THE PHARMACOLOGY OF MIDAZOLAM AND THIOPENTAL WITH REGARD TO THE LETHAL INJECTION PROTOCOL IN THE STATE OF MISSISSIPPI

Re: Mississippi Lethal Injection Case

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Sections

1. Background and Qualifications of the Author

Craig W. Stevens, Ph.D., is the author of this report. He performed the medical and pharmacological literature research, the pharmacological calculations used to determine the blood levels of thiopental and midazolam, and completed the writing of the entirety of this report. Dr. Stevens is a Professor of Pharmacology, a full-time faculty member in the department of Pharmacology and Physiology at the College of Osteopathic Medicine, a unit of the Oklahoma State University, Center for Health Sciences campus in Tulsa, Oklahoma.

After receiving his Ph.D. in Pharmacology from the Mayo Clinic, in Rochester, Minnesota, Dr. Stevens completed a 2 year postdoctoral fellowship at the University of Minnesota Medical School in Minneapolis, Minnesota, and secured a position as an Assistant Professor of Pharmacology with his present employer in 1990. He advanced through the academic ranks to Associate Professor of Pharmacology in 1993, and Professor of Pharmacology in 2000.

Besides his regular duties of teaching medical students, pursuing research and scholarly activities, and serving on college committees, Dr. Stevens works part-time as a litigation consultant/expert witness on cases involving pharmacological issues. He has consulted in both civil and criminal cases, working with both the prosecution or plaintiff and the defendant. With regard to the pharmacological issues of lethal injection, he has consulted with the State as well as with Federal Public Defenders representing condemned inmates.

Dr. Stevens was asked to investigate the pharmacological nature of midazolam regarding its use as a lethal injection drug and specifically (a) whether midazolam can be characterized as an "other similar drug" to an ultra short-acting barbiturate, such as thiopental (the original first drug used in the MS three drug lethal injection protocol), and (b) whether the use of midazolam as the first drug in Mississippi's three-drug lethal injection protocol creates a substantial risk of serious harm and severe pain to the condemned prisoner.

Dr. Stevens' *curriculum vitae* (CV) is attached as Appendix A to this report.

2. Midazolam and Thiopental are not Pharmacologically Equivalent

A. Pharmacological Equivalency and Pharmacological Substitution

Each drug has a unique chemical (atomic) structure and exerts a unique profile of pharmacological effects. Drugs are classified both by their chemical structures and by their therapeutic uses. Drugs that have very similar chemical structures are grouped together based on that structure. Drugs that have similar therapeutic uses are also grouped together by their therapeutic or pharmacological effects.

Pharmacological equivalency is present when two or more drugs exhibit the same or closely similar pharmacological properties. It is a working principle used by physicians who often substitute drugs due to drug allergies or for reasons of cost. Pharmacological equivalency is also the guiding principle for the FDA to accept a generic version of the same branded drug (e.g.

Walgreen's ibuprofen, the generic form, is *pharmacologically equivalent* to Advil®, the branded formulation of ibuprofen. See *Meredith 2003, Borgheini 2003*).

Pharmacological substitution is the act of using one drug in the place of another. It is axiomatic that in order to maintain the same pharmacological and therapeutic effect of two drugs, the drug that is substituted must have pharmacological equivalency to the new drug.

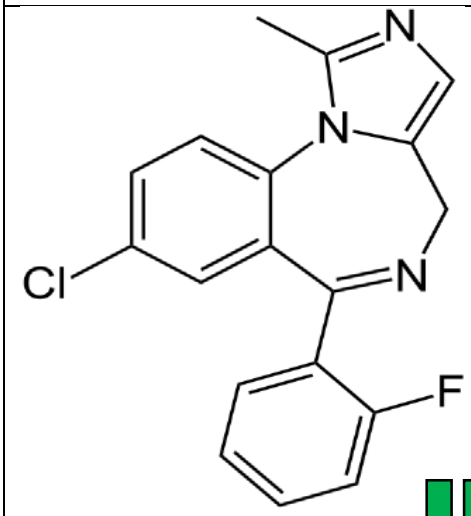
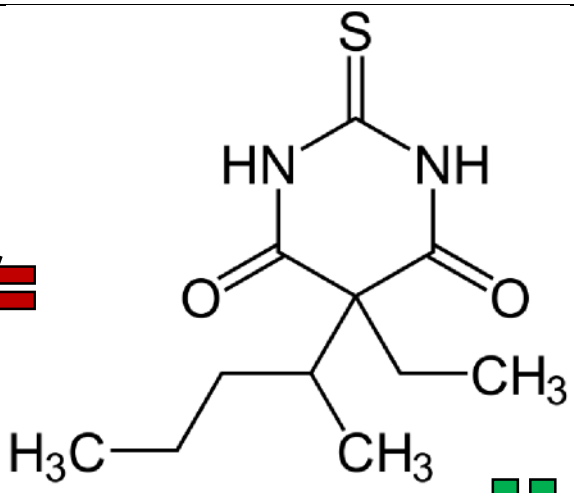
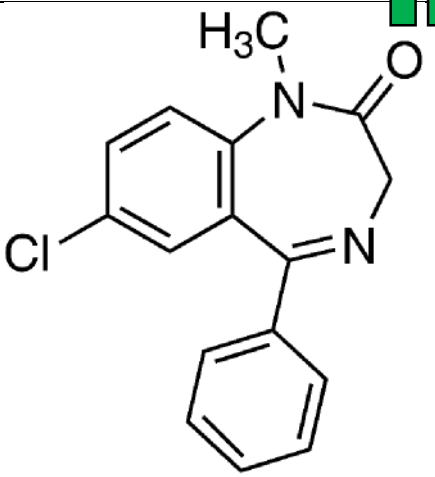
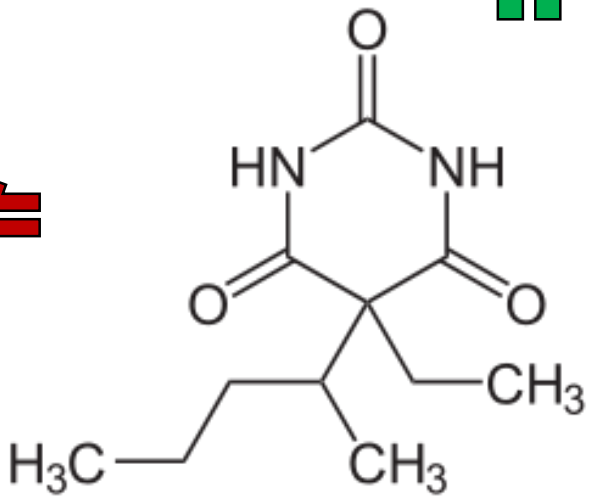
There is no question that midazolam and thiopental are different drugs. The key question in substituting drugs for lethal injection is one of a pharmacological nature: Does midazolam have *pharmacological equivalency* to thiopental such that a valid pharmacological substitution can be made? Pharmacological equivalency between midazolam, a benzodiazepine, and thiopental, a barbiturate, is examined herein with respect to **pharmacological classification by chemical (atomic) structure, mechanisms of action, partial and full effects of these agents and the 'ceiling effect', therapeutic uses, and DEA scheduling of these agents.**

B. Pharmacological Classification of Midazolam and Thiopental

Midazolam belongs to the class of drugs called benzodiazepines and thiopental is a member of the barbiturate class of drugs (*Brenner and Stevens, 2013*). The chemical structure of midazolam and thiopental are shown in the first row of Table 1 below (next page) to provide an accessible first exposure to the differences between the two drugs. The untrained eye clearly recognizes that midazolam and thiopental do not have similar structures and are not close analogs. The second row in Table 1 (previous page) shows examples of other drugs from the same class of drugs as midazolam and thiopental. Most notably, at the center of the benzodiazepines there is 7-sided ring with two nitrogen atoms (N) attached to a 6-sided ring with one chloride atom (Cl). Quite differently, the two barbiturates do not contain such a core structure and instead consist of a single 6-sided ring containing two nitrogen atoms. The non-expert can see that the chemical structure of the benzodiazepine, midazolam is similar to diazepam (Valium®), and the chemical structure of the barbiturate, thiopental, is similar to pentobarbital (Nembutal®). There is an irrefutable difference between midazolam and thiopental at the atomic level.

In summary, Table 1 (next page) shows that **pharmacological equivalency by consideration of chemical structures is NOT met when employing midazolam as a substitute for thiopental**.

Table 1. Visual comparison of benzodiazepine and barbiturate chemical structures.

BENZODIAZEPINES	BARBITURATES
	
Midazolam (Versed®)	Thiopental (Pentothal®)
	
Diazepam (Valium®)	Pentobarbital (Nembutal®)

C. Mechanism of Action of Midazolam and Thiopental

The description of the pharmacology of drugs range from effects on the whole organism, to effects on specific tissues or organs, down to the actual mechanism of action at the molecular level. For many drugs, the action at the molecular level can be traced upward to the effect on the whole organism, yielding a nearly complete description of drug action.

Starting at the molecular level, both midazolam and thiopental act on the $GABA_A$ receptor-chloride ion channel complex (henceforth $GABA_A$ receptor). GABA is the acronym for gamma-aminobutyric acid, an inhibitory neurotransmitter in the brain that is the natural activator of $GABA_A$ receptors (*Sigel and Steinmann 2012, Sieghart 2015*). When inhibitory neurons of the brain release GABA onto other brain neurons, the recipient neurons are inhibited and become more quiescent. This is an ongoing neurotransmitter action, occurring without the presence of any drugs or exogenous substances in the brain. The $GABA_A$ receptor is shaped like a funnel

with a lid on it. When GABA binds to the receptor, the lid opens and chloride ions rush from the outside of the neuron to the inside. The chloride ions rushing inside the neuron causes the neuron to decrease its electrical activity.

Benzodiazepines act at the GABAA receptor on brain neurons where GABA itself acts (*Chang et al. 1981, Sigel and Barnard 1984*). Midazolam and all benzodiazepines do not increase the synthesis of the inhibitory neurotransmitter GABA but enhance the effect of GABA at the $GABA_A$ receptor (*Greenblatt et al. 1983*). Benzodiazepines bind to the $GABA_A$ receptor at a different site than GABA binds (*Cromer et al. 2002, Ernst et al. 2003*). GABA must be released by inhibitory neurons and be acting on the $GABA_A$ receptor at the same time as the benzodiazepine for drugs like midazolam to enhance GABA inhibition (*D'Hu!st et al. 2009, Sieghart et al. 2012*). GABA acts on the receptor and opens the lid to the chloride ion channel (funnel) and midazolam increases the frequency that the lid opens (*Study and Barker 1981, Rogers et al. 1994*). In that way, midazolam helps GABA have a greater inhibitory effect, however without GABA present, midazolam does not activate the inhibitory $GABA_A$ receptor.

Barbiturates such as thiopental also act at the $GABA_A$ receptor on brain neurons where GABA itself acts (*Olsen and Snowman 1982, Greenfield LJ 2013*). Barbiturates bind to a different spot on the $GABA_A$ receptors than benzodiazepines (*Cestari et al. 1996*). Unlike midazolam, thiopental and other barbiturates enhance GABA inhibition by increasing the time that the ion channel lid remains in the open position (*Study and Barker 1981*). Contrary to the mechanism of action of midazolam, thiopental, like all barbiturates, can cause neuronal inhibition even when GABA is not present (*Mathers and Barker 1980, Jackson et al. 1982*). Barbiturates therefore can open the lid on the ion channel by themselves and keep it open longer than benzodiazepines (*MacDonald et al. 1989, Sancar and Czajkowski 2011*). As a result, the flow of chloride ions into the neuron is not limited to enhancement only when GABA is present, but barbiturates can increase the rush of chloride ions into the neuron in the absence of GABA so that the activity of the neuron is completely shut down. Thus, barbiturates are more potent drugs at the $GABA_A$ receptor than benzodiazepines.

In summary, a large body of pharmacological research on the mechanisms of action of midazolam and thiopental **clearly demonstrates that benzodiazepines, like midazolam, and barbiturates, such as thiopental, do NOT exhibit pharmacological equivalency with regard to their detailed mechanism of action**. Compared to barbiturates, benzodiazepines bind to a different site on the $GABA_A$ receptor, need GABA to co-activate the $GABA_A$ receptor to work, and increase the frequency of the opening of the chloride ion channel not the time it remains open.

D. The Pharmacology of the Partial Agonist, Midazolam, and the Full Agonist, Thiopental

Most drugs that are used clinically do something to cells or neurons that they affect. They bind to (act on) a target receptor and the receptor does something, like open an ion channel. These types of drugs that do something are called agonists. Other types of clinically-used drugs, like the antihypertensive drugs called 'beta-blockers', bind to a receptor and prevent another substance from doing something. These drugs are called antagonists.

Agonists are further subdivided into partial agonists and full agonists. As their name suggests, full agonists produce a full pharmacological effect and partial agonists only produce a partial pharmacological effect. The difference between one drug being a partial agonist and another drug being a full agonist arises from the two drugs differing mechanism of action.

As noted above, midazolam, like all benzodiazepines, increases the frequency (not the duration) of ion channel opening only when GABA is present. As GABA is a neurotransmitter synthesized by inhibitory brain neurons, the amount of GABA released onto $GABA_A$ receptors is limited. Because midazolam depends on the co-activation of GABA to produce its effects, midazolam effects on the brain is therefore also limited. In this regard, **midazolam is a partial agonist**.
Thiopental, to the contrary, does not need co-activation by GABA to produce its effects. In this regard, the neuronal inhibition produced by barbiturates is not limited. In this regard, **thiopental is a full agonist**.

By definition, partial agonists will exhibit a 'ceiling effect' in which greater doses will not produce a greater pharmacological effect. The ceiling effect of benzodiazepines, and the lack of ceiling effect for barbiturates, is so well-accepted that many medical pharmacology textbooks contain a Figure illustrating this fact. Fig. 1 below shows one such example.

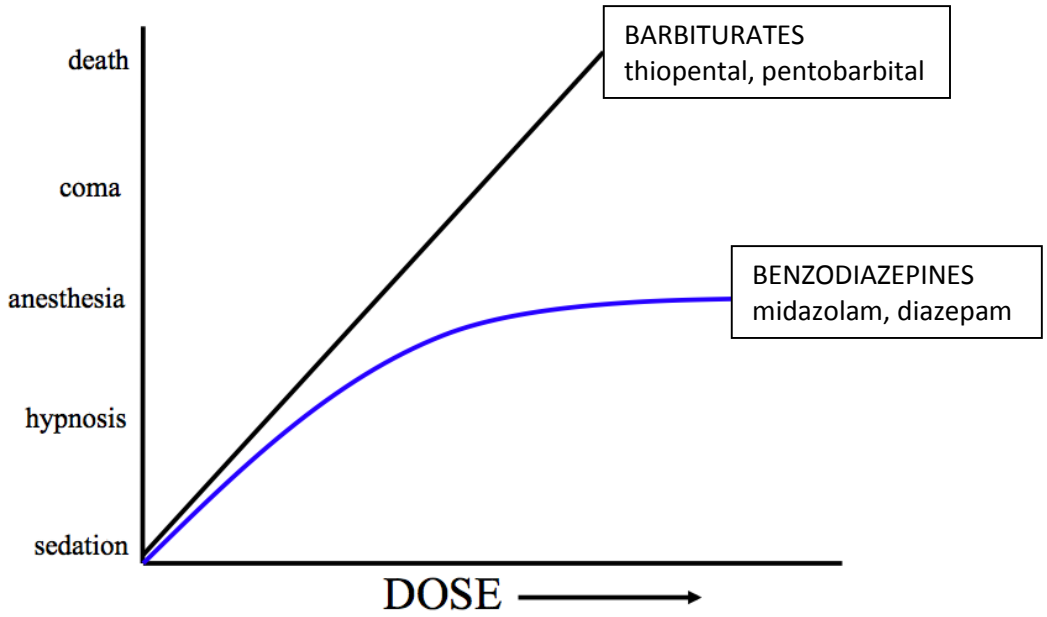


Fig. 1. Typical textbook example of a graph showing the differences between barbiturates (top line) and benzodiazepines (bottom line). The dose increases along the horizontal axis as you move to the right; the effects in humans increases as you move up the vertical axis. Note that the ceiling effect shown for benzodiazepines versus lack of ceiling effect for barbiturates As the dose of benzodiazepine increases, a plateau ('ceiling') is reached before reliable general anesthesia is obtained. Increasing doses of barbiturates reliably produce anesthesia, coma, and death. Note: the term 'hypnosis' is medical terminology for 'sleep'. Adapted from *Brenner and Stevens 2013*.

In summary, **the fact that midazolam is a partial agonist, and that thiopental is a full agonist, arises directly from their mechanisms of action as barbiturates can act in the absence of GABA and increase the inhibition of brain neurons whereas midazolam and other benzodiazepines are limited with their effect only when GABA is present and thus cannot inhibit neurons as much as barbiturates. This pharmacological fact, demonstrates that pharmacological equivalency is NOT met by substitution of a barbiturate with a benzodiazepine**. The ceiling effect of a midazolam and other benzodiazepines, and the lack of ceiling effect with the use of thiopental and other barbiturates, is beyond controversy and taught to all medical and pharmacology students.

E. Therapeutic Uses of Benzodiazepines and Barbiturates

The therapeutic use of a drug is a direct result of the drug's pharmacological properties, including, most importantly, a drug's mechanism of action. As noted above, while both benzodiazepines and barbiturates act on the $GABA_A$ receptor, they do so in very different ways. Because of the difference in their mechanism of action, the clinical use of benzodiazepine and barbiturate drugs are for different therapeutic reasons.

Table 2 is a list of therapeutic uses for benzodiazepines and barbiturates. Entries marked with a 'YES' indicate that the class of drugs is FDA-approved for this indication and show which particular drug(s) is approved for this therapeutic use.

Table 2. Comparison of therapeutic uses for five benzodiazepines and five barbiturates.

Therapeutic Use	**Benzodiazepines**	**Barbiturates**
Anxiety disorders	YES, alprazolam, diazepam, lorazepam	YES but only for 'sedation' with butabarbital
Panic Disorder	YES, alprazolam, clonazepam	NO
Acute Alcohol Withdrawal	YES, diazepam	NO
Skeletal Muscle Spasm	YES, diazepam	NO
Seizure Disorders	YES, clonazepam, diazepam	YES, pentobarbital (IV), phenobarbital (IV), thiopental (IV)
Preoperative Sedation	YES, midazolam (IM/IV)	YES, pentobarbital (IV), secobarbital
Outpatient Sedation	YES, midazolam (IV)	NO
Anesthesia Induction	YES, midazolam (IV)	YES, thiopental (IV)
Sole Anesthesia (brief)	NO	YES, thiopental (IV)
Sedation for Intubated Ptx	YES, midazolam (IV cont.)	NO
Co-Anesthesia (Adjunct)	YES, midazolam (IV)	YES, thiopental (IV)
Insomnia (short-term)	NO	YES, butabarbital, secobarbital, pentobarbital (IV)
Induce Coma in Brain Trauma	NO	YES, thiopental (IV)
Psychiatric Use (Narcoanalysis)	NO	YES, thiopental (IV)

Notes: Benzodiazepine data of therapeutic uses are from the FDA-approved Prescribing Information labels of alprazolam (Xanax®), clonazepam (Klonopin®), diazepam (Valium®), lorazepam (Ativan®), and midazolam (Versed® injection). Barbiturate data are from the current FDA-approved labels for butabarbital (Butisol®), pentobarbital (Nembutal® injection), phenobarbital (Luminal®), secobarbital (Seconal®) except the discontinued label for thiopental (Pentothal®) which is no longer marketed. All drug formulations are oral tablets except where noted; IV=intravenous, IM=intramuscular.

As shown in Table 2 above, there are 14 therapeutic uses for the benzodiazepine and barbiturate drugs. Among these 14 therapeutic uses, only 5 (or 35.7%) are common to both benzodiazepines and barbiturates. These shared indications are Anxiety Disorders, Seizure Disorders, Preoperative Sedation, Anesthesia Induction, and Adjunct/Co-Anesthesia (used with a general anesthetic). It should be noted that benzodiazepines for the treatment of Anxiety Disorders have almost universally supplanted the older barbiturate drugs for this use (*Howie 1975*, *Pieters and Snelders 2007*). Five indications are for the use of benzodiazepines only; Panic Disorder, Acute Alcohol Withdrawal, Skeletal Muscle Spasms, Outpatient Sedation, and Sedation for Intubated Patients. Four indications are for the use of barbiturates only; Sole Anesthesia (for brief procedures), Insomnia (for short-term treatment of 2 weeks), Induce Coma in Brain Trauma, and the Psychiatric Use (Narcoanalysis), which is the limited and historical use of thiopental to get a therapy patient to talk, as in 'truth serum'.

With regards to specific drugs, out of five indications for midazolam, midazolam shares only two therapeutic uses with thiopental – anesthesia induction and co-anesthesia.

The demonstration that benzodiazepines and barbiturates, and more specifically midazolam and thiopental, have different therapeutic uses **shows that pharmacological equivalency of barbiturates and benzodiazepines is NOT met considering the criteria of approved therapeutic uses.** Most importantly, midazolam was not approved for use as a Sole Anesthetic. In contrast, thiopental, was approved as a Sole Anesthetic for brief procedures.

F. DEA Scheduling of Midazolam and Thiopental

Most prescription drugs are safe and without the potential for abuse and dependence. Thus the vast majority of drugs prescribed by physicians do not come under the purview of the Drug Enforcement Administration (DEA). Drugs that pose a special danger of abuse or drug dependence are tightly regulated by the DEA and are called controlled substances.

Midazolam and thiopental are controlled substances according to the DEA, as promulgated by the Controlled Substances Act of 1970. The DEA places dangerous drugs into five schedules, with Schedule I drugs being the most dangerous drugs with no approved medical use. Schedule II-V are drugs with medical uses but with decreasing danger of abuse and dependence. Midazolam, as with most of the other benzodiazepines like diazepam (Valium®) and lorazepam (Ativan®) are placed into Schedule IV. Thiopental is deemed a more dangerous drug than midazolam as thiopental is a Schedule III controlled substance. This is evidence that midazolam is deemed safer to use by the DEA, with less evidence of abuse and drug dependence than thiopental. Simply put, the DEA decision to schedule midazolam and thiopental differently **reflects the DEA finding that midazolam and thiopental do NOT exhibit pharmacological equivalency in causing drug dependence and abuse.**

G. Summary

Pharmacological equivalency between benzodiazepines and barbiturates, and more specifically between midazolam and thiopental, was investigated by examining key aspects of the pharmacology of the two drugs and their drug classes. The findings from this section are:

i. There is no pharmacological equivalency between midazolam and thiopental using the criterion of chemical structures for benzodiazepines and barbiturates

ii. There is no pharmacological equivalency when examining the different mechanisms of action of benzodiazepines (midazolam) and barbiturates (thiopental).

iii. There is no pharmacological equivalency between the magnitude of pharmacological effects produced by benzodiazepines (partial agonists) and barbiturates (full agonists). In particular, it is well-known that midazolam has a ceiling effect that is not present in thiopental.

iv. There is little pharmacological equivalency when examining the different therapeutic uses of benzodiazepines and barbiturates, or between midazolam and thiopental.

v. There is no pharmacological equivalency in the drug abuse and dependence properties of midazolam and thiopental as confirmed by the different scheduling of these drugs by the DEA.

3. Dosage and Characteristics of Thiopental Used in Lethal Injection

A. Therapeutic, Toxic, and Lethal Blood Concentrations of Thiopental

Barbiturates are a class of sedative-hypnotic drugs, largely replaced in clinical therapeutics by the benzodiazepine class of sedative-hypnotics (*Brenner and Stevens 2013*). Examples of common barbiturate drugs are thiopental, pentobarbital, phenobarbital, and methohexital.

Clinical studies and forensic toxicology studies have determined the therapeutic, toxic, and lethal blood concentrations of thiopental, pentobarbital, midazolam, and diazepam (*Musshoff et al. 2004; Regenthal et al. 1999; Schulz 2012; Winek et al. 2001*). These values are given in blood concentration ranges from the most recent paper, as shown in Table 3 below.

Table 3. Therapeutic, toxic, and lethal ranges of thiopental, pentobarbital, midazolam, and diazepam blood concentrations. Concentrations given in mg/L (milligram per Liter). Half-life ($t_{1/2}$) is the time in hours it takes for half the amount of drug to be eliminated. From Schulz et al. 2012.

Substance/Class	**Blood-plasma concentration (mg/L)**			**Half-life, $t_{1/2}$ (hours)**
	Therapeutic	**Toxic**	**Comatose-Fatal**	
BARBITURATES				
Thiopental	1-5	7	10-15	3-8 h
Pentobarbital	1-10	10-19	15-25	20-40 h
BENZODIAZEPINES				
Midazolam	0.04-0.25	1-1.5		1.5-3.0 h
Diazepam	0.1-0.25	3-5		24-48

Table 3 above shows that there are known therapeutic and toxic blood concentrations for the barbiturates, thiopental and pentobarbital, and for the benzodiazepines, midazolam and

diazepam. However, there are only Comatose-Fatal concentrations given for thiopental and pentobarbital. The Comatose-Fatal concentration for midazolam (or diazepam) is not known.

Given the fatal blood concentrations for thiopental above, it is of considerable interest to calculate the blood concentration that results from the IV administration of 2 grams thiopental used in the 3-drug lethal injection protocol. Once a reasonable estimate is made of the thiopental blood concentration after a 2 gram IV thiopental dose, this blood concentration obtained can be compared to fatal thiopental concentration range as shown in Table 3, above.

B. Thiopental Blood Levels following a 2 gram dose of IV Thiopental in Humans

There are no clinical studies determining the lethal dose of IV thiopental in humans for obvious reasons. However, there is an early report from 1950 that used IV thiopental doses of 1, 2 and 3.8 grams administered over 5 minutes (two lower doses) or 50 minutes (3.8 g dose) to human volunteers (*Brodie et al. 1950*). While initial blood concentrations of thiopental were not determined in these volunteers, the authors note that following these large doses of IV thiopental, the volunteers were deeply asleep and had to be on an a respirator until spontaneous ventilation was deemed adequate. Such studies could not be performed today due to safety and ethical concerns, but it is clear that 1-3.8 grams of IV thiopental was a lethal dose in this study as it caused the volunteers to stop breathing on their own.

The study of drug movement after administration is called pharmacokinetics. The pharmacokinetics of thiopental are characterized by a rapid distribution of thiopental from the bloodstream to the tissues of the body and into the brain. With direct IV administration, there is no absorption phase of the drug like when a pill is swallowed. For this reason, the peak plasma concentration of IV thiopental is observed with the first time point of sampling after the IV bolus injection.

As mentioned above, there are no studies in the literature that give the initial blood concentrations of thiopental following a 2 gram IV dose as this is higher than approved clinical doses. However it is possible to examine the thiopental blood concentrations in humans from studies following the administration of lower doses of IV thiopental. The data from these clinical studies can then be used to model the blood concentrations of thiopental after a 2 gram IV dose.

An early clinical study examined the relationship between IV thiopental doses and blood concentrations of thiopental in surgical patients with renal failure compared to age-matched normal controls *(Burch and Stanski 1982*). These authors found that renal patients had a larger unbound fraction of thiopental in their blood. In another clinical study, an IV bolus dose of 300 mg thiopental gave a peak blood concentration of approximately 40 mg/L (*Morgan et al. 1981*). In a study comparing ages of patient groups, the administration of 285 mg of IV thiopental gave an initial thiopental blood concentration of approximately 35 mg/L (*Avram et al. 1990*). Although sufficient clinical data are lacking to assure a linear relationship between the administered doses of IV thiopental and resulting thiopental blood levels, the above studies and

the one highlighted next, show that the relationship between IV thiopental dose and thiopental blood concentrations is at least dose-dependent.

The graph below (Fig. 2, top of next page) shows the blood concentrations of thiopental from a study of surgical patients following a 400 mg IV thiopental dose given in 5 seconds (*Burch and Stanski 1983*). The maximum (peak) concentration of thiopental was approximately 60 mcg/mL (equal to 60 mg/L) at 30 seconds after administration. By 10 mins after administration, thiopental blood levels are within the therapeutic range at 5 mg/L (see Table 3 above).

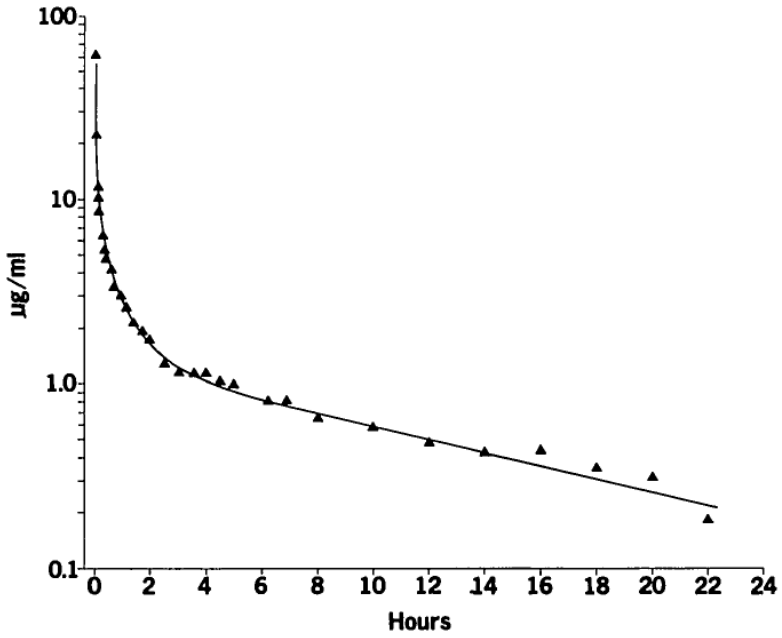


Fig. 2. Blood levels of thiopental after rapid IV injection of 400 mg thiopental. From (*Burch and Stanski 1983*). Note: µg/mL (mcg/mL) is equal to mg/L.

Given that a 400 mg IV dose of thiopental gave an initial thiopental blood concentration of 60 mg/L , to a first approximation, it follows that a 2,000 mg (=2 gram) IV dose of thiopental would give an initial thiopental blood concentration of 300 mg/L. This is calculated from the fact that a 2,000 mg IV dose is 5 times greater than the 400 mg IV dose and 5 times 60 mg/L equals 300 mg/L. By examining therapeutic, toxic, and fatal blood levels given in Table 3 above, this initial thiopental blood concentration of 300 mg/L after a 2 gram IV dose of thiopental is 20 to 30 times greater than the fatal blood concentration for thiopental listed as 10-15 mg/L.

The above calculation that shows that a dose of 2 grams of IV thiopental yields an initial blood concentration of 300 mg/L, which quickly decreases over the next hour, as shown in Fig. 2 above. It can be seen from Figure 2 above that the fall of thiopental blood concentrations occurs in two parts; the decrease in thiopental occurs more rapidly for the first hour, then the concentration of thiopental changes slowly from the thiopental levels seen at one hour. The first rapid phase of the decrease in thiopental concentrations is due to the rapid distribution of thiopental from the blood to the brain and other tissues. The second, slower phase in the decrease of thiopental is due to a slower distribution of thiopental to the tissues and the elimination of thiopental from the blood by metabolism and excretion. The time it takes for the

thiopental blood level to decrease by one-half is called the 'half-life' ($t_{1/2}$). The first rapid phase of thiopental decrease has a smaller half-life than the half-life of the second slower phase of thiopental decrease.

In order to determine the fall of thiopental concentrations over time, it is necessary to use the half-life data for IV thiopental from the pharmacokinetic studies cited above. Pharmacokinetic studies of IV thiopental show a rapid distribution half-life of 4.6 min and an elimination half-life of 11.5 hours (*Morgan et al. 1981*). Using these half-life values, the pharmacokinetic modeling of a 2 gram (2,000 mg) IV thiopental dose was done using an Excel® spreadsheet, as noted previously in the scientific literature (*Chamberlain 2003*).

The resulting graph of the decrease in thiopental blood levels after IV injection of 2 grams (2,000 mg) is shown in Figure 3 below. This graph shows that with an initial plasma concentration of 300 mg/L thiopental, the blood levels of thiopental decrease to 13 mg/L after 120 minutes. Within the first 5 minutes, the blood levels decrease to 140 mg/L (inset graph, Figure 3, below). Comparing these blood levels of thiopental with the fatal concentrations summarized in Table 3 above, after the first 5 minutes, the 2 gram IV dose of thiopental yields blood levels of thiopental (140 mg/mL) that are 9.3 to 14 times higher than fatal thiopental blood concentrations (10-15 mg/L). After 120 minutes, the 2 gram thiopental dose gives blood levels (13 mg/mL) that remain in the range of fatal thiopental concentrations.

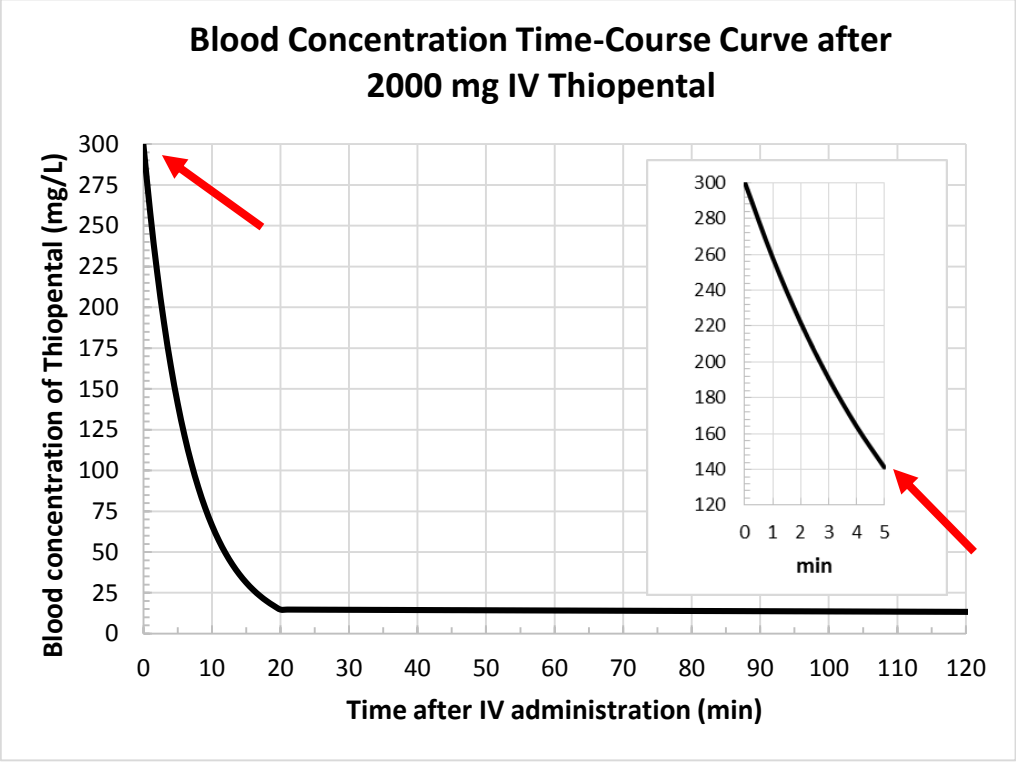


Fig. 3. Blood levels of thiopental following IV injection of 2 grams (2,000 mg) as modeled by available data. The initial plasma concentration was 300 mg/L (at left arrow). The rapid decrease used a half-life of 4.6 min that lasted for 20 min; the slower elimination phase used a half-life of 11.5 hours (*Morgan et al. 1981*). Inset graph in upper right corner shows an enlargement of the first 5 minutes after IV injection (right arrow).

C. Summary

The findings from this section are:

i. The normal therapeutic blood concentration of thiopental ranges from 1-10 mg/L. Toxic blood concentrations of thiopental occur at 7 mg/L and fatal concentrations of thiopental range from 10-15 mg/L and higher.

ii. A 2 gram IV bolus dose of thiopental produces initial thiopental blood concentrations of about 300 mg/L, which is 20 to 30 times greater than the fatal blood concentration range of thiopental. After 5 minutes, the blood concentration of thiopental decreases to about 140 mg/mL which is 9.3 to 14 times greater than the fatal blood concentrations of thiopental. After 2 hours, the blood concentration of thiopental remains within the fatal blood concentration range for thiopental.

4. Calculation of the 'Ceiling Effect' Dosage of Midazolam Used in Lethal Injection

A. Introduction to the Issue of the 'Ceiling Effect' With an IV Bolus Dose of Midazolam

In the denial of the Petitioners' appeal in Oklahoma's *Glossip et al. v. Gross et al* case, the Supreme Court of the United States makes a point of the ceiling effect and the importance of knowing the dosage of midazolam wherein the ceiling effect occurs (Slip Opinion, *Glossip et al. v. Gross et al.* No. 14-7955, Argued April 29, 2015-Decided June 29, 2015):

> "What matters for present purposes is the dosage at which the ceiling effect kicks in, not the biological process that produces the effect." (p. 25)

Therefore, the determination of the midazolam IV dosage that reaches the ceiling effect, and a comparison of the concentration of midazolam that produces a ceiling effect in research studies and the concentration of midazolam in the brain of the condemned inmate after receiving a dose of 500 mg IV midazolam, is detailed in this section.

A 500 mg IV dose of midazolam is examined because the current Lethal Injection Protocol embedded in the Mississippi Department of Corrections (MDOC) Policy "Capital Punishment Procedures" (Doc. 38-2, filed 7/28/2015) was amended to include the use of midazolam as an alternative first drug (if thiopental and pentobarbital are not available) in a 3-drug protocol with midazolam given at an IV dose of 500 mg.

In light of the revised MDOC's lethal injection protocol, the present determination is based on whether the ceiling effect of midazolam is reached at or below the brain concentration of midazolam produced immediately after the IV bolus administration of 500 mg midazolam dose and the brain concentration up to 5 minutes after IV midazolam administration. There is no reference in the MDOC Protocol to a time point when the effect of midazolam will be assessed after IV administration of 500 mg midazolam.

The 'ceiling effect' refers to the fact that greater amounts or doses of midazolam do not produce a greater pharmacological effect. The ceiling effect is well-known for midazolam and all similar drugs in the class called benzodiazepine sedative-hypnotics. By way of contrast, there is no ceiling effect seen with barbiturate sedative-hypnotics like thiopental and pentobarbital.

To determine the midazolam dose which produces a ceiling effect in humans is not easy, as it is ethically not possible to experiment on humans and administer doses greater than those used clinically. Therefore, the approach used in this report is to first examine the midazolam concentrations used in studies done *in vitro* (using cells in a laboratory dish) and determine at which concentration of midazolam that the ceiling effect occurs. Secondly, a calculation of the plasma (blood) concentration of midazolam following a 500 mg IV bolus dose (bolus means a single IV injection all at one time as opposed to continuous infusion at a lower rate) will be made based on blood concentrations of midazolam following clinically-used doses. Thirdly, based on the pharmacological data of midazolam crossing into the brain in preclinical studies, the extent of the 500 mg midazolam dose that enters the brain will be calculated. Fourthly, published studies will be researched to calculate the concentration of midazolam in the brain after a 500 mg IV dose. Finally, by comparing the concentration of midazolam that produces a ceiling effect in studies done *in vitro* and in the clinic, with the calculated concentration of midazolam in the human brain after a 500 mg dose, conclusions will be reached to determine if this 500 mg dose is above or below a midazolam concentration shown to produce a ceiling effect.

B. Ceiling Effect of Midazolam and Other Benzodiazepines Observed In Vitro

As detailed in §2C above, the mechanism of action of midazolam and other benzodiazepines is enhancing the inhibitory effect of the neurotransmitter, GABA, on brain neurons. The decrease in neuronal activity produced by the inhibitory neurotransmitter, GABA, is not 'all or none'. GABA simply decreases the ongoing activity of neurons by a graded amount, depending on how much GABA is present. GABA is a limited resource in the brain as it is made and released by inhibitory brain neurons, which are finite in number. The concentration of GABA around brain neurons is reported to be 10-400 nM (*Houston et al. 2012*). This information on the concentration of GABA is important in calculating the ceiling effect of midazolam (see below), as midazolam has to have GABA present to exert its pharmacological effect.

A little more pharmacology of benzodiazepine's mechanism of action and an analogy is needed. Midazolam and other benzodiazepines potentiate the binding of GABA at the $GABA_A$ receptor, but at a site different than where GABA binds. This is called allosteric modulation. To use an analogy, the allosteric action of midazolam might be thought of as a Boy Scout helping an elderly woman (GABA) across the street. The woman can cross the street without the Boy Scout (midazolam) but his presence and assistance helps the elderly woman move faster. Midazolam and other benzodiazepines can only enhance GABA action and have no inhibitory action on brain neurons on their own. Benzodiazepines by this allosteric mechanism of action have an innate 'ceiling effect' and can only produce a limited plateau effect. Using our analogy, the Boy Scout can move the elderly woman across the street only so fast, the act of getting the woman

across the street is still limited by the ability of the woman to ambulate on her own two legs. There is a 'ceiling effect' in how fast the woman can cross the street, even if two or more Boy Scouts were to help her.

The ceiling effect of midazolam and other benzodiazepines is not controversial and is portrayed in many introductory pharmacology textbooks (see Fig. 1 above). The remainder of this section will highlight studies from the scientific literature that show the ceiling effect of midazolam and other benzodiazepines and provide specific threshold drug concentrations from these studies when the ceiling effect was reached. This ceiling effect with benzodiazepines, including diazepam (Valium®) and midazolam (Versed®) was observed early and consistently in the research studies that determined the mechanism of action for benzodiazepine drugs. Samples of figures from these original research papers are reproduced below (next two page) so that it will be obvious that a ceiling effect is documented and pervasive in the scientific and pharmacological literature.

The studies shown on the next two pages and others are summarized in Table 4 below showing the threshold dose(s) that produced the observed ceiling effect. Most studies of diazepam show a ceiling effect threshold at 100 nM and <u>all three studies of midazolam gave 100 nM as the concentration producing a ceiling effect</u>.

Table 4. Summary of selected studies showing ceiling effect of diazepam and midazolam

Benzodiazepine	**Ceiling effect at:**	**Preparation**	**Reference**
Diazepam	10 nM[a]	Cell culture (mouse spinal neurons)	*Skerritt and Macdonald (1984)*
Diazepam	100 nM	Cell culture (oocytes)	*Sigel and Baur (1988)*
Diazepam	50-100 nM	Cell culture (mouse spinal neurons)	*Rogers et al. (1994)*
Diazepam	100 nM	Cell culture (HEK cells)	*Li et al. (2013)*
Diazepam	100 nM	Cell culture (oocytes)	*Rüsch and Forman (2005)*
Midazolam	100 nM	Brain slices (rat)	*Rovira and Ben-Ari (1999)*
Midazolam	100-200 nM	Brain slices (rat)	*Bai et al. (2001)*
Midazolam	100 nM	Cell culture (oocytes)	*Rüsch and Forman (2005)*

[a] nM stands for 'nanomolar' which is a concentration term relating the number of drug molecules in a liter of solution.

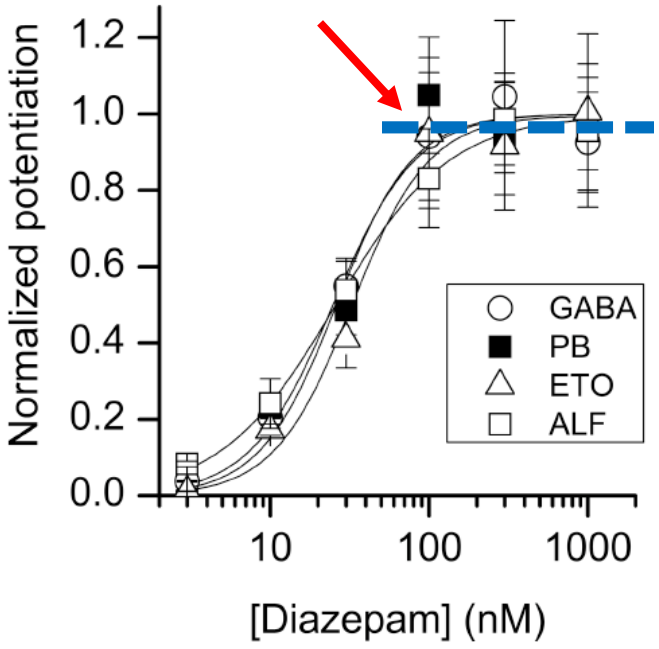


Fig. 4. Various doses of the benzodiazepine, Diazepam, were added with GABA (open circles) and other drugs and the current measured on the vertical scale (Y-axis). Arrow shows ceiling effect threshold at 100 nM. Horizontal dash line shows the ceiling effect. From Fig. 4 in *Li et al. 2013.*

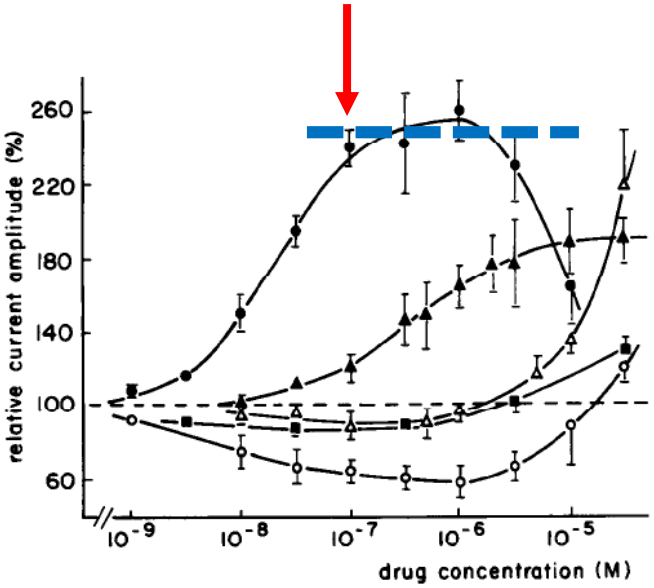


Fig. 5. Various doses of the benzodiazepine, Diazepam (closed circle, top curve) were applied to cells in the presence of GABA and the current measured on the vertical scale (Y-axis). Arrow shows ceiling effect threshold at 10^{-7} M which is equal to 100 nM. Horizontal dash line shows the ceiling effect. From Fig. 4 in *Sigel and Baur 1988.*

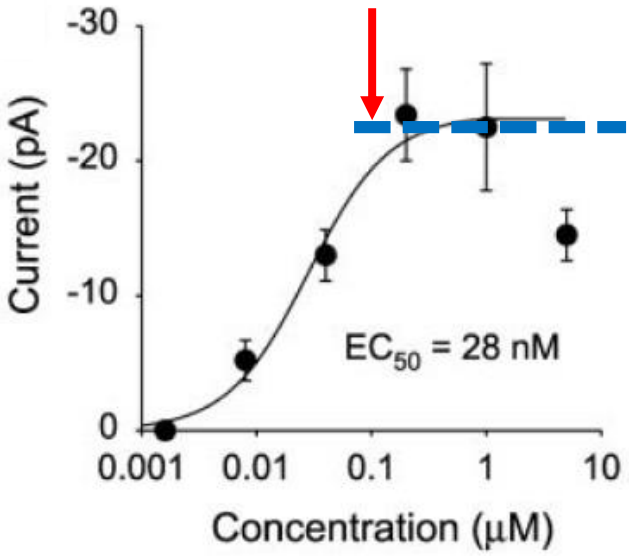


Fig. 6. Various doses of Midazolam (closed circle, top curve) along the horizontal scale (x-axis) were applied to cells in the presence of GABA and current measured on the vertical scale (Y-axis). Arrow shows ceiling effect threshold at 0.1 µM which is equal to 100 nM. Horizontal dash line shows ceiling effect. From Fig. 5B in *Bai et al. 2001.*

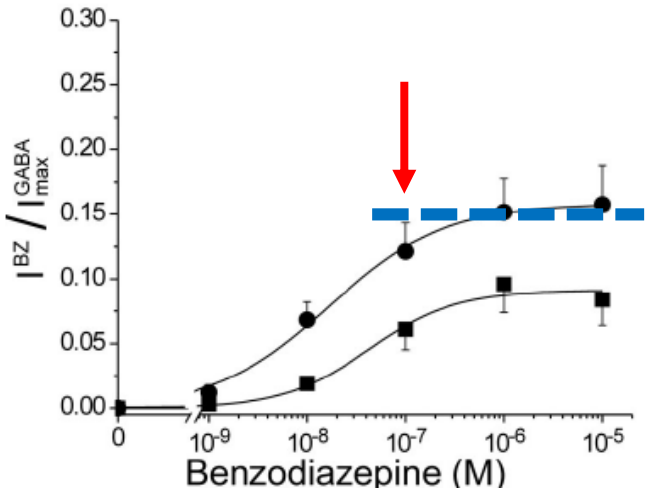


Fig. 7. Various doses of Midazolam (closed circle, top curve) or Diazepam (closed squares, bottom curve) along the horizontal scale (x-axis) were applied to cells in the presence of GABA and current measured on the vertical scale (Y-axis). Arrow shows ceiling effect threshold at 10^{-7} M which is equal to 100 nM. Horizontal dash line shows ceiling effect. From Fig 2A in *Rüsch and Forman 2005.*

C. Blood Levels of 500 Mg Midazolam after IV Bolus Dose in Humans

As mentioned above, there are no studies in the literature that give the plasma concentrations of midazolam following a 500 mg IV dose in humans as this is higher than approved clinical doses. However, it is possible to review the plasma concentrations in humans from studies examining the plasma concentrations after clinical doses of IV midazolam. The data from these studies can then be used to model the plasma concentrations of midazolam after a 500 mg IV dose.

A clinical study measured the peak amount of midazolam in the plasma after IV bolus administration of 5 mg midazolam in eight healthy volunteers (*Schwagmeier et al. 1998*). This study gave peak plasma concentrations of nearly 120 ng/mL (nanogram per milliliter) after a 5 mg IV dose. It follows then that with a 500 mg IV dose, the initial amount after direct IV bolus infusion is 100 times of what occurred with the 5 mg dose, which gives an initial plasma concentration of 120,000 ng/mL of midazolam after a 500 mg IV dose.

A direct linear modeling of the 500 mg IV dose from the 5 mg dose is supported by other studies. In a more recent study using half of the above 5 mg IV dose, a 2.5 mg IV dose of midazolam, the peak plasma concentration of 51.2 ng/mL which is about half the peak plasma concentration seen in the above clinical study using a 5 mg IV dose of midazolam (*Veldhorst-Janssen et al. 2011*). Therefore it is not unreasonable to use this linear relationship to extrapolate from the 5 mg giving 120 ng/mL and one-hundred times that dose (500 mg) giving one-hundred times the initial blood concentration for a result of 120,000 ng/mL.

Given the estimate that the initial concentration of midazolam in the plasma after a 500 mg IV bolus dose is 120,000 ng/mL, the next determination is to model the fall of midazolam plasma concentration over time to determine the amount of midazolam that is available for transfer to the brain during the first 5 minutes.

In order to determine the midazolam plasma concentrations over time, it is necessary to have established pharmacokinetic data for IV midazolam. A key paper in this regard examined the pharmacokinetic data after dosing volunteers with 0.1 mg/kg midazolam IV infusions after 1 minute, 1 hour, and 3 hour lengths of infusion (*Greenblatt et al. 2004*). The dosing of midazolam with a 1 minute bolus infusion comes closest to the method to be used by the Oklahoma Department of Corrections (see above). The Greenblatt study found that midazolam IV dose given in 1 minute had a half-life of immediate distribution ($t_{½\ alpha}$) of 21 min and a half-life of elimination ($t_{½\ beta}$) of 171.6 minutes. Using these two parameters, it was possible to model the plasma concentration curve over time following the IV dose of 500 mg midazolam (see Fig. 6 next page). The modeling of the blood concentration curve following a 500 mg IV midazolam dose was done using an Excel spreadsheet, as noted in the scientific literature (*Chamberlain 2003*) and was done above in §3B.

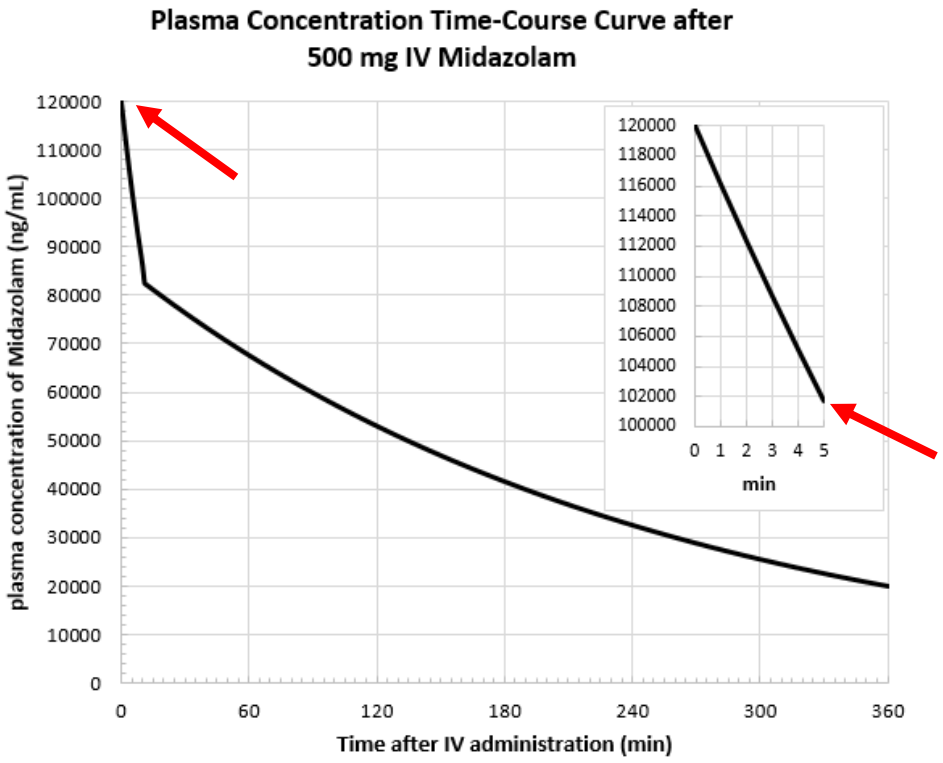


Fig. 8. Plasma concentration curve following a single IV bolus dose of 500 mg midazolam. Inset shows the region of the plot from 0-5 minutes. See text for further details. Arrows denote the initial blood concentration of midazolam and midazolam concentration after 5 minutes (inset).

<u>The key parameters calculated above are that following the 500 mg IV dose of midazolam, the initial highest concentration of midazolam is 120,000 ng/mL and after 5 minutes, the concentration of midazolam is 102,000 ng/mL</u>.

D. Extent of Midazolam Entering the Human Brain after an IV Bolus Dose

Studies that show the amount or extent of midazolam that enters the human brain would be best done by administering an IV dose and then sampling brain tissue at various time points after administration in numerous people. These studies, of course, cannot be done. However, there have been a number of preclinical studies in non-human animals that provide the fraction of midazolam that crosses into the brain from the blood to give reliable data. These studies are reviewed next and will provide a value that can be used to determine the amount or extent of midazolam that enters the human brain after a 500 mg IV dose.

However, it should first be noted that drugs in the plasma or blood bind to plasma proteins such as albumin and gamma-globulins and the amount of protein binding varies with each drug. This is important as only the free (unbound) drug is available to cross from the blood into the brain to exert its effect. Midazolam is a drug with high plasma protein binding, on the order of 94-97% (*Fragen 1997*). Using 95% as an estimate, this gives only 5% of the amount of

midazolam in the blood available for crossing the blood-brain barrier and entering the brain. Taking this into account for the two key parameters of interest noted above, a 500 mg IV bolus of midazolam gives an initial free drug blood plasma concentration of 6,000 ng/mL (120,000 X 0.05) and a free drug blood concentration at 5 minutes of 5,100 ng/mL (102,000 X 0.05).

Preclinical studies of the fraction of midazolam that enters the brain after an IV dose are done by sampling the cerebrospinal fluid (CSF) along with the plasma at various times after midazolam administration (*Arendt et al. 1983, Jones et al. 1988*). The CSF is a good surrogate for the fluid surrounding the brain cells as it is relatively protein-free so there is little to no binding of drugs to proteins like that which occurs in the blood. The CSF circulates around and through the brain and spinal cord, bathing the CNS (*Lin 2008*). Fig. 9 below (next page) shows the concentration of midazolam in the blood and in and brain CSF at the same time points from the paper by *Arendt 1983*.

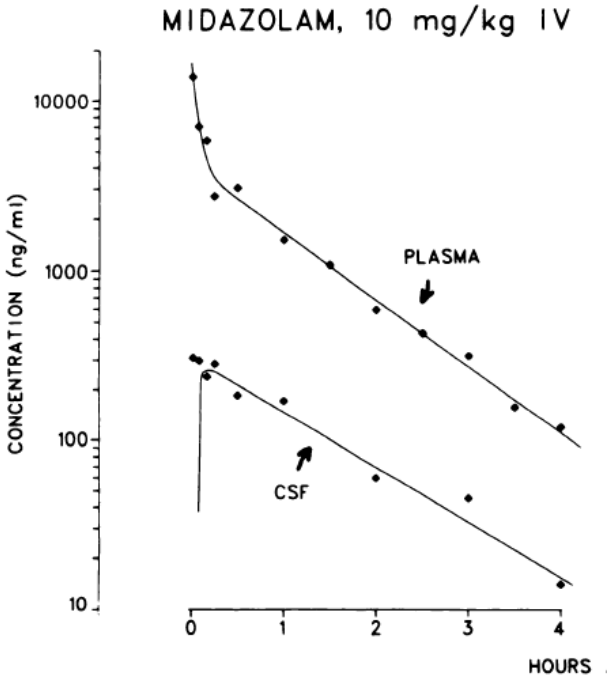


Fig. 9. Midazolam concentrations curve in plasma (top curve) and in brain CSF (bottom curve) after a single 10 mg/kg IV bolus dose. Note that the CSF concentration is much less than plasma at all time points but mirrors the plasma curve. From Fig 2 (left panel) in *Arendt et al. (1983).*

The calculations performed in the study shown in Fig. 9 yielded a brain CSF/plasma concentration ratio of 0.14 or 14% (*Arendt et al. 1983*). This ratio can be used in our determinations of brain concentration after 500 mg IV dose of midazolam to calculate that an initial plasma concentration of 6,000 ng/mL midazolam equals 840 ng/mL in the brain (6000 X 0.14) and at 5 minutes after start of infusion, the plasma concentration of 5,100 ng/mL is equal to 714 ng/mL (5100 X 0.14) in the brain.

E. Dosage of IV Midazolam That Produces a Ceiling Effect in Humans

The above data gave the measurement of midazolam in blood in the units of ng/mL, or nanogram per milliliter (ng/mL is a weight per volume measure, like mixing a teaspoon of salt in a glass of water). However, the existing data on the concentration of midazolam that produces a ceiling effect from *in vitro* studies reviewed above gave a value of 100 nM (nanomolar) which is in different units. The brain concentration of midazolam (in ng/mL) calculated in the last section above needs to be converted to nanomolar terms (nM) to compare it with the existing *in vitro* data showing that midazolam's ceiling effect occurs at a midazolam concentration of 100 nM. This conversion is done by using the molecular weight of midazolam which gives the relationship between grams and moles[1]. For example, a concentration of midazolam of 32.6 ng/mL in the brain equals 100 nM in nanomolar terms.

The calculated values of the brain concentrations of midazolam following a 500 mg IV dose give an estimate of 840 ng/mL when the infusion begins and 714 ng/mL after 5 minutes elapsed since the start of the infusion. These two values expressed in nM are: 840 ng/mL = 2,579 nM and 714 ng/mL = 2,272 nM.

Given that midazolam shows ceiling effects at 100 nM concentration (see Table 1 above), the estimated brain concentrations for midazolam under the current MDOC Mississippi lethal injection protocol using a 500 mg IV dose of midazolam as the first drug are about 20 to 25 times higher than the concentration of midazolam that produces a ceiling effect. Furthermore, the concentration of the inhibitory neurotransmitter, GABA, in the vicinity of neurons in the brain is reported as ranging from 10-400 nM (*Houston et al. 2012*). Taking the high-end value of the GABA concentration at 400 nM, when the midazolam brain concentration produced by a 500 mg IV dose of midazolam is at 2,579 nM, there is about 6.45 times more midazolam than GABA (calculated by 2,579/400). As midazolam cannot by itself work without GABA present, once midazolam has worked with all the GABA that is available, there is about 5.45 times extra midazolam that cannot exert a pharmacological effect. Going back to the analogy of Boy Scouts (midazolam) helping little old ladies (GABA) cross the street, there are about 6 Boy Scouts to every one lady, so adding the extra 5 Boy Scouts to the corner did not serve any purpose.

The midazolam dose that results in a 100 nM concentration of midazolam, the ceiling effect concentration, is obtained by using the values of brain concentration obtained with a 500 mg IV dose above. A 500 mg IV dose gives a brain concentration of 2,579 nM (call it 2,500 nM) which is 25 times the ceiling effect concentration of 100 nM. Therefore, a dose that is 25 times less than 500 mg is 20 mg. Thus, a 20 mg IV dose of midazolam would be expected to reach the threshold concentration of midazolam to produce a ceiling effect.

In the clinic, the range of midazolam IV doses for intravenous sedation is 5 to 15 mg IV, with a standard patient weighing 100 kg or about 220 pounds (*Reves et al. 1985*). Even when used at higher doses for induction of anesthesia, the range is 15 to 40 mg IV. The analysis presented here suggest that the highest clinically-used doses approach the ceiling effect dosage but that

[1] Calculations were assisted by the Molar solution concentration calculator found at *www.physiologyweb.com.*

the usual clinical midazolam IV doses produce brain concentrations that are below the ceiling or plateau effect. This is consistent with clinical rationale whereby greater doses of drugs are not given if there is no greater pharmacological effect observed.

Most telling is the lack of a fatal blood level range for midazolam in the latest compendium of therapeutic, toxic, and fatal blood levels of over 1,000 drugs (*Schulz et al. 2012*). **Table 5 below (which is a repeat of Table 3 above) highlights in bold lines the blank space for the fatal blood levels of midazolam (and for diazepam)**. **This shows that there are few reported fatalities and no consensus whether fatal effects occur with midazolam and at what dosage range they may occur.**

Table 5. Therapeutic, toxic, and lethal ranges of thiopental blood concentrations. Concentrations given in mg/L (milligram per Liter) which is equal to mcg/mL (microgram per milliLiter). Half-life ($t_{1/2}$) is given in the last column and is the time in hours it takes for half the amount of drug to be cleared from the bloodstream. From Schulz et al. 2012.

Substance/Class	**Blood-plasma concentration (mg/L)**			
	Therapeutic	**Toxic**	**Comatose-fatal**	**Half-life, $t_{1/2}$ (hours)**
BARBITURATES				
Thiopental	1-5	7	10-15	3-8 h
Pentobarbital	1-10	10-19	15-25	20-40 h
BENZODIAZEPINES				
Midazolam	0.04-0.25	1-1.5		1.5-3.0 h
Diazepam	0.1-0.25	3-5		24-48

F. Summary

The findings from this section are:

i. The ceiling effect of midazolam is a direct result of midazolam's mechanism of action. Thiopental and other barbiturates have a different mechanism of action and therefore do not exhibit a ceiling effect.

ii. Research done *in vitro* show that the ceiling effect of midazolam occurs at a concentration of 100 nM.

iii. An IV bolus dose of 500 mg midazolam produces a brain concentration of 2,579 nM after dosing and 2,272 nM after 5 minutes.

iv. An IV bolus dose of 500 mg midazolam produces a brain concentration that is 20-25 times higher than the concentration that midazolam produces a ceiling effect.

v. An IV bolus dose of 20 mg midazolam is sufficient to reach the threshold of midazolam's ceiling effect.

5. Comparison of the Effects of Midazolam and Thiopental on Consciousness

A. Translation of 'Unconsciousness' to a Drug-Induced State of General Anesthesia

Anesthesia is the loss of all feeling and is generally meant to be in a state of unconsciousness. General anesthesia is often used to contrast with the term local anesthesia, which is the loss of feeling in only part of the body (*Brenner and Stevens 2013*).

Science demands measurement. The pharmacological data that is the essence of drug characterization is based on numbers and measured parameters. Using a scientific approach to determine the relative potency of midazolam or thiopental to produce 'unconsciousness', first the linkage between unconsciousness and general anesthesia must be examined because 'unconsciousness' *per se* cannot be measured but one can measure to a certain degree the depth (magnitude) of general anesthesia.

Scientific models of consciousness rely on the measurement of activity in different areas of the brain and the known functions associated with them. When a general anesthetic is given, there is inhibition of the activity in the higher-order association areas of the brain more so than primary processing areas of the brain (*MacDonald et al. 2015*). Most telling, as patients come out of general anesthesia there is dramatic and sudden activation of the higher-order association areas of the brain regions that correlates with patient responding to verbal commands (*Långsjö et al. 2012*). To a first approximation, consciousness is correlated to activity in brain association areas and therefore unconsciousness is correlated to lack of activity in these brain association areas.

Clinical experience with non-responsive patients shows that a cautious approach to the risk evaluation of midazolam's ability to produce anesthesia should be taken. Patients that are non-responsive are diagnosed of being in a vegetative state after repeated tests of consciousness show no evidence of sustained, reproducible, purposeful, or voluntary behavioral response to visual, auditory, tactile, or noxious stimuli (*MacDonald et al. 2015).* These tests in non-responsive patients are the same as tests used by anesthesiologist to detect the surgical plane of anesthesia. In the non-responsive patients, studies show that up to 43% of these patients that are diagnosed as vegetative are actually aware or conscious. This finding and the numerous studies documenting the lack of unconsciousness during surgery, called 'awareness during anesthesia' (*Escallier et al. 2014*) in some patients even when using strong general anesthetics like thiopental or inhalation agents, mandates a conservative approach to questions of the first drug used in a 3-drug lethal injection protocol. In other words, even under the best circumstances, clinicians assessing non-responsive patients and anesthesiologists inducing general anesthesia appear to get it wrong a significant percentage of the time and their patients are not unconscious (or anesthetized) as often as they think. In the case of lethal injection using a 3-drug protocol, it is even more crucial to insure general anesthesia by the action of the first drug due to the intolerable effects of the second drug (muscle paralytic) and third drug (potassium chloride) if the condemned inmate is not unconscious after the first drug.

B. The Potency of Thiopental to Induce General Anesthesia

In general, thiopental or other barbiturates are more potent than midazolam or other benzodiazepines in inducing anesthesia because thiopental produces a dose-dependent depression of the central nervous system while midazolam is limited by a ceiling effect (*Rosenberg and Weaver 1991*).

Researchers and clinicians developed a way to measure the depth of general anesthesia using EEG recordings of the frontal lobe brain and computer processing called bispectral analysis or BIS (*Escallier et al. 2014*). BIS gives a single number, on the scale from 100 (completely awake and alert) to 0 (coma and total EEG burst suppression). Clinical signs of anesthesia correlate moderately well with BIS scores (*Weaver et al. 1970*). BIS values of less than 60 are targeted during anesthesia procedures as that is the depth of anesthesia associated with lack of anesthesia awareness (*Weaver et al. 1970*). In this study, BIS values of 60 correlated with general anesthesia, 65 with deep sedation and 80 to moderate sedation. Using thiopental doses to induce (but not maintain general anesthesia) gave BIS values as low as 60 (*Yoo et al. 2012*).

C. The Inability of Midazolam to Induce General Anesthesia

There are general characteristics that differentiate the use of midazolam from thiopental in use as an anesthetic induction agent. Midazolam has a significantly slower onset of action than thiopental (*White 1982*). Midazolam also does not produce the early activation of EEG that is seen with thiopental and other IV general anesthetics (*Kuizenga et al. 2001*).

There are few research reports from the medical and pharmacological literature looking at the level of anesthesia after midazolam by measuring the BIS. Generally, midazolam is used as a premedicant before general anesthesia or for regional anesthesia (*Khanderia and Pandit 1987*). Midazolam is a less reliable induction agent than thiopental and induction of anesthesia using midazolam alone is unpredictable. Clinically, benzodiazepines such as midazolam are not used as much for anesthesia or induction of anesthesia but for conscious sedation (*Giovannitti and Trapp 1991*). Conscious sedation is a drug-induced state of relaxation where the patient remains conscious with reflexes intact and little effect on cardiovascular or respiratory function. Midazolam is often used with an opioid analgesic in outpatient procedures such as colonoscopy and oral surgery.

In light of the lesser potency of midazolam compared to thiopental, most studies have investigated the relation of BIS values to levels of anesthesia. BIS values of in the range of 77-92 were reported after repeated IV doses of midazolam in a surgical outpatient study (*Sandler 2000*). In surgery patients, the lowest BIS score for IV midazolam was 65, whereas the inhalational agent, sevoflurane, and the intravenous anesthetic, propofol, produced low BIS scores ranging from 32-40 (*Ibrahim et al. 2001*). In a clinical study using adult healthy volunteers, IV midazolam was infused until patients become unresponsive to mild prodding or shaking (*Lui et al. 1996*). Midazolam at the greatest dose decreased the BIS to the lowest value of 69. All the above studies support the finding that midazolam does not induce general anesthesia which is stated to occur at BIS values less than 60.

D. Summary

The findings from the section are:

i. Studies show a link between unconsciousness, anesthesia, and decreased activity in brain association areas.

ii. Thiopental and other barbiturate anesthetics decrease activity in these brain association areas, and are potent in decreasing the BIS value which is associated with depth of anesthesia.

iii. There are few studies of midazolam's depth of anesthesia because midazolam cannot produce the same anesthetic effects as thiopental on the brain, and midazolam is less potent in reducing BIS values.

iv. Scientific studies show that a cautious and conservative approach is warranted in positing an 'anesthetic' action of midazolam, as a significant number of patients are found to be under-anesthetized and conscious during surgery even when using the strongest general anesthetic agents are used.

v. For these reasons, it is my opinion, to a reasonable degree of scientific certainty, that the use of midazolam in the Mississippi three-drug protocol creates a substantial risk of serious harm and severe pain to the condemned prisoner.

6. Overall Summary and Conclusions

TITLE 99 - CRIMINAL PROCEDURE of the Mississippi Code, Chapter 19 - Judgment, Sentence, and Execution, § 99-19-51 "Manner of execution of death sentence" states:

> "The manner of inflicting the punishment of death shall be by continuous intravenous administration of a lethal quantity of an ultra short-acting barbiturate or other similar drug in combination with a chemical paralytic agent until death is pronounced by the county coroner where the execution takes place or by a licensed physician according to accepted standards of medical practice."

The Mississippi Department of Corrections (MDOC) "Capital Punishment Procedures" (version date 3/7/2012) listed as the first drug in a 3-drug protocol, the use of 2 grams of Sodium Pentothal® (thiopental) or, if not available, the use of 5 grams of Sodium Nembutal® (pentobarbital). For the second drug, the use of 50 mg Pavulon® (pancuronium) or, if not available, the use of 40 milligrams of Norcuron® (vecuronium). The third drug to be used in the lethal injection protocol is 50 milliequivalents of Potassium Chloride.

MDOC Amended "Capital Punishment Procedures" (Document 38-2, filed 7/28/2015) was revised solely to include 500 mg of Versed® (midazolam) as the first drug in the 3-drug protocol if both thiopental and pentobarbital are not available.

It is my opinion, to a reasonable degree of scientific certainty, that midazolam is not an "other similar drug" to an ultra short-acting barbiturate as required by Mississippi Code § 99-19-51, the manner of execution statute.

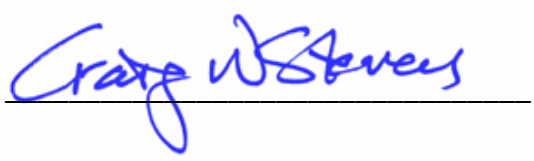
A lethal quantity of an ultra-short acting barbiturate or other similar drug means that another drug that is pharmacologically equivalent to thiopental (which is an ultra short-acting barbiturate) can be used instead of thiopental. **Midazolam, a benzodiazepine, has a fast onset but is not an 'ultra short-acting' drug and is not a barbiturate.** The fact that thiopental is not pharmacologically equivalent to midazolam is evidenced by midazolam and thiopental failing the tests of equivalency detailed in §2A-F; the supporting fact that lethal levels of thiopental are obtained after a 2 gram IV bolus dose as calculated in §3B and that midazolam produces a ceiling effect and does not produce a fatal blood level after 500 mg bolus IV dose as shown in §4E; and the supporting fact that midazolam does not produce general anesthesia nor a depth of anesthesia equal to thiopental in clinical studies detailed in §5A-C. By using midazolam, which is neither ultra short-acting, nor a barbiturate, and therefore cannot be considered a similar drug, the current MDOC Lethal Injection Protocol is in violation of the Mississippi State Statute § 99-19-51 "Manner of execution of death sentence."

In conclusion, the decision by the Mississippi Department of Corrections to substitute midazolam for an ultra short-acting barbiturate as the first drug in the 3-drug lethal injection protocol was made without sound medical or scientific reasoning or expert pharmacological advice. Pharmacological substitution is a legitimate method to provide equal pharmacological effects when one drug is no longer be available. However, it is not permissible to pharmacologically substitute one drug, such as the barbiturate thiopental, with another drug, such as the benzodiazepine midazolam, where no such pharmacological equivalency exists.

It is therefore my opinion, to a reasonable degree of scientific certainty, that (a) midazolam is not an "other similar drug" to an ultra short-acting barbiturate, and that (b) the use of midazolam in the Mississippi three-drug protocol creates a substantial risk of serious harm and severe pain to the condemned prisoner.

I reserve the right to amend this report if further information becomes available that may alter the findings in this report.

I declare under penalty of perjury that I have examined this report and all statements contained herein, and to the best of my knowledge and belief, they are true, correct and complete. My opinions stated herein are based on reasonable degree of scientific and medical certainty.



Date: 01/15/2016

Craig W. Stevens, Ph.D.

7. References Cited

Al-Halawani M, Sen P, Abdeen Y, Shaaban H, Klukowicz AJ, Miller RA (2015) Continuous intravenous flumazenil infusion in a patient with chlordiazepoxide toxicity and hepatic encephalopathy. J Emerg Trauma Shock 8:58-60.

Arendt RM, Greenblatt DJ, deJong RH, Bonin JD, Abernethy DR, Ehrenberg BL, Giles HG, Sellers EM, Shader RI (1983) In vitro correlates of benzodiazepine cerebrospinal fluid uptake, pharmacodynamic action and peripheral distribution. J Pharmacol Exp Ther. 227:98-106.

Avram MJ, Krejcie TC, Henthorn TK (1990) The relationship of age to the pharmacokinetics of early drug distribution: the concurrent disposition of thiopental and indocyanine green. Anesthesiology 72:403-411.

Bai D, Zhu G, Pennefather P, Jackson MF, MacDonald JF, Orser BA (2001) Distinct functional and pharmacological properties of tonic and quantal inhibitory postsynaptic currents mediated by gamma-aminobutyric acid(A) receptors in hippocampal neurons. Mol Pharmacol. 59:814-824.

Borgheini G (2003) The bioequivalence and therapeutic efficacy of generic versus brand-name psychoactive drugs. Clin Ther. 25:1578-1592.

Brenner GM, Stevens CW (2013) *Pharmacology*, 4th edition. Pharmacology textbook for medical and health professional students, Saunders/Elsevier, Philadelphia/London.

Brodie BB, Mark LC, Papper EM, Lief PA, Bernstein E, Rovenstine EA (1950) The fate of thiopental in man and a method for its estimation in biological material. J Pharmacol Exp Ther. 98:85-96.

Bruhn J, Myles PS, Sneyd R, Struys MM (2006) Depth of anaesthesia monitoring: what's available, what's validated and what's next? Br J Anaesth. 97:85-94.

Burch PG, Stanski DR (1982) Decreased protein binding and thiopental kinetics. Clin Pharmacol Ther. 32:212-217.

Burch PG, Stanski DR (1983) The role of metabolism and protein binding in thiopental anesthesia. Anesthesiology 58:146-152.

Campo-Soria C, Chang Y, Weiss DS (2006) Mechanism of action of benzodiazepines on $GABA_A$ receptors. Br J Pharmacol. 148:984-990.

Cestari IN, Uchida I, Li L, Burt D, Yang J (1996) The agonistic action of pentobarbital on $GABA_A$ beta-subunit homomeric receptors. Neuroreport 7:943–947.

Chamberlain J (2003) The use of spreadsheets for pharmacokinetic simulations. Scientific World Journal 3:265-278.

Chang L-R, Barnard E, Lo MS, Dolly JO (1981) Molecular sizes of benzodiazepine receptors and the interacting GABA receptors in the membrane are identical. FEBS Lett. 126:309-312.

Cromer BA, Morton CJ, Parker MW (2002) Anxiety over $GABA_A$ receptor structure relieved by AChBP. Trends Biochem. Sci. 27:280–287.

D'Hulst C, Atack JR, Kooy RF (2009) The complexity of the $GABA_A$ receptor shapes unique pharmacological profiles. Drug Disc. Today 14:866-875.

Ernst M, Brauchart D, Boresch S, Sieghart W (2003) Comparative modeling of $GABA_A$ receptors: limits, insights, future developments. Neuroscience 119:933–943.

Escallier KE, Nadelson MR, Zhou D, Avidan MS (2014) Monitoring the brain: processed electroencephalogram and peri-operative outcomes. Anaesthesia 69:899-910.

Fragen RJ (1997) Pharmacokinetics and pharmacodynamics of midazolam given via continuous intravenous infusion in intensive care units. Clin Ther. 19:405-419.

Giovannitti JA, Trapp LD (1991) Adult sedation: oral, rectal, IM, IV. Anesth Prog. 38:154-71.

Greenblatt DJ, Shader RI, Abernathy DR (1983) Current status of benzodiazepines. N Engl J Med. 309:354-358.

Greenblatt DJ, Ehrenberg BL, Culm KE, Scavone JM, Corbett KE, Friedman HL, Harmatz JS, Shader RI (2004) Kinetics and EEG effects of midazolam during and after 1-minute, 1-hour, and 3-hour intravenous infusions. J Clin Pharmacol. 44:605-611.

Greenfield LJ (2013) Molecular mechanisms of antiseizure drug activity at $GABA_A$ receptors. Seizure 22:589-600.

Houston CM, McGee TP, Mackenzie G, Troyano-Cuturi K, Rodriguez PM, Kutsarova E, Diamanti E, Hosie AM, Franks NP, Brickley SG (2012) Are extrasynaptic $GABA_A$ receptors important targets for sedative/hypnotic drugs? J Neurosci. 32:3887-3897.

Howie JG (1975) Psychological medicine. Psychotropic drugs in general practice. Br Med J. 2:177-179.

Ibrahim AE, Taraday JK, Kharasch ED (2001) Bispectral index monitoring during sedation with sevoflurane, midazolam, and propofol. Anesthesiology 95:1151-1159.

Jackson MB, Lecar H, Mathers DA, Barker JL (1982) Single channel currents activated by gamma-aminobutyric acid, muscimol, and (-)-pentobarbital in cultured mouse spinal neurons. J Neurosci. 2:889-894.

Jaggi P, Schwabe MJ, Gill K, Horowitz IN (2003) Use of an anesthesia cerebral monitor bispectral index to assess burst-suppression in pentobarbital coma. Pediatr Neurol. 28:219-222.

Jones DR, Hall SD, Jackson EK, Branch RA, Wilkinson GR (1988) Brain uptake of benzodiazepines: effects of lipophilicity and plasma protein binding. J Pharmacol Exp Ther. 245:816-822.

Khanderia U, Pandit SK (1987) Use of midazolam hydrochloride in anesthesia. Clin Pharm. 6:533-547.

Kuizenga K, Wierda JM, Kalkman CJ (2001) Biphasic EEG changes in relation to loss of consciousness during induction with thiopental, propofol, etomidate, midazolam or sevoflurane. Br J Anaesth. 86:354-360.

Långsjö JW1, Alkire MT, Kaskinoro K, Hayama H, Maksimow A, Kaisti KK, Aalto S, Aantaa R, Jääskeläinen SK, Revonsuo A, Scheinin (2012) Returning from oblivion: imaging the neural core of consciousness. J Neurosci. 32:4935-4943.

Lavoie AM, Twyman RE (1996) Direct evidence for diazepam modulation of $GABA_A$ receptor microscopic affinity. Neuropharmacology 35:1383-1392.

Li P, Eaton MM, Steinbach JH, Akk G (2013) The benzodiazepine diazepam potentiates responses of α1β2γ2L γ-aminobutyric acid type A receptors activated by either γ-aminobutyric acid or allosteric agonists. Anesthesiology 118:1417-1425.

Lin JH (2008) CSF as a surrogate for assessing CNS exposure: an industrial perspective. Curr Drug Metab. 9:46-59.

MacDonald RL, Rogers CJ, Twyman RE (1989) Barbiturate regulation of kinetic properties of the $GABA_A$ receptor channel of mouse spinal neurones in culture. J Physiol. 417:483-500.

Mathers DA, Barker JL (1980) Pentobarbital opens ion channels of long duration in cultured mouse spinal neurons. Science 209:507-509.

Meredith P (2003) Bioequivalence and other unresolved issues in generic drug substitution. Clin Ther. 25:2875-2890.

Morgan DJ, Blackman GL, Paull JD, Wolf LJ (1981) Pharmacokinetics and plasma binding of thiopental. I: Studies in surgical patients. Anesthesiology 54:468-473.

Musshoff F, Padosch S, Steinborn S, Madea B (2004) Fatal blood and tissue concentrations of more than 200 drugs. Forensic Sci Int 2004, 142: 161-210.

Olsen RW, Snowman AM (1982) Chloride-dependent enhancement by barbiturates of gamma-aminobutyric acid receptor binding. J Neurosci. 2:1812-1823.

Pieters T, Snelders S (2007) From King Kong pills to mother's little helpers--career cycles of two families of psychotropic drugs: the barbiturates and benzodiazepines. Can Bull Med Hist. 24:93-112.

Regenthal R, Krueger M, Koeppel C, Preiss R (1999) Drug levels: therapeutic and toxic serum/plasma concentrations of common drugs. J Clin Monit Comput 15: 529-544

Reves JG, Fragen RJ, Vinik HR, Greenblatt DJ (1985) Midazolam: pharmacology and uses. Anesthesiology 62:310-324.

Rogers CJ, Twyman RE, Macdonald RL (1994) Benzodiazepine and beta-carboline regulation of single GABAA receptor channels of mouse spinal neurones in culture. J Physiol. 475:69-82.

Rosenberg M, Weaver J (1991) General anesthesia. Anesth Prog. 38:172-186.

Rovira C, Ben-Ari Y (1999) Developmental study of miniature IPSCs of CA3 hippocampal cells: modulation by midazolam. Brain Res Dev Brain Res. 114:79-88.

Rüsch D, Forman SA (2005) Classic benzodiazepines modulate the open-close equilibrium in alpha1beta2gamma2L gamma-aminobutyric acid type A receptors. Anesthesiology 102:783-792.

Sancar F, Czajkowski C (2010) Allosteric modulators induce distinct movements at the GABA-binding site interface of the GABA-A receptor. Neuropharmacology. 60:520-528.

Sandler NA (2000) Additional clinical observations utilizing bispectral analysis. Anesth Prog. 47:84-86.

Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A (2012) Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. Crit Care. 16:R136.

Schwagmeier R, Alincic S, Striebel HW (1998) Midazolam pharmacokinetics following intravenous and buccal administration. Br J Clin Pharmacol. 46:203-206.

Sieghart W (2015) Allosteric modulation of GABAA receptors via multiple drug-binding sites. Adv. Pharmacol. 72:53-96.

Sieghart W, Ramerstorfer J, Sarto-Jackson I, Varagic Z, Ernst M (2012) A novel $GABA_A$ receptor pharmacology: drugs interacting with the $\alpha^+\beta^-$ interface. Brit. J. Pharmacol. 166:476-485.

Sigel E, Baur R (1988) Allosteric modulation by benzodiazepine receptor ligands of the GABAA receptor channel expressed in Xenopus oocytes. J Neurosci. 8:289-295.

Sigel E, Barnard EA (1984) A gamma-aminobutyric acid/benzodiazepine receptor complex from bovine cerebral cortex. J. Biol. Chem. 259:7219-7223.

Sigel E, Steinmann ME (2012) Structure, function, and modulation of GABAA receptors. J. Biol. Chem. 287:40224-40231.

Skerritt JH, Macdonald RL (1984) Benzodiazepine receptor ligand actions on GABA responses. Benzodiazepines, CL 218872, zopiclone. Eur J Pharmacol. 101:127-134.

Study RE, Barker JL (1981) Diazepam and (-)-pentobarbital: Fluctuation analysis reveals different mechanisms for potentiation of y-aminobutyric acid responses in cultured central neurons. Proc. Natl. Acad. Sci. USA 78:7180-7184.

Veldhorst-Janssen NM, Fiddelers AA, van der Kuy PH, Theunissen HM, de Krom MC, Neef C, Marcus MA (2011) Pharmacokinetics and tolerability of nasal versus intravenous midazolam in healthy Dutch volunteers: a single-dose, randomized-sequence, open-label, 2-period crossover pilot study. Clin Ther. 33:2022-2028.

Weaver CS, Hauter WH, Duncan CE, Brizendine EJ, Cordell WH (2007) An assessment of the association of bispectral index with 2 clinical sedation scales for monitoring depth of procedural sedation. Am J Emerg Med. 25:918-924.

White PF (1982) Comparative evaluation of intravenous agents for rapid sequence induction--thiopental, ketamine, and midazolam. Anesthesiology 57:279-284.

Winek CL, Wahba WW, Winek CL Jr, Balzer TW (2001) Drug and chemical blood-level data 2001. Forensic Sci Int 122: 107-123.

Woodcock J, Ropper AH, Kennedy SK (1982) High dose barbiturates in non-traumatic brain swelling: ICP reduction and effect on outcome. Stroke 13:785-757.

Yoo KY, Jeong CW, Jeong HJ, Lee SH, Na JH, Kim SJ, Jeong ST, Lee J (2012) Thiopental dose requirements for induction of anaesthesia and subsequent endotracheal intubation in patients with complete spinal cord injuries. Acta Anaesthesiol Scand. 56:770-776.