the fluoroacetate toxicity.

The intracranial experiments with fluorocitrate make it possible to interpret some of these effects. Since it was proved earlier that fluorocitrate is not synthesized in the rat brain, the convulsions must be caused by fluorocitrate entering the brain, which has been synthesized elsewhere in the body. We have shown here that monoacetin had no action on fluorocitrate in the brain and did not act as antidote when given i.p. to fluorocitrate injected by the intracerebral route. Hence, it is concluded that the absence of convulsions with monoacetin indicates that no fluorocitrate synthesized from

TABLE 1

Experiment	No. of animals	Weight average (g)	Treatment	No. of animals convulsive	Time of onset of convulsion (min)	No. animal dead	Time of death (min)
1	3	135	Monoacetin 0·0005 ml/ i.c. (in Arabic gum)	none	—	none	—
2	4	145	Monoacetin 0·002 ml i.c. (in H_2O)	none	—	none	—
3	3	150	Monoacetin 0·002 ml/i.c. (in Arabic gum)	none	—	none	—
4	4	150	Monoacetin 0·004 ml/i.c. (in Arabic gum)	none	—	none	—
5	4	150	F. cit. 15 μg/i.c. + monoacetin 0·004 ml/i.c.	3/4	47–55–60	4/4	55–72–100–280
6	3	150	Monoacetin 0·5 ml/i.p. (4·0 g/kg)	1/3	20	1/3	270
7	3	125	Monoacetin 0·6 ml/i.p. (6·0 g/kg)	2/3	12–14	2/3	21
8	4	150	F. cit. 15 μg/i.c. + monoacetin 0·3 ml/i.p.	4/4	35–40	4/4	57–106–151–77
9	6	150	F. acetate 10 mg/kg/i.p.	6/6	30–40	6/6	66–57–101–55–53–53
10	6	155	F. Acetate 10 mg/kg/i.p. + after 15 min Monoacetin 0·3 ml/i.p.	1/6	51	6/6	between 4–22 hr
11	6	170	F. Acetate 10 mg/kg/i.p. +, after 20 min, Monoacetin 0·3 ml/i.p.	0/6	—	6/6	between 5–22 hr
12	6	150	F. Acetate 10 mg/kg/i.p. +, after 20 min, Vaseline oil as control	6/6	40–65	6/6	58–65–70–73–42–213

i.c. = Intracerebral injection. i.p. = Intraperitoneal injection.

intraperitoneal fluoroacetate reaches the brain. There could be two reasons for this; either monoacetin interferes as such with the penetration of fluorocitrate through the blood-brain barrier, or much more probably less fluorocitrate is synthesized in the body in presence of monoacetin and this also explains the prolongation of the time to death. In presence of monoacetin, even after an interval of 20 min less fluorocitrate is formed from the fluoroacetate. Since it was shown *in vitro*[2] that 0·4 mM acetate interfered with the synthesis of fluorocitrate from 6·7 mM fluoroacetate in kidney mitochondria, the idea is reasonable[1] that monoacetin acts by a liberation of intracellular acetate.

The convulsions did not occur after giving monoacetin; so the question arises, "what causes the muscular weakness and the final death?" It is known that fluoroacetate (and fluorocitrate) induce large changes in the kidneys, e.g. citrate accumulation and in the liver. We did not find any striking difference in the behaviour of hepatectomized rats. Buffa, Azzone, Carafoli and Muscatello[6] have

detailed some of the changes in muscle and liver, for instance the gradual diminution in the concentration of energy-rich phosphate compounds due to fluoroacetate poisoning. Though pathological and biochemical effects on these organs are very important, it is unlikely that these kill the animals at this stage. On the other hand, there are visible effects on the peripheral blood vessels in the ears and feet; the heart rate is also much slowed. Peripheral vasoconstriction could be due either to a direct toxic effect on the vessels or to a reflex closure of the bed of circulation to sustain a failing heart. In 3 groups of rats, 14 animals in all dosed with 10 mg/kg Na fluoroacetate and monoacetin at +20′, we have tried a slow perfusion of noradrenaline; in one group phenoxybenzamine was also injected. Though the results were not decisive, in no case was the condition of the animals improved; the rats appeared to die more quickly. From this we incline to the view that the peripheral vasoconstriction is the reflex attempt to counteract the failing heart though we cannot exclude a direct action on the vessels. Since convulsions can be averted, we feel that the most urgent problem now is to find a compound. which can penetrate the heart mitochondria and reverse the fluorocitrate block in these.

DISCUSSION

Some years ago in experiments (unpublished) with mitochondria from guinea pig kidney, one of us (R.A.P.) observed no action of monoacetin upon the inhibition of aconitase by fluorocitrate. This is again consistent with the view that monoacetin has no antidote action on fluorocitrate once formed. In view of this we do not know the meaning of the remarkable effect seen by Chenoweth *et al.*[1] on the heart of a monkey poisoned with fluoroacetate. They reported that monoacetin (intramuscular) injected within 7 min reversed the commencing caridac irregularities due to 15 mg/kg fluoroacetate. This suggests a reversal of fluorocitrate, though it was only temporary as it regressed in 176 min to a ventricular fibrillation in 242 min. It is an interesting corollary of the finding that monoacetin prolongs life, even when given 20 min after poisoning, that the synthesis of all the fluoroacetate injected to fluorocitrate must take longer than 20 min after intraperitoneal injection.

In summary: (1) Monoacetin injected by the intracerebral route in rats is not toxic at a dose of 3·8 g/kg. (2) Monoacetin (3·8 g/kg) does not act as antidote to an $LD_{100} \times 3$ of fluorocitrate given by the intracerebral route. (3) Monoacetin given by the intraperitoneal route can be toxic at a level of 4·0–6·0 g/kg, confirming earlier work of others. (4) Monoacetin (0·3 ml) given intraperitoneally does not alter the toxicity of intracerebral fluorocitrate. (5) Monoacetin (0·3 ml) given intraperitoneally 15 or 20 min after an i.p. injection of Na fluoroacetate 10 mg/kg, reduces considerably the number of rats convulsing and prolongs life for many hours.

It is considered that (5) is due to a reduction of the amount of fluorocitrate synthesized, so that little if any fluorocitrate reaches the brain. Death (after administration of fluoroacetate and monoacetin) is considered to be due to the failing heart.

Acknowledgement—We are grateful to Prof. S. Garattini and Prof. L. Valzelli for facilities provided. We also thank Drs. Jori and Paglialunga for performing the perfusion experiments, Prof. Palma for preparing hepatectomized rats, and Dr. Marcucci for the chromatographic examination of monoacetin.

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