BIOMARKERS, 2001, VOL. 6, NO. 1, 72–76

Development of antidotes for sodium monofluoroacetate (1080)

CHRISTIAN J. COOK[1*], CHARLES T. EASON[2], MARK WICKSTROM[2†] and CHRIS D. DEVINE[1]

[1] Technology Development Group, HortResearch, Private Bag 3123, Hamilton, New Zealand.

[2] Landcare Research, PO Box 69, Lincoln, New Zealand

Baits containing sodium monofluoroacetate (1080) are commonly used in New Zealand during feral pest control operations. However, each year, a number of domestic dogs are unintentionally killed during these control operations, and a suitable antidote to 1080 intoxication is required. The primary toxic mechanism of 1080 is well known. However, as with other pathologies where energy deprivation is the main effect of intoxication, the cascade of effects that arises from this primary mechanism is complex. At present, putative antidotes for 1080 are generally unable to address the primary mechanism of intoxication but such agents may be able to control the cascade of secondary effects, which can result during intoxication. Part of the reason for this is that targeting the cascade can provide a longer window of time for antidote success. We have undertaken studies that identified some of the central nervous system (CNS) and systemic pathophysiological cascades caused by 1080 intoxication. Using this information we designed antidotes, on the basis of preventing different steps in this cascade. In the chicken model targeting systemic changes, in particular reducing effects of nitric oxide derivatives generated in cardiac muscle, proved successful in reducing fatality associated with 1080. In rats and sheep, targeting the CNS with a number of compounds including: glutamate; calcium and dopamine antagonists; gamma amino butyric acid agonists, and astressin-like compounds reduced fatalaties. However, to be successful in the rat and sheep model a given antidote needed to move quickly from systemic circulation across the blood brain barrier and into the CNS. The work also suggests ways in which specific biomarkers of 1080 exposure may be developed with respect to different species.

Keywords: 1080, antidotes, secondary poisoning.

Introduction

Baits containing sodium monofluoroacetate (1080) have proven one of the mainstays in vertebrate control operations in New Zealand (Eason *et al*. 1993, Livingstone 1994). However, as with most bait-associated toxins, 1080 can cause both primary and secondary poisoning of some non-target species (Anitra Schultz *et al*. 1982, Rammell *et al*. 1985), particularly domestic dogs.

The primary mode of action of 1080 in animals has been extensively examined (Savarie 1984, Eason *et al*. 1994). It is generally agreed that this involves conversion of fluoroacetate to fluorocitrate which competitively inhibits the actions of the tricarboxylic acid enzyme aconitate hydratase, resulting in citrate accumulation and energy deprivation. Fluorocitrate may also inhibit citrate transport into and out of mitochondria (Kirsten *et al*. 1978). Developing an antidote to prevent this primary mechanism is difficult, as effective antagonism of the 1080 actions is in itself likely to

* Corresponding author: Christian J. Cook, Technology Development Group, HortResearch, Private Bag 3123, Hamilton, New Zealand. e-mail: ccook@hort.cri.nz

† Present address: Toxicology Centre, University of Saskatchewan, Saskatoon SK S7N5B3, Canada.

Biomarkers ISSN 1354-750X print/ISSN 1366-5804 online © 2001 Taylor & Francis Ltd
http://www.tandf.co.uk/journals

severely compromise the homeostasis of the animals. Furthermore the time window available for this treatment is limited. A number of potential antidote candidates have been tried with mixed success (Rammell *et al*. 1985, Omara and Sisodia 1990). None as yet reported provide an adequate treatment for most poisoning cases.

The energy deprivation resulting from intoxication by compounds such as 1080 ultimately produces a sequence of increasing detrimental effects, and a cascade of physiological changes which may contribute to the death of brain and systemic organ cells (Sapolsky *et al*. 1986, Olney 1990). Amelioration of these secondary changes in conjunction with supportive therapies may increase survival of intoxicated animals. We have shown previously (Cook *et al*. submitted) in rats that 1080 intoxication produces an increase in excitatory amino acid and dopamine neurotransmission in the brain. This can produce an overexcitation of the brain manifested in convulsions (Sapolsky *et al*. 1990). Combined with increased calcium influx neurones swell and die, a necrotic processes. A number of hours after this apoptosis of other cells begins. This is common to many energy deprivation situations and can result in death (Akira *et al*. 1994, Bonfoco *et al*. 1995). However, there appears to be differences between species in organ vulnerability to 1080 intoxication (Chenoweth 1949). Most species show some CNS dysfunction with 1080, however impact on cardiac function, independent of CNS depression, seems to vary (Chenoweth 1949). We have shown that chickens are more vulnerable to cardiac damage (Cook *et al*. submitted) and this seems to involve the generation of excess nitric oxide systemically.

This present study had the aim of designing putative antidotes based upon the above information.

Methods

Thirty male and 30 female rats (*Rattus norvegicus*, liveweights 300–500g) were housed individually in cages and maintained in a light:dark cycle of 12 h:12 h at a temperature of 20°C. Animals were given daily fresh feed consisting of 60 g standard cereal pellets (Diet 86, Sharpes Grain and Seeds Ltd, Lower Hutt, New Zealand) and 250 ml of water. Sixteen laying hens (*Gallus gallus*, liveweight 2.2–2.8 kg) were also housed in individual cages and fed a standard grain mix of 500 g daily and 500 ml of water. Twelve sheep (*Ovis aries*, ewes, 35–45kg) were run together as a flock and had *ad libitum* paddock and water access.

The rats and chickens were divided into four equal groups, the sheep into two. All were administered an oral dose of 1080 at 8.0 mg kg^{-1} and also received an intra-peritoneal injection of diazepam at 3 mg kg^{-1}. Diazepam was administered to reduce convulsions, part of the ethical requirements of this study. Two of the rat and chicken, and one of the sheep, groups also received oral saline (1 ml for rats and chickens, 10 ml for sheep) at the time of 1080 administration and 6h following the administration (Control groups). The remaining two groups (rats and chickens) received 1 ml of one of two putative antidotes immediately following 1080 administration and 6h following the administration (antidote groups 1 and 2). The remaining sheep group received only antidote 2 at 10 ml. Both antidotes contained a mix of the following agents in equimolar ratios:

I.	dextromethorphan	—	glutamate antagonist
II.	MK-801	—	glutamate antagonist
III.	Memantine	—	glutamate antagonist
IV.	N^g - nitro -L-arginine	—	Nitric oxide synthase antagonist
V.	Allopregnanlone	—	GABA modulator
VI.	Haloperidol	—	dopaminergic antagonist
VII.	17-β-oestradiol	—	oestrogen analog and anti-oxidant
VIII.	GR89696	—	Kappa opioid agonist
IX.	Salicylate	—	anti-inflammatory agent and activator of NF-χB
X.	flunarazine	—	calcium channel antagonist
XI.	astressin	—	an anti-corticotrophin releasing hormone agent

Both antidotes were made up in 0.9% saline containing 45% w/w 2-hydroxy propyl-β-cyclodextrin, a

non-toxic solubilizer. The antidotes were administered at 0.5 mg kg^{-1}. All drugs were obtained from Research Biochemicals Inc. (Natick, MA 01760-2447, USA).

The second antidote (antidote 2) also contained a pyrrolopyrimidine agent (U–101033E). Pyrrolopyrimidines are known lipid membrane transfer facilitators for muscle and possibly the blood–brain barrier.

The potential antidotes were designed to prevent some of the cascading consequences of 1080 intoxication, secondary to its primary effect. A multi-targeted approach was taken to target various changes within this cascade. A number of glutamate antagonists (compete with endogenous glutamate for receptor sites and in doing so reduce the effect of endogenous glutamate) aimed at preventing the excitatory effects of excess glutamate were included in the mix. As there are at least three different sites on which glutamate can act we chose three different types of antagonist to target these sites. A GABA modulator was used to increase endogenous GABA, as this may be protective, and a nitric oxide synthase inhibitor (to reduce synthesis of nitric oxide). We also chose a calcium channel inhibitor, flunarazine, which supposedly has less systemic side effects than other calcium inhibitors (Fadda 1989). Haloperidol was chosen to reduce the effects of excess dopamine release in response to the 1080 intoxication, and 17-β-oestradiol was chosen because of its reputed powerful anti-oxidant effects (Behl *et al*. 1995). The Kappa opioid agonist (GR89696) and salicylate were chosen because they appear to have anti-inflammatory properties in energy deprivation insult situations (Grilli *et al*. 1996). Earlier work by us (data not shown) showed that high doses of calcium channel and dopamine antagonists compromised recovery, however, complete removal of these agents reduced antidote success. Therefore a low dose of each was chosen. The addition of astressin was in an attempt to reduce secondary apoptosis.

Survival at 12, 24, 36 and 72 h was measured for each group. In addition animals that survived at 72 h were again examined at 14 and 28 days subsequent to 1080 administration. In each group data were analysed by the use of a two way ANOVA with an independent factor of antidote (n = 3, antidote 1; antidote 2; no antidote). Species were also independently evaluated. In rats separate analysis of males and females showed no significant differences so the data were pooled.

Results and discussion

Administration of antidote 1 significantly (p<0.01, ANOVA) reduced fatalaties, from 1080 intoxication in both chicken and rats compared to no antidote (Control) for each species (table 1). Antidote 2, which contained the pyrrolopyrimidine, had a significant effect, on reducing fatalities, in chickens, rats and sheep (p<0.01, ANOVA) compared with no antidote same species comparison groups (tables 1 and 2). In rats antidote 2 was more successful (p<0.01, ANOVA) than Antidote 1 at preventing fatalities. In chickens there was no significant difference between antidote 1 and 2. All animals that survived 72 h post-1080 administration were still alive at 14 and 28 days post-administration.

Table 1. Reduction of fatalities in rats and chicken with administration of antidote 1.

1080 administered at 8.0 mg kg^{-1} orally	Control, chicken (n = 4)	Control, rat (n = 15)	Antidote 1, chicken (n = 4)	Antidote 1, rat (n = 15)
% animals surviving after 72 h	25	13	75	40

Table 2. Reduction of fatalities in rats, sheep and chicken with administration of antidote 2.

1080 administered at 8.0 mg kg^{-1} orally	Control, chicken (n = 4)	Control, rat (n = 15)	Control, sheep (n = 6)	Antidote 2, chicken (n = 4)	Antidote 2, rat (n = 15)	Antidote 2, sheep (n = 6)
% animals surviving after 72 h	25	20	33	75	87	100

The pattern of CNS-related changes following 1080 intoxication, in rats, mirrors that seen in many other forms of energy deprivation insult and cyanide poisoning (Kanthasamy *et al*. 1994). Such changes include an excess of excitatory neurotransmitters, and resultant over-excitation and exacerbated energy compromisation, an excess of reactive free radicals, and changes in calcium ion regulation. Loss of membrane integrity, oedema and inflammation follow as do peptidase-induced cell breakdown (see Choi 1998; Schwartz and Milligan 1996 for a review of these processes).

Diazepam was administered across all animal groups; both control and antidote treated, to reduce the incidence of convulsions, an ethical design requirement of the experiments. On its own diazepam did not provide an antidote effect, however it could easily have been synergistic to the effect of the other compounds. This speculation was neither proved nor disproved in this set of studies. The antidotes were designed on the basis of compounds that could potentially alleviate some of the energy deprivation-related changes described above. As we wished to target numerous points in the cascade of events, we chose a multi-drug approach. GABA modulators, calcium inhibitors and 17-β-oestradiol have previously (Cook, unpublished data) been tried on their own with a small degree of success as antidotes. A similar overall mix has also been tried without the calcium inhibitors and produced results less effective in antidote success (unpublished data) than those described here. The contribution of any other individual compounds in the mix has not yet been elucidated. It is conceivable that one of these compounds could be responsible for the antidote without the need for the other compounds. A more economical antidote may well result from further studies on the individual compounds.

In the chicken the antidotes were equally successful irrespective of the presence of pyrrolopyrimidines (antidote 1 versus antidote 2). Previous work by us (Cook *et al*. submitted) has suggested that systemic vulnerability to 1080 intoxication is greater than CNS vulnerability in chickens and thus antidote 1 may not have needed to penetrate the brain tissue quickly to be effective. In contrast rats, and sheep, appear to show greater CNS vulnerability to 1080 and the addition of pyrrolopyrimidines may have allowed a faster transfer of the antidote to brain tissue, explaining the greater effectiveness in rats of antidote 2. Measurement studies made in brain tissue of the drugs at different times following their administration suggest, in rats, that the presence of pyrrolopyrimidines does speed up transfer (data not shown).

While the main thrust of this study was to design successful antidotes for 1080, that design also allows a pharmacological approach to bio-marking 1080 exposure. The data suggest that the choice of biomarkers for 1080 exposure could differ depending on the species used in measurement. CNS markers are likely to be of more potential in rats than chickens, cardiac tissue markers showing a reverse pattern of usefulness. By understanding the pathophysiological processes that underlie the intoxication in different species it may be possible to design very specific biomarkers.

While we have provided convincing evidence of an 'antidote-like effect' in rats, chickens and sheep extrapolation to other species must be undertaken with caution, particularly given the observations herein of species differences in antidote response. However, the response to 1080 intoxication of the rat, and both dogs and humans is similar suggesting that our antidotes may also be applicable for use to dogs and humans.

Acknowledgements

We acknowledge funding from the Foundation for Research, Science and Technology, New Zealand and from HortResearch (Cook and Devine).

References

Akira, T., Henry, D., Baldwin, R. A. and Wasterlain, C. G. 1994, Nitric oxide participates in excitotoxin mechanisms induced by chemical hypoxia. *Brain Research*, **645**, 285–290.

Anitra-Schultz, R., Coetzer, J. A., Kellerman, T. S. and Naude, T. W. 1982, Observations on the clinical, cardiac and histopathological effects of fluoroacetate in sheep. *Onderstepoot Journal of Veterinary Research,* **49**, 237–245.

Behl, C., Widmann, M., Trapp, T. and Holsboer, F. 1995, 17-β-estradiol protects neurons from oxidative stress induced cell death in vitro. *Biochemical and Biophysical Research Communications*, **216**(2), 473–482.

Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P. and Lipton, S. A. 1995, Apoptosis and necrosis: two district events induced, respectively, by mild and intense insults with NMDA or nitric oxide/superoxide in cortical cell cultures. *Proceedings of the National Academy of Sciences, USA*, **92**, 7162–7166.

Chenoweth, M. B. 1949, Monofluoroacetic acid and related compounds. *Pharmacological Reviews*, **1**, 383–424.

Choi, D. W. 1998, Glutamate neurotoxicity and diseases of the nervous system. *Neuron,* **1**(8), 623–634.

Cook, C. J., Eason, C. T. and Devine, C. D. Sodium monofluoroacetate (1080) produces different patterns of intoxication in rats and chickens. *Research in Veterinary Science* (submitted).

Eason, C. T., Frampton, C. M., Henderson, R., Thomas, M. D. and Morgan, D. R. 1993, Sodium monofluoroacetate and alternative toxins for possum control. *New Zealand Journal of Zoology,* **20**, 329–334.

Eason, C. T., Gooneratne, R. and Rammell, C. G. 1994, A review of the toxicokinetics and toxicodynamics of sodium monofluoracetate in animals. In *Proceedings of the Science Workshop on 1080*, A. A. Seawright and C. T. Eason, eds, The Royal Society of New Zealand, Misc. Series No. 28, pp. 82–89. New Zealand.

Fadda, E. 1989, Differential effects of the calcium antagonists nimodipine and flunarazine on dopamine metabolisms in the rat brain. *Journal of Neural Transmission,* **75**, 195–204.

Grilli, M., Pizzi, M., Memo, M. and Spano, P. 1996, Neuroprotection by aspirin and sodium salicylate through blockade of NF-Kβ activation. *Science,* **274**, 1383–1385.

Kanthasamy, A. G., Borowitz, J. L., Pavlakovic, G. and Isom, G. E. 1994, Dopaminergic neurotoxicity of cyanide: neurochemical, histological and behavioural characterisation. *Toxicology and Applied Pharmacology*, **126**, 156–163.

Kirsten, E., Sharma, M. L. and Kun, E. 1978, Molecular toxicity of (–) erythro-fluorocitrate: selective inhibition of citrate transport in mitochondria and the binding of fluorocitrate to mitochondrial proteins. *Molecular Pharmacology*, **14**, 172–184.

Livingstone, P. G. 1994, The use of 1080 in New Zealand. In *Proceedings of the Science Workshop on 1080*, A. A. Seawright and C. T. Eason, eds, The Royal Society of New Zealand, Misc. Series No. 28, pp. 1–9. New Zealand.

Olney, J. 1990, Excitotoxin mediated neuron death in young and old age. *Progress in Brain Research,* **86**, 37–49.

Omara, F. and Sisodia, C. S. 1990, Evaluation of potential antidotes for sodium monofluoroacetate in mice. *Veterinary and Human Toxicology,* **32**, 427–431.

Rammell, C. A., Hoogenboom, J. J. and Julian, A. F. 1985, Treatment of 1080 poisoning in dogs with glycerol monoacetate. *New Zealand Veterinary Journal,* **33**, 149–150.

Sapolsky, R., Krey, L. and McEwen, B. 1986, The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocrine Reviews,* **7**, 284–301.

Savarie, P. J. 1984, Toxic characterisation of fluorocitrate, the toxic metabolite of compound 1080. *Proceedings of the 11th Vertebrate Pest Conference*, pp. 132–137.

Schwartz, L. M. and Milligan, C. E. 1996, Cold thoughts of death: the role of ICE proteases in neuronal cell death. *Trends in Neuroscience,* **19**(12), 555–562.