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Investigation into the Potential for Acquired Cross Tolerances of Amphetamines

Benjamin Weldon

Investigation into the Potential for Acquired Cross Tolerances of Amphetamines

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By

Benjamin Weldon

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Abstract

In the United States, the treatment of ADHD through use of psychostimulants in adolescents is a growing reality that many people know today and many more will know in the coming years. Although the effectiveness of psychostimulants is a known asset, the benefits of these medications may not be a permanent solution, suggesting not only the presence of a tolerance build up, but as many stimulants are prescribed in a joint effort to combat ADHD, a possible cross tolerance between two prescribed medications. There are minimal studies published that have addressed the topic of cross stimulation tolerance buildup between prescription amphetamines.

This proposal aims to probe the potential for a cross tolerance build up between multiple commonly prescribed stimulants to a given patient. By varying the concentration of a psychostimulant for a given period of time prior to injection of a structurally-similar amphetamine, this experiment will attempt to effectively determine the extent to which a cross tolerance is acquired between common ADHD combatant drugs. It is expected that rats treated with an initial, constant dose of Amphetamine will demonstrate a concentration-dependent decrease in stimulation within the central nervous system when given methylphenidate when compared to a control group treated with placebo pills followed by the injection of methylphenidate (MPD). This experiment will provide a basis for a possible future experiment using human subjects in the place of rats to establish the presence of a cross tolerance of methylphenidate and amphetamine within humans.

Introduction

On June 18th, 1971, President Richard Nixon famously announced the start of the "War on Drugs".[35] This was the beginning of the world-wide effort, led by the United States, to combat the production, distribution, and consumption of illegal drugs that had been flooding into the United States and other countries around the globe. The War on Drugs implemented various laws and policies through the *Controlled Substances Act* (CSA) that made drug trafficking increasingly difficult in an attempt to decrease criminal behavior and potential fatalities surrounding addictive substances.[36]

After the passing of the CSA, dozens of substances were deemed controlled, including Amphetamine.

There exists a subgenre of chemistry known as stereochemistry. This field is the study of molecular and atomic arrangement relative to other molecules and atoms, centering around the study of chirality, stereoisomers, and stereocenters.

A chiral molecule or atom is defined as being non-superimposable on its own mirror image, regardless of rotations and translations.[1] Any given chiral molecule will be present in one of two forms known as stereoisomers. If a molecule's stereoisomers are perfect, non-superimposable mirror images of each other, they are known as enantiomers[2]. A common example of enantiomers is the way person's hands are mirror images of each other, and reorientation cannot make them appear identical or superimposable.

Enantiomers are (separate) non-superimposable, chemically identical molecules that are distinguished by their unique trait of having ALL stereocenters differ between the two mirror image enantiomers (known as enantiomorphs)[3]. **Figure 1** provides an example of two enantiomers of lactic acid. Enantiomers will have neither mirroring planes nor equal/opposite halves, and they will possess indistinguishable physical properties[4]. When a mixture is present that contains a 1:1 ratio between enantiomorphs, this mixture is a racemate or a racemic mixture[5]. Within these mixtures, the net rotation is zero due to an equal amount of positive and negative rotation.

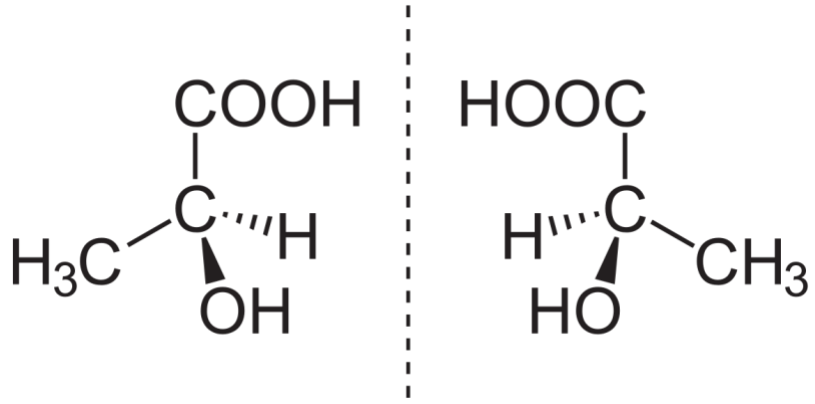

Figure 1: Enantiomers of lactic acid: (S)-(+) lactic acid (left) with (R)-(-) lactic acid (right)[9]

While enantiomers are defined as being non-superimposable mirror images, diastereomers are defined as non-superimposable NON mirror image stereoisomers[6]. **Figure 2** provides an example of two diastereomers of But-2-ene. The presence of a diastereomer is only possible when a molecule not only has multiple stereocenters, but more than one of them have different configurations[7]. Diastereomers vary from enantiomers in that while enantiomorphs must differ in ALL stereocenters and are non-superimposable "mirror images" of each other, diastereomers are not.[8] Rather, diastereomers have the same molecular formula and same connectivity but different arrangement, meaning they are non-superimposable NON-mirror images of each other. While two enantiomorphs will have identical physical properties, diastereomers will not. They differ in both physical and chemical properties and reactivity.

It is possible for a molecule that contains multiple stereocenters to contain enantiomers as well as diastereomers depending on the spatial orientation surrounding the stereocenters.

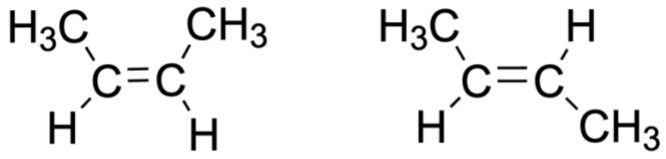

Figure 2: Two diastereomers of but-2-ene: cis-but-2-ene (left) with trans-but-2-ene (right)[10]

Molecules such as carbohydrates, DNA, and proteins are chiral compounds, and the understanding of stereochemistry and orientation are critical in the study of organic chemistry or how organisms are able to live. It is known that two enantiomorphs typically differ in pharmacodynamics and enzyme metabolism activity.[11] This leads to a preference in organic pathways for one enantiomer over the other.[11] In pharmaceutical drugs, an organism's preference for a single enantiomer will often lead to varying levels of effectiveness between enantiomers of the same drug.

Amphetamine is a potent central nervous system (CNS) stimulant. The amphetamine molecule exists as one of two non-superimposable optical isomers also known as enantiomers. The two enantiomers are named *d*-amphetamine and *l*-amphetamine, also known as levoamphetamine and dextroamphetamine. Both isomers are found in the racemic amphetamine prescribed today. Despite equal abundance in prescription amphetamine, dextroamphetamine is ground to have more pronounced effects when compared to its levoamphetamine counterpart by 3 to 5 times[53]. The term amphetamine is specific to a chemical where each pure amine enantiomer exists in equal parts, but the term has been informally broadened to include either, both, or a combination of the enantiomers.

Amphetamine and derivatives of it are commonly used in the medical community for a variety of purposes, prescribed in low, controlled dosages. Common medical uses of amphetamine include treatment of attention deficit hyperactivity disorder (ADHD), narcolepsy, and obesity. Side effects include alertness, improved reaction time, improved cognitive control, fatigue resistance.[37] The CSA deemed amphetamine a controlled substance due to its potential to cause addiction and dependence.[38] Despite this, amphetamine and its derivatives are commonly used today recreationally, in dosages much larger than medically recommended. Physical side effects of large recreational consumption of amphetamine(s) can include rapid muscle breakdown, hypertension, psychosis (delusion and paranoia), hypotension, erectile dysfunction (in males), constipation, diarrhea, nausea, appetite loss, nosebleeds, profuse sweating, weight loss, and more.[39] Mental side effects of large recreational consumption of amphetamines can include alertness, apprehension,

concentration, self-confidence, mood swings, insomnia, grandiosity, irritability, and an increase of obsessive behaviors.[40]

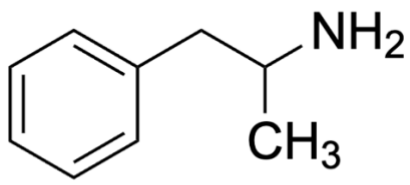

Figure 3: General structure of an Amphetamine[13]

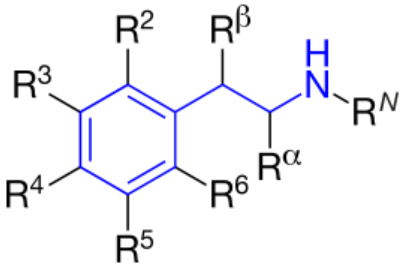

Figure 4: General structure of a substituted phenethylamine[12]

Amphetamines (Figure 3) are part of the substituted phenethylamine (Figure 4) chemical class and the parent compound of the substituted amphetamine chemical class. The substituted phenethylamine class is a chemical class composed of molecules with the shared structural base known as a phenethylamine. The structure of any substituted phenethylamine is made of a phenyl ring connected to an amino group through the use of two carbon side chains.[59] The substituted phenethylamine class and the substituted amphetamine class slightly differ on their base structure, with substituted amphetamines being a more specific type of substituted phenethylamines.[59] A substituted amphetamine structure contains the base amphetamine

structure ($C_9H_{13}N$).[58] The carbon atom adjacent to the primary amine is a stereogenic center. Any compound synthesized through replacement or substitution of one or more of the hydrogen atoms on the amphetamine base structure is classified as a substituted amphetamine.

Attention Deficit Hyperactivity Disorder (ADHD)

Attention Deficit Hyperactivity Disorder (ADHD) is a common neurodevelopmental disorder affecting approximately 5% of the population, although some figures suggest a higher number closer to 18%.[41] ADHD is often identified during adolescence and is characterized by inattention, hyperactivity, impulsivity, and troubles with concentration further exaggerated when in a group or academic setting[42]. Studies suggest a ratio of 3:1 of boys afflicted to girls afflicted.[56] While ADHD is often first observed during adolescence, symptoms of ADHD are still common in adults previously diagnosed as children. The existence of ADHD in adults is a topic of discussion without a definitive conclusion, with symptoms often decreasing as a child ages into adulthood. ADHD is associated with traits often leading to substance abuse.[43] Interestingly enough, patients diagnosed with ADHD and treated with psychostimulants (e.g., methylphenidate or amphetamine) do not demonstrate increased rates of dependance. Given this, ADHD treatment has widely been treated with the application of psychostimulants. Three leading stimulants widely used are methylphenidate (brand name: Ritalin), amfetamine (brand name: Adderall), and dex-methylphenidate. Once- or twice-daily oral consumption of these medications has been shown to effectively combat ADHD.

ADHD is associated with functional impairments of the neurotransmitters involved with the uptake of dopamine and norepinephrine in the prefrontal cortex and locus coeruleus. By preventing both the dopamine active transporter (DAT) and norepinephrine active transporter (NAT), methylphenidate acts as a norepinephrine-dopamine reuptake inhibitor (NDRI). The disruptive action to both the dopamine and norepinephrine transporters results in an increase in the extracellular concentrations of both dopamine and

norepinephrine, ultimately increasing the longevity and neurotransmission of both chemicals. The increased activity within the prefrontal cortex and locus coeruleus additionally result in the symptoms commonly associated with methylphenidate/amphetamines, such as alertness, improved attention, greater cognitive control, and reduced fatigue. MRI studies have shown methylphenidate to produce a much higher effect upon DAT when compared to NAT. Additionally, studies have shown continuous, long-term treatment of ADHD with methylphenidate decreases abnormalities in the structure of the brain in individuals diagnosed with ADHD.

Another drug, commonly branded as an alternative to *d*-threo-methylphenidate, is its enantiomer, dexmethylphenidate. Having been shown to not only affect the brain in very similar ways, but also have a very high risk of addiction, dexmethylphenidate ($C_{14}H_{19}NO_2$) is another strong central nervous system stimulant that has been shown to be an effective alternative in the treatment of ADHD. Studies have shown that when comparing two methylphenidate (MPH) isomers, *d*-mph and *l*-mph, chirality is important, as the two chemicals not only work differently, but may hinder the effectiveness of each other[17].

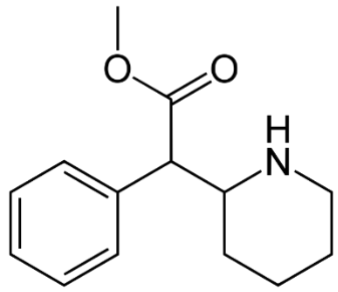

Figure 5: Skeletal structure of Methylphenidate (MPH)[14]

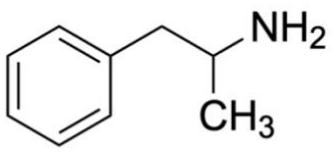

Figure 6: Skeletal structure of Amphetamine[15]

Methylphenidate

Originally developed in 1944, MPH (Figure 5) was first used as an analeptic before being recognized as an effective psychostimulant.[44] Containing two different chiral centers, MPH exists as one of four stereo-isomers: *d*-threo-methylphenidate, *l*-threo-methylphenidate, *d*-erythro-methylphenidate and *l*-erythro-methylphenidate. Upon investigation, it was found that neither erythro- isomer produced the stimulating effect desired within the central nervous system.[45] Once discovered, prescribed MPH was made to only contain *d*- or *l*-threo-methylphenidate. As of today, nearly all prescribed ADHD medication is also racemic containing a 1:1 ratio of *d*-threo-methylphenidate and *l*-threo-methylphenidate. Recently, labs have been working with the *d*-threo-methylphenidate isomer in an attempt to improve upon the "pharmacologically active" isomer, also known as dexmethylphenidate (brand name: Focalin).

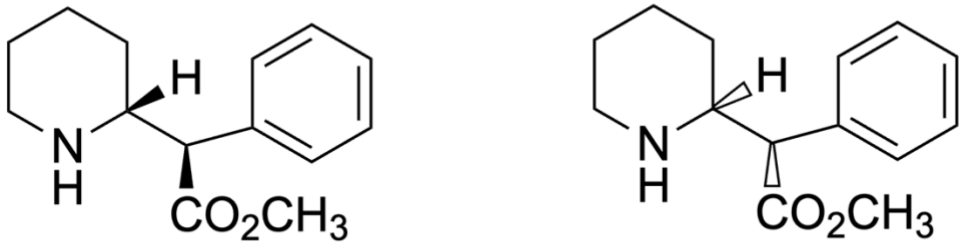

Figure 7: skeletal structure of *d*-threo-methylphenidate (left) and l-threo-methylphenidate[52] (right).

Metabolism

Typically administered through capsule form orally to a patient, Methylphenidate ($C_{14}H_{19}NO_2$) is a psycho-stimulant drug acting upon the central nervous system.[16] In humans, *dl*-threo-methylphenidate

absorption is near 100% despite having relatively low bioavailability[17]. This is due to a phenomenon known as the "First Pass Effect". The First Pass effect is an event that occurs in the metabolic pathway of some drugs. During the metabolic process, a drug gets consumed and processed at a specific location within an organism, which results in a lowered concentration of said drug within the organism's circulation. This phenomenon is often associated with common sites of metabolism, such as the liver.[17] Following consumption of MPH, the drug undergoes hydrolysis (catalyzed by carboxylesterase 1) to produce *dl*-threo-ritalinic acid.[17] The process of de-esterification is the primary pathway for the processing of MPH, which produces Ritalinic acid (as seen in Figure 8).

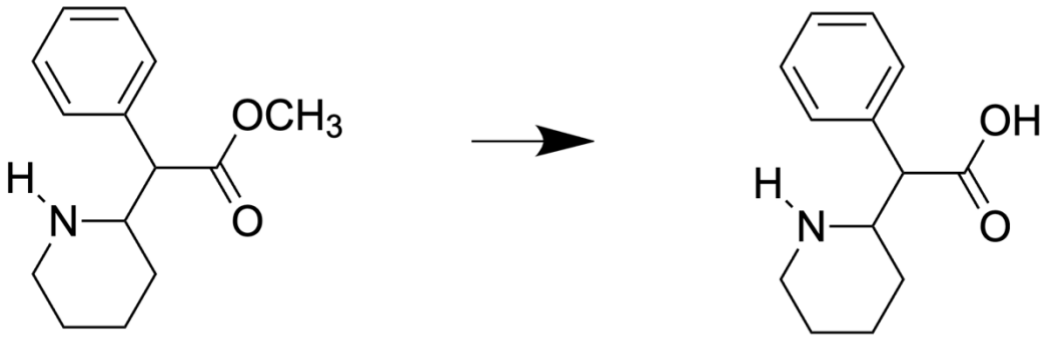

Figure 8: Methylphenidate undergoing hydrolysis to form Ritalinic acid

Mechanism of Action

Orally consumed and processed by ritalic acid in the liver, methylphenidate (MPH) works through use of non-competitive blocking to prevent the reuptake of dopamine and noradrenaline back into a neuron's terminal by blocking both the noradrenaline transporter (NAT) as well as the dopamine transporter (DAT). The inhibition methylphenidate produces is largely more effective than other amphetamines. By

inhibiting transport of norepinephrine (NE) and dopamine (DA) out of the neurons, the concentration of NE and DA are both increased. Increased levels of NE and DA within the synaptic cleft leads to stimulation within the central nervous system and brain.[17]

Pharmacology of MPH

The primary mechanisms involved in the movement of MPH through the CNS are monoamine release and monoamine reuptake inhibition.[17] The two differ from one another in that monoamine release acts within the terminal while monoamine reuptake inhibition acts outside of the terminal. The transport of the monoamines is prevented by the reuptake inhibitors binding due to the binding that occurs on the outside of the nerve's terminal. This produces an extended effect from the monoamine. Furthermore, it should be noted that releasing agents are not enzymes, but rather substrates utilized for reuptake carrying. The releasing agents play a key role in the uptake process, as they remove unwanted monoamine neurotransmitters and disrupt them from their storage sites.

Acquired tolerance to MPH

As previously mentioned, a key factor in the treatment of ADHD involves continuous prescription of stimulant medication. Studies suggest that up to 25% of patients develop tolerance to psychostimulant medication as treatment continues. Unfortunately, due to lack of clinical research, there is a lack of data to highlight what could be a growing problem.[57]

Tachyphylaxis is a medical term to describe rapidly diminishing response to successive usage of a drug, rendering a decrease in desired effect. Tachyphylaxis is often related to a depletion of neurotransmitters. Studies have shown an onset of acute tachyphylaxis with MPH using PET scans where MPH was administered intravenously.[57] A review of retrospective data suggested that among patients with ADHD being treated with MPH, patients prescribed more than 60 mg of MPH per day developed a

tolerance, suggesting that an MPH tolerance can be developed in a timeframe ranging from days up to a year.[57]

Proposal

Cross tolerance is an observable phenomenon of tolerance acquisition that can occur between two similar pharmaceutical compounds. After review of recent literature, it has been observed that there is documentation on the development of a tolerance to stimulant medication for ADHD.[46] The next question to ask is given the possibility for tolerance acquisition, is there also a possibility for the development of cross tolerance between multiple commonly prescribed psychostimulants? This study aims to propose a method to answer this question. Laboratory rats will be separated into four groups: one control group and three experimental groups. The three experimental groups will be exposed to varying levels of amphetamine before extended exposure to methylphenidate, while the control group will not be exposed to amphetamine prior to its methylphenidate treatment. This experiment plans to determine if there is a decrease in physical stimulation after extended exposure to different amounts of stimulating drugs in hopes of addressing the existence and degree of an acquired cross tolerance.

Materials and Methods

General method

Four-month-old, adult, male Sprague Dawley Rats (n=400) weighing an average of 500 grams that have not been exposed to either methylphenidate or any other type of amphetamine will be obtained from a breeder. Only male rats will be used in this study given the possibility of variance between male and female rats due to hormonal differences or other unknown confounding factors. All rats will be housed in

a controlled environment consisting of a 12 hour “day” (light) cycle followed by a 12 hour “night” (dark) cycle that will simulate standard days. Water and standard food consisting of fruits, vegetables, and seeds will be provided and readily available for consumption.

Rats will be split into four groups (n = 100 per group) and housed in identical environments (i.e., identical light cycles and food). The experimental group will be denoted by the names “Group A”, “Group B” and “Group C”, with the control group being denoted by the name “Group D”.

For 100 days (light cycles), Groups A, B, and C will be administered amphetamine delivered via injection. This period will be referred to as the treatment period. It is known that the rats will develop a resistance to the daily administered amphetamine[47]. In order to create a range, administration rates will be dosed at 1 mg/kg for Group A, 2.5 mg/kg for Group B, and 5 mg/kg for Group C. Given the 0.5 kg weight of the test subjects, administered amounts will be 0.5 mg, 1.25 mg and 2.5 mg for groups A, B and C respectively. Administration will continue for the duration of the treatment period. Following the 100 days, the amphetamine injections will be replaced with methylphenidate injections at a constant dosage of 0.125 mg per rat/per day. At this point, vital signs of all rats will be monitored before administration of MPH and 30 minutes after administration. Vitals to be monitored will include blood pressure (via tail-cuff method),[48] heart rate bpm (via cardiometer), and dopamine levels within the brain (via microdialysis).[49] This data will be recorded via computer collection.

This experiment will contain one control group (n = 100), called Group D. This group will receive placebo pills in place of amphetamine for the first 100-day period of the experiment. Following these 100 days, Group D will be administered 0.125 mg of methylphenidate per rat/ per day. Again, vitals to be monitored will include blood pressure (via tail-cuff method), heart rate bpm (via cardiometer), and lastly dopamine levels within the brain (via microdialysis).[48,49] This data will be recorded via computer collection.

Upon completion of the treatment period, all rats will be administered a non-amphetamine stimulant drug called Modafinil via tablet that will mitigate amphetamine withdrawal symptoms across all groups and help wean test subjects off amphetamines.[50]

Discussion

This experiment is intended to understand the relationship between two chemically similar pharmaceutical drugs, amphetamine and methylphenidate, in hopes of being able to more accurately prescribe medication to humans as well as gain insight into the development of cross tolerances between pharmaceuticals. The varying concentrations of amphetamine are intended to provide starting points for the rats' bodies to be accustomed to constant stimulation.

The 100-day treatment phase of the experiment will provide rats with varying, constant doses of amphetamine. It is expected that increased amounts of amphetamine will lead to increased tolerance within experimental groups. It is expected given varying levels of treatment (0.5 mg, 1.25 mg and 2.5 mg) to observe different levels of tolerance to the drug. It would then be reasonable to assume that the rats administered 0.5 mg amphetamine per day will record a larger change in blood pressure, heart rate increase, and dopamine levels (within the brain) than rats in a group with 1.25 mg of amphetamine administered per day, and even less than the rats in the group with 2.5 mg of amphetamine administered per day once full tolerance is established. The expectation is that a larger change in physiological vital signs, including blood pressure, heart rate and dopamine levels, will reflect a smaller tolerance to the newly administered drug in the second half of the test. Test subjects demonstrating little response to new stimuli indicate(?) acquired tolerance to past stimuli.

It is expected that Groups A, B, and C will all experience varying levels of blood pressure increase, heart rate increase, and dopamine increase after the administration of methylphenidate, but much less than the changes observed in Group D, which was not administered amphetamine for 100 days prior to the administration of methylphenidate. Most rats should develop some amount of tolerance to amphetamine, as demonstrated in previous studies.[51]

This data will show that prior exposure to a chemically similar compound (amphetamine) will yield a weaker response to methylphenidate after continuous exposure. There are multiple ways in which future studies can build upon the findings in this experiment. First, the experiment needs to be repeated with

human subjects in the place of rats. This would provide insight into the prescription of ADHD medication to numerous patients globally. Additionally, this experiment would provide insight into the possible differences of metabolism and amphetamine response when comparing rats to humans.

A second possible study could be varying the treatment period's length to see if tolerance plateaus at a certain point. What would happen if the treatment period was 10 days? 300 days? Do the findings vary as time is changed? What is the minimal time of prior exposure to demonstrate an observable cross tolerance?

A third possible study that may be worth conducting would be to repeat the study entirely but substitute the four-month-old, adult, male Sprague Dawley Rats with Four-month-old, adult, female Sprague Dawley Rats and compare results to gather insight if varying the sex of the rat varies the result.

A final possible study that could be conducted would be a comparison of other chemically similar drugs. For example, methylphenidate and methamphetamine. Since both of these drugs are actively used in the medical world,[54,55] insight into their relationship with each other could give medical providers a more accurate method of assistance

Conclusion

This experiment will provide insight into the existence of an observable cross tolerance between amphetamine and methylphenidate, and if it exists, if varying amounts of prior exposure to amphetamines varies the strength of the observed cross tolerance. Control group D is expected to lack all tolerance to MPH after an initial treatment period due to lack of administered Amphetamine. Groups A, B, and C are expected to develop a mild tolerance following the initial treatment period, with Group A having the smallest built up tolerance, Group B having a mild amount, and Group C having the largest tolerance to the new drug.

This experiment could provide an effective base for future research that could determine the interaction between various types of psychostimulant drug and the potential for an acquired cross tolerance.

References

1. Bruice, Paula Yurkanis. *Organic Chemistry*. Pearson/Prentice Hall, 2004.
2. McNaught, Alan D., and Andrew Wilkinson. *Compendium of Chemical Terminology: IUPAC Recommendations*. Blackwell Science, 1997.
3. McNaught, Alan D., and Andrew Wilkinson. *Compendium of Chemical Terminology: IUPAC Recommendations*. Blackwell Science, 1997.
4. Tester, R.F., and J. Karkalas. "Enantiomer." *Enantiomer - an Overview | ScienceDirect Topics*, https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/enantiomer.
5. McNaught, Alan D., and Andrew Wilkinson. *Compendium of Chemical Terminology: IUPAC Recommendations*. Blackwell Science, 1997.
6. The International Union of Pure and Applied Chemistry (IUPAC). "Diastereoisomerism." *IUPAC*, 2019, https://goldbook.iupac.org/terms/view/D01679.
7. Garrett, Reginald H., and Charles M. Grisham. *Biochemistry*. Thomson/Brooks/Cole, 2005.
8. The International Union of Pure and Applied Chemistry (IUPAC). "Diastereoisomerism." *IUPAC*, 2019, https://goldbook.iupac.org/terms/view/D01679.
9. "Enantiomer." *Wikipedia*, Wikimedia Foundation, 15 Mar. 2023, https://en.wikipedia.org/wiki/Enantiomer.
10. Raut, Bimal. "Diastereomers: Definition, Characteristics, and Examples." *Chemistry Notes*, 23 Apr. 2022, https://chemistnotes.com/organic/diastereomers-definition-characteristics-and-examples.
11. Lu, Hong. "PubMed." *National Center for Biotechnology Information*, U.S. National Library of Medicine, 2007, https://pubmed.ncbi.nlm.nih.gov/?db=PubMed.
12. "Result Filters." *National Center for Biotechnology Information*. U.S. National Library of Medicine. Web. 07 Jan. 2014. <http://www.ncbi.nlm.nih.gov/pubmed/18160646>.
13. "File:Amfetamin.svg." *Wikipedia*, Wikimedia Foundation, https://en.wikipedia.org/wiki/File:Amfetamin.svg.
14. "File:Methylphenidate-2d-Skeletal.svg." *Wikipedia*, Wikimedia Foundation, 2016, https://en.wikipedia.org/wiki/File:Methylphenidate-2D-skeletal.svg.
15. "File:Dexmethylphenidate Structure.svg." *Wikipedia*, Wikimedia Foundation, https://en.wikipedia.org/wiki/File:Dexmethylphenidate_structure.svg.
16. Gahr, Maximilian, and Paul L Plener. "Methylphenidate." *Methylphenidate - an Overview | ScienceDirect Topics*, 2016,

https://www.sciencedirect.com/topics/neuroscience/methylphenidate#:~:text=Methylphenidate%20is%20a%20psychostimulant%20that,for%20the%20management%20of%2n.d.HD.&text=Methylphenidate's%20mode%20of%20action%20appears,via%20inhibition%20of%20the%20DAT.

17. Heal, David J, and David M Pierce. “Methylphenidate and Its Isomers: Their Role in the Treatment of Attention-Deficit Hyperactivity Disorder Using a Transdermal Delivery System.” *CNS Drugs*, U.S. National Library of Medicine, https://pubmed.ncbi.nlm.nih.gov/16953648/.
18. Bruice, Paula Yurkanis. *Organic Chemistry*. Pearson/Prentice Hall, 2004.
19. McNaught, Alan D., and Andrew Wilkinson. *Compendium of Chemical Terminology: IUPAC Recommendations*. Blackwell Science, 1997.
20. McNaught, Alan D., and Andrew Wilkinson. *Compendium of Chemical Terminology: IUPAC Recommendations*. Blackwell Science, 1997.
21. Tester, R.F., and J. Karkalas. “Enantiomer.” *Enantiomer - an Overview | ScienceDirect Topics*, https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/enantiomer.
22. McNaught, Alan D., and Andrew Wilkinson. *Compendium of Chemical Terminology: IUPAC Recommendations*. Blackwell Science, 1997.
23. The International Union of Pure and Applied Chemistry (IUPAC). “Diastereoisomerism.” *IUPAC*, 2019, https://goldbook.iupac.org/terms/view/D01679.
24. Garrett, Reginald H., and Charles M. Grisham. *Biochemistry*. Thomson/Brooks/Cole, 2005.
25. The International Union of Pure and Applied Chemistry (IUPAC). “Diastereoisomerism.” *IUPAC*, 2019, https://goldbook.iupac.org/terms/view/D01679.
26. “Enantiomer.” *Wikipedia*, Wikimedia Foundation, 15 Mar. 2023, https://en.wikipedia.org/wiki/Enantiomer.
27. Raut, Bimal. “Diastereomers: Definition, Characteristics, and Examples.” *Chemistry Notes*, 23 Apr. 2022, https://chemistnotes.com/organic/diastereomers-definition-characteristics-and-examples.
28. Lu, Hong. “PubMed.” *National Center for Biotechnology Information*, U.S. National Library of Medicine, 2007, https://pubmed.ncbi.nlm.nih.gov/?db=PubMed.
29. "Result Filters." *National Center for Biotechnology Information*. U.S. National Library of Medicine. Web. 07 Jan. 2014. <http://www.ncbi.nlm.nih.gov/pubmed/18160646>.
30. “File:Amfetamin.svg.” *Wikipedia*, Wikimedia Foundation, https://en.wikipedia.org/wiki/File:Amfetamin.svg.
31. “File:Methylphenidate-2d-Skeletal.svg.” *Wikipedia*, Wikimedia Foundation, 2016, https://en.wikipedia.org/wiki/File:Methylphenidate-2D-skeletal.svg.

32. “File:Dexmethylphenidate Structure.svg.” *Wikipedia*, Wikimedia Foundation, https://en.wikipedia.org/wiki/File:Dexmethylphenidate_structure.svg.
33. Gahr, Maximilian, and Paul L Plener. “Methylphenidate.” *Methylphenidate - an Overview | ScienceDirect Topics*, 2016, https://www.sciencedirect.com/topics/neuroscience/methylphenidate#:~:text=Methylphenidate%20is%20a%20psychostimulant%20that,for%20the%20management%20of%2n.d.HD.&text=Methylphenidate's%20mode%20of%20action%20appears,via%20inhibition%20of%20the%20DAT.
34. Heal, David J, and David M Pierce. “Methylphenidate and Its Isomers: Their Role in the Treatment of Attention-Deficit Hyperactivity Disorder Using a Transdermal Delivery System.” *CNS Drugs*, U.S. National Library of Medicine, https://pubmed.ncbi.nlm.nih.gov/16953648/.
35. Foster, Anne L. “The Long War on Drugs.” *Oxford Research Encyclopedia of American History*, 2017, https://doi.org/10.1093/acrefore/9780199329175.013.402.
36. DEA. "The Controlled Substances Act."*Drug Enforcement Agency*, 25 July, 2018, https://www.dea.gov/drug-information/csa Accessed 13 April, 2023.
37. Stahl, Stephen M., et al. “Amphetamine.” *Stahl's Essential Psychopharmacology: Prescriber's Guide*, Cambridge University Press, Cambridge, United Kingdom, 2021, pp. 45–51.
38. “Controlled Substance Schedules.” *DEA Diversion Control Division*, https://www.deadiversion.usdoj.gov/schedules/.
39. “Amphetamine Effects-Short-Term and Long-Term Use.” Edited by Patrick Condron, *American Addiction Centers DrugAbuse.com*, 19 Dec. 2022, https://drugabuse.com/stimulants/amphetamine/effects-use/.
40. Scher, Lorin M. “Amphetamine-Related Psychiatric Disorders.” *Medscape*, 27 Dec. 2022, https://emedicine.medscape.com/article/289973-overview.
41. Song, Peige, et al. “The Prevalence of Adult Attention-Deficit Hyperactivity Disorder: A Global Systematic Review and Meta-Analysis.” *Journal of Global Health*, vol. 11, 2021, https://doi.org/10.7189/jogh.11.04009.
42. “Attention-Deficit/Hyperactivity Disorder in Children and Teens: What You Need to Know.” *National Institute of Mental Health*, U.S. Department of Health and Human Services, https://www.nimh.nih.gov/health/publications/attention-deficit-hyperactivity-disorder-in-children-and-teens-what-you-need-to-know#:~:text=ADHD%20is%20a%20developmental%20disorder,the%20teen%20years%20and%20adulthood.

43. Davis, Caroline, et al. “Attention-Deficit/Hyperactivity Disorder in Relation to Addictive Behaviors: A Moderated-Mediation Analysis of Personality-Risk Factors and Sex.” *Frontiers in Psychiatry*, vol. 6, 2015, https://doi.org/10.3389/fpsyt.2015.00047.
44. Panizzon L. The preparation of pyridines- and piperidine-arylacetonitriles and the alkylation products of transformations (Pt I). Helv Chim Acta 1944; 27: 1748–56
45. Szporny, L., and P. Görög. “Investigations into the Correlations between Monoamine Oxidase Inhibition and Other Effects Due to Methylphenydate and Its Stereoisomers.” *Biochemical Pharmacology*, vol. 8, no. 3, 1961, pp. 263–268., https://doi.org/10.1016/0006-2952(61)90100-9.
46. Handelman, Kenneth, and Fernando Sumiya. “Tolerance to Stimulant Medication for Attention Deficit Hyperactivity Disorder: Literature Review and Case Report.” *Brain Sciences*, vol. 12, no. 8, 2022, p. 959., https://doi.org/10.3390/brainsci12080959.
47. Götestam, K. Gunnar. “Acute Tolerance to Amphetamine in Rats.” *Psychopharmacology*, vol. 49, no. 1, 1976, pp. 113–115., https://doi.org/10.1007/bf00427481.
48. Fritz, M., and G. Rinaldi. “Blood Pressure Measurement with the Tail-Cuff Method in Wistar and Spontaneously Hypertensive Rats: Influence of Adrenergic- and Nitric Oxide-Mediated Vasomotion.” *Journal of Pharmacological and Toxicological Methods*, vol. 58, no. 3, 2008, pp. 215–221., https://doi.org/10.1016/j.vascn.2008.08.002.
49. Chefer, Vladimir I., et al. “Overview of Brain Microdialysis.” *Current Protocols in Neuroscience*, vol. 47, no. 1, 2009, https://doi.org/10.1002/0471142301.ns0701s47.
50. Greenblatt, Karl, and Ninos Adams. “Modafinil.” *National Center for Biotechnology Information*, National Library of Medicine, https://www.ncbi.nlm.nih.gov/books/NBK531476/.
51. Götestam, K. Gunnar. “Acute Tolerance to Amphetamine in Rats.” *Psychopharmacology*, vol. 49, no. 1, 1976, pp. 113–115., https://doi.org/10.1007/bf00427481.
52. Heal, David J, and David M Pierce. “Methylphenidate and Its Isomers.” *CNS Drugs*, vol. 20, no. 9, 2006, pp. 713–738., https://doi.org/10.2165/00023210-200620090-00002.
53. Lori A. Knackstedt, et al. “Levoamphetamine.” *Levoamphetamine - an Overview | ScienceDirect Topics*, 2013, https://www.sciencedirect.com/topics/neuroscience/levoamphetamine#:~:text=Amphetamine%20exists%20in%20two%20active,et%20al.%2C%201978).
54. “Methylphenidate: Uses, Dosage, Side Effects.” *Drugs.com*, 2 Apr. 2023, https://www.drugs.com/methylphenidate.html.
55. IBM micromedex. “Methamphetamine (Oral Route) Description and Brand Names.” *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 1 Feb. 2023,

https://www.mayoclinic.org/drugs-supplements/methamphetamine-oral-route/description/drg-20071824.

56. Skogli, Erik Winther, et al. “ADHD in Girls and Boys--Gender Differences in Co-Existing Symptoms and Executive Function Measures.” *BMC Psychiatry*, U.S. National Library of Medicine, 9 Nov. 2013, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3827008/.
57. Handelman, Kenneth, and Fernando Sumiya. “Tolerance to Stimulant Medication for Attention Deficit Hyperactivity Disorder: Literature Review and Case Report.” *Brain Sciences*, U.S. National Library of Medicine, 22 July 2022, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9332474/.
58. National Center for Biotechnology Information. "PubChem Compound Summary for CID 3007, Amphetamine" *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Amphetamine. Accessed 14 April, 2023.
59. Catharine Montgomery, and Carl A. Roberts. “Substituted Phenethylamine.” *Substituted Phenethylamine - an Overview | ScienceDirect Topics*, https://www.sciencedirect.com/topics/neuroscience/substituted-phenethylamine.