ASIA PACIFIC JOURNAL of MEDICAL TOXICOLOGY
APJMT 4;1 http://apjmt.mums.ac.ir March 2015

REVIEW ARTICLE

Translational Antidote Research: A Bedside to Bench Tale

JEFFREY BRENT*

School of Medicine and Colorado School of Public Health, University of Colorado, Aurora, USA

Abstract

Although antidote development should proceed in an orderly fashion from observation, to experimental and safety studies, to clinical trials, this sequence is not always precisely followed. The development of fomepizole as an antidote for toxic alcohol and glycol poisoning is an example of how this may not be the case. Interest in the development of fomepizole was spurred in the 1960s. Shortly thereafter studies characterized by administration to humans commenced. The potential value of fomepizole as an antidote for methanol poisoning was highlighted by primate experiments. Simultaneously, the utility of fomepizole was shown in an experimental model of ethylene glycol poisoning. Further studies on humans showed effectiveness of fomepizole in the treatment of disulfiram-alcohol reactions and ethylene glycol poisoning. In addition, in primate experiments, the safety of fomepizole was established as the subjects tolerated serum fomepizole concentrations over 150 times higher than therapeutic target levels. Subsequent studies have validated the efficacy of fomepizole in the treatment of ethylene glycol and methanol poisonings. Fomepizole has been found to be associated with fewer complications than the alternative alcohol dehydrogenase inhibitor, ethanol. In serious cases of methanol toxicity, fomepizole has been shown to improve survival compared to that obtained with ethanol.

Keywords: Antidote; Fomepizole; Methanol; Poisoning; Translational Medical Research

How to cite this article: Brent J. Translational Antidote Research: A Bedside to Bench Tale. Asia Pac J Med Toxicol 2015;4:9-12.

INTRODUCTION

The development of new antidotes may proceed in the counterintuitive fashion. A fundamental premise, although sometimes more theoretical than real, is that by virtue of an observation, or a series of observations, a hypothesis about the potential utility of an antidote is generated, which then leads to hypothesis testing studies. If the foundational concepts underlying the hypothesis are validated, preclinical safety studies can be undertaken, initially on animals, and subsequently, in the absence of major safety concerns, on humans (1). Ultimately, if it felt to be promising, clinical efficacy studies can be done to validate the utility of the antidote in a clinical setting (Figure 1). If we look at the development of fomepizole (4-methyl pyrazole, 4-MP); however, an interesting tale emerges demonstrating that this theoretical paradigm does not always apply.

The Early Development of Fomepizole

Much of the early work on the development of fomepizole can be traced back to studies at the Karolinska Institute in Stockholm. There, Hugo Theorell, a pioneering enzymologist who won the Nobel Prize for his description of enzymatic oxidation reactions, brought attention to the effect of 4-MP on reactions catalyzed by the alcohol dehydrogenase (ADH). Theorell, building on the early studies of von Wratburg, reported that 4-MP was an effective inhibitor of ADH at submicromolar concentrations *in vitro* (2). Theorell et al reviewed the effects of a number of heterocyclic compounds on the enzymatic activity of hepatic ADH and noted that of 31 compounds tested, 4-MP had the lowest inhibitory constant, reported to be 0.08 micromolars (2). This represents approximately 8,000 times greater binding affinity of 4-MP for human ADH than for ethanol. At about the same time, studies in rats by David Lester, at Rutgers University, demonstrated that 4-MP, and other 4-substituted pyrazoles, inhibited ethanol oxidation *in vivo* (3).

The year following Theorell's publication (2), the first published administration of 4-MP to humans was reported by Blomstrand and Theorell (4), who demonstrated that doses up to 10 mg/kg administered intravenously to 7 human volunteers, two of whom were alcoholic, had a dose-dependent inhibitory effect on ethanol oxidation. At the 10 mg/kg dose there was an approximately 50% inhibition of the rate of ethanol metabolism. In 1973, Blomstrand and Kager showed that in eight volunteers an intravenous dose of 180 mg (approximately 2.6 mg/kg) of 4-MP prevented the inhibition of fatty acid oxidation by ethanol (5). Meanwhile, Bjorkhem et al, from the Karolinska group, published their work on the development of a mass spectroscopy technique for the analytical determination of 4-MP in serum and demonstrated that this can be done following the intravenous administration of 4-MP to one subject and subsequent collection of serum (6).

It is important to note that during this era of early human experimentation, there were no published safety studies

*Correspondence to: Jeffrey Brent; MD, PhD. Department of Internal Medicine (Clinical Pharmacology and Toxicology), School of Medicine, University of Colorado, Denver, CO, USA.
Tel: +1 303 765 3800, E-mail: jeffrey.brent@ucdenver.edu
Received 16 December 2014; Accepted 28 February 2015

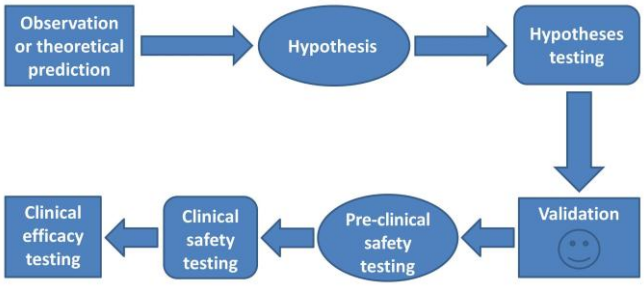

Figure 1. The usual and expected sequence of events in the early life history of an antidote under development

providing reassurance that whether or not major adverse effects would be unlikely to occur with 4-MP administration. However, published papers during this time period from Karolinska made reference to unpublished animal studies on rats and dogs reportedly showing an absence of significant toxicity (4). It was not until 1974; however, that the first published toxicity study, described as a long-term study but actually involved 12-week oral administration in rats and therefore best characterized as a sub-chronic study, reported on the safety of 4-MP as evidenced by data on complete blood counts, serum chemistries, and histopathology (7).

Because methanol, similar to ethylene glycol, is toxic due to formation of acidic metabolites in a pathway that begins with the enzyme alcohol dehydrogenase, the potential of 4-MP as an antidote for methanol poisoning was realized by McMartin, first studying under Tephly at the University of Iowa, subsequently a postdoctoral fellow at Karolinska. The McMartin studies form the basis for our understanding of the use of 4-MP as an antidote for the clinical management of methanol toxicity. These studies were done on monkeys and validated that model for studying on human methanol poisoning. McMartin and colleagues demonstrated that the acidosis and toxicity following methanol administration were the result of it being metabolized to formic acid, and that a dose of 15 mg/kg 4-MP or greater, can inhibit methanol metabolism and thereby prevent both the development of metabolic acidosis and toxicity (8,9). These doses were associated with plasma 4-MP concentrations of $\geq$ 9 millimolar (mM), a concentration used as the target in subsequent fomepizole validation studies. While the studies of McMartin were underway, Clay and Murphy in the United States were doing similar studies showing that 4-MP inhibits the metabolism and toxicity of ethylene glycol (10).

The Introduction of Fomepizole into Clinical Practice

Although there was extremely limited human experience with the administration of 4-MP, the first published clinical use of this antidote was by Lindros et al from Finland in 1981 (11). They took note of the potential severe manifestations of disulfiram-alcohol interactions, which had been reported to cause respiratory depression, cardiac dysrhythmias, myocardial infarction, acute congestive heart failure, alterations of consciousness and seizures. At the time of their publication, there had already been 20 disulfiram-related deaths reported, 13 of which involved excessive doses. They, therefore, undertook an evaluation of the utility of 4-MP in the treatment of these reactions. In the course of doing so, they reported its clinical use on a 36-year-old man who presented to the University Hospital in Helsinki with flushing, tachycardia, nausea, emesis and chest pain. He was a chronic alcoholic with a history of relapsing pancreatitis, and despite his young age, was diagnosed with ischemic cardiovascular disease requiring nitroglycerine. A day prior to admission, his wife had secretly given him disulfiram. The following morning he drank two bottles of red wine causing him to present with flushing, tachycardia and chest pain. While being monitored, blood samples were drawn for ethanol and acetaldehyde determinations at three-minute intervals, a dose of 7 mg/kg of 4-MP was given intravenously, and these parameters were followed for the subsequent three hours. During that time period, the intensity of his flushing markedly reduced and ST-depressions on his electrocardiogram resolved. Within 30 minutes of receiving the 4-MP, his tachycardia normalized and, importantly, his blood acetaldehyde concentration, which was constant at 60-70 mM for the 9 minutes prior to the dose of 4-MP, dropped to less than 10 mM three minutes later.

Recognizing the potential for 4-MP in the treatment of these reactions, Lindros et al studied 4 human volunteers who were given 0.2 g/kg of ethanol followed by a dose of calcium carbimide, an aldehyde dehydrogenase inhibitor (11). Thirty minutes after the ethanol infusion, but prior to the calcium carbimide, an unspecified dose of 4-MP or saline was given intravenously and blood acetaldehyde and ethanol concentrations were sequentially determined using headspace chromatography. It should be noted that despite the small dose of ethanol given, corresponding to approximately 150 mL of red wine, subjects exhibited facial flushing and tachycardia associated with elevations of blood acetaldehyde concentrations up to the range of 70-80 mM. However, in those individuals given 4-MP, the concentration of acetaldehyde rapidly fell to 5-7 mM post-administration.

The first reported clinical use of 4-MP in the treatment of toxic alcohol or glycol poisoning was published in 1986 when Baud et al, from a toxicology unit in Paris, described their use of oral 4-MP in three individuals using a loading dose of 15 mg/kg, followed by 5 mg/kg twelve hours later and 10 mg/kg every 12 hours subsequently (12). This regimen caused a drop in plasma oxalate concentrations, reduction of urine oxalate excretion, preservation of renal function, and resolution of metabolic acidosis (12). However, it was not until 1988 that Jacobsen et al, working in McMartin's laboratory, published the first major human safety study, using ascending 4-MP doses (13). They showed that subjects given up to 100 mg/kg, and generating 4-MP plasma concentrations of nearly 1,500 mM, tolerated the drug without significant adverse effects. These investigators also characterized the human pharmacokinetics of fomepizole (14). Those studies led to the development of an investigational drug application to the U.S. Food and Drug Association (FDA) and subsequent funding by that agency for a prospective study on the use of 4-MP in the treatment of ethylene glycol and methanol poisoning (15,16).

Validation of the Efficacy of Fomepizole

The FDA funded study was a multi-center clinical trial by a group constituted as the Methyl-Pyrazole for Toxic Alcohols Study Group (the META study) which demonstrated that fomepizole treatment was associated with a rapid decline in plasma glycolate concentrations following ethylene glycol poisoning and that was accompanied by a normalization of arterial pH (15). Patients who had normal renal function at the time of administration of fomepizole maintained their renal function without abnormality. However, the uncertainty of the efficacy of this antidote at the beginning of the trial required the researchers to use a series of triggers (renal dysfunction, significant metabolic acidosis, serum ethylene glycol concentrations $\geq$ 50 mg/dl) for simultaneous hemodialysis. At approximately the same time as the publication of the META trial results on ethylene glycol toxicity, the Paris Group presented their retrospective experience and reported similar results (17). However, the French investigators did not utilize adjunctive hemodialysis, which we know today is an unnecessary treatment in most cases if renal function is preserved.

Subsequently, the published findings of META trial could conclusively demonstrate fomepizole efficacy in the treatment of methanol poisoning (16). With fomepizole administration, plasma formic acid concentrations rapidly normalized and patients tended to survive, unless they were moribund at the time of presentation. Meanwhile, the French Group reported their experience in the treatment of methanol poisoning with fomepizole revealing similar results (18). One challenge in the treatment of methanol poisoning that was noted in the META trial was that once its metabolism is inhibited by fomepizole, methanol has a half-life of approximately 54 hours (16). This is due the fact that unlike ethylene glycol, methanol does not have major alternate routes of clearance (18). Thus, while ethylene glycol toxicity can generally be treated with fomepizole in the absence of hemodialysis, except in cases of extreme metabolic derangements or renal failure, the same cannot be said about methanol poisoning.

Superiority of Fomepizole over Ethanol

There has only been one controlled clinical trial comparing ethanol treatment to fomepizole and that was in the veterinary literature, where a randomized clinical trial was done on dogs following ethylene glycol poisoning (20). That study showed that while both ethanol and fomepizole attenuated the metabolic acidosis and was efficacious in preventing nephrotoxicity, ethanol was associated with severely increased central nervous system depression. A similar retrospective clinical experience on humans reported by Lepik et al showed a markedly lower incidence of adverse reactions in patients treated with fomepizole than those treated with ethanol (21). Ethanol-treated patients were reported to develop coma, agitation (which could be severe), cardiovascular toxicity and potentially life-threatening respiratory depression and hypotension at a greater frequency than seen with fomepizole (21).

Although there are no prospective controlled clinical trial on humans comparing ethanol and fomepizole, a multi-center retrospective study on methanol poisoned patients has revealed that among those patients who were sick enough to develop compensatory hyperventilation from a metabolic acidosis, there was a significantly greater mortality in ethanol-treated individuals compared those receiving fomepizole (22).

The Future

Although fomepizole place in the treatment of ethylene glycol and methanol poisoning is well entrenched, we know less about its efficacy in its use for other glycols, such as diethylene glycol. Future research on fomepizole as an antidote should also revolve around assessing its utility as an oral agent, thus facilitating many cases of ethylene glycol or methanol poisoning, which heretofore required treatment in intensive care units, to be treated as outpatients.

CONCLUSION

Once the potential clinical utility of the chemical 4-MP was recognized, it was quickly evaluated in humans. Given its apparently good safety profile in limited animal studies, and the lack of adverse effects after administration to only a few humans, it was quickly utilized clinically, first in the treatment of an ethanol-disulfiram reaction, and then in a small series of patients with ethylene glycol poisoning. Hence the usual sequence of antidote research was diverged. It was not until 1999, 18 years after its first use clinically, that the first prospective study of the safety and efficacy of fomepizole was published. The experience to date indicates that fomepizole is a safer and more efficacious antidote in the treatment of ethylene glycol and methanol poisoning than is ethanol.

Conflict of interest: None to be declared.

REFERENCES

1. Food and Drug Administration, HHS. Requirements on content and format of labeling for human prescription drug and biological products. Final rule. Fed Regist 2006;71:3921-97.
2. Theorell H, Yonetani T. On the effects of some heterocyclic compounds on the enzymic activity of liver alcohol dehydrogenase. Acta Chemica Scandinavica 1969;23: 255-260.
3. Lester D, Keokosky WZ, Felzenberg F. Effect of pyrazoles and other compounds on alcohol metabolism. Q J Stud Alcohol 1968;29:449-54.
4. Blomstrand R, Theorell H. Inhibitory effect on ethanol oxidation in man after administration of 4-methylpyrazole. Life Sci 1970;9:631-40.
5. Blomstrand R, Kager L. The combustion of triolein-1-14C and its inhibiton by alcohol in man. Life Sci 1973;13:113-23.
6. Björkhem I, Blomstrand R, Lantto O, Svensson L. Determination of 4-methylpyrazol in serum with mass fragmentography. Biochem Med 1975;12: 205-12.
7. Kager L, Ericcson JLE. Long term toxicity study with alcohol and 4-methylpyrazole in rat. Acta Pathol Microbiol Scand A 1974;82:534-8.
8. McMartin KE, Makar AB, Martin G, Palese M, Tephly TR. Methanol poisoning I. The role of formic acid in the development of metabolic acidosis in the monkey and the reversal by 4-methylpyrazole. Biochem Med 1975;13: 319-33.
9. McMartin KE, Hedström KG, Tolf BR, Ostling-Wintzell H, Blomstrand R. Studies on the metabolic interactions between 4-methylpyrazole and methanol using the monkey as an animal model. Arch Biochem Biophys 1980;199:606-14.

10. Clay KL, Murphy RC. On the metabolic acidosis of ethylene glycol intoxication. Toxicol Appl Pharmacol 1977;39:39-49.
11. Lindros KO, Stowell A, Pikkarainen P, Salaspuro M. The disulfiram (Antabuse)-alcohol reaction in male alcoholics: Its efficient management by 4-methylpyrazole. Alcohol Clin Exp Res 1981;5:528-30.
12. Baud FJ, Bismuth C, Garnier R, Galliot M, Astier A, Maistre G, et al. 4-methylpyrazole may be an alternative to ethanol therapy for ethylene glycol intoxication in man. J Toxicol Clin Toxicol. 1986-1987;24:463-83.
13. Jacobsen D, Sebastian CS, Blomstrand R, McMartin KE. 4-methylpyrazole: A controlled study of safety in healthy human subjects after single, ascending doses. Alcohol Clin Exp Res 1988;12:516-22.
14. Jacobsen D, Barron SK, Sebastian CS, et al: Non-linear kinetics of 4-methylpyrazole in healthy human subjects. Eur J Clin Pharmacol 1989;37:599-604.
15. Brent J, McMartin K, Phillips S, Burkhart KK, Donovan JW, Wells M, et al. Fomepizole for the treatment of ethylene glycol poisoning. Methylpyrazole for Toxic Alcohols Study Group. N Engl J Med 1999;340:832-8.
16. Brent J, McMartin K, Phillips S, Aaron C, Kulig K; Methylpyrazole for Toxic Alcohols Study Group. Fomepizole for the treatment of methanol poisoning. N Engl J Med 2001;344:424-9.
17. Borron SW, Megarbane B, Baud FJ. Fomepizole in treatment of uncomplicated ethylene glycol poisoning. Lancet 1999;354:831.
18. Mégarbane B, Borron SW, Trout H, Hantson P, Jaeger A, Krencker E, et al. Treatment of acute methanol poisoning with fomepizole. Intensive Care Med. 2001;27:1370-8.
19. Brent J. Fomepizole for ethylene glycol and methanol poisoning. N Engl J Med 2009;360;2216-23.
20. Grauer GF, Thrall MA, Henre BA, Hjelle JJ. Comparison of the effects of ethanol and 4-methylpyrazole on the pharmacokinetics and toxicity of ethylene glycol in the dog. Toxicol Lett 1987;35:307-14.
21. Lepik KJ, Levy AR, Sobolev BG, Purssell RA, DeWitt CR, Erhardt GD, et al. Adverse drug events associated with the antidotes for methanol and ethylene glycol poisoning: a comparison of ethanol and fomepizole. Ann Emerg Med 2009;53:439-450.e10.
22. Paasma R, Hovda KE, Hassanian-Moghaddam H, Brahmi N, Afshari R, Sandvik L, et al. Risk factors related to poor outcome after methanol poisoning and the relation between outcome and antidotes--a multicenter study. Clin Toxicol (Phila) 2012;50:823-31.