



## Xenobiotica

the fate of foreign compounds in biological systems

ISSN: 0049-8254 (Print) 1366-5928 (Online) Journal homepage: <https://www.tandfonline.com/loi/ixen20>

# Ascending single dose pharmacokinetics of cytosine in healthy adult smokers

Soo Hee Jeong, Janie Sheridan, Chris Bullen, David Newcombe, Natalie Walker & Malcolm Tingle

To cite this article: Soo Hee Jeong, Janie Sheridan, Chris Bullen, David Newcombe, Natalie Walker & Malcolm Tingle (2018): Ascending single dose pharmacokinetics of cytosine in healthy adult smokers, *Xenobiotica*, DOI: [10.1080/00498254.2018.1557760](https://doi.org/10.1080/00498254.2018.1557760)

To link to this article: <https://doi.org/10.1080/00498254.2018.1557760>



Accepted author version posted online: 11 Dec 2018.



Submit your article to this journal [↗](#)



Article views: 33



View Crossmark data [↗](#)

# **Ascending single dose pharmacokinetics of cytisine in healthy adult smokers**

Soo Hee Jeong<sup>a,b\*</sup>, Janie Sheridan<sup>a,b</sup>, Chris Bullen<sup>b,c</sup>, David Newcombe<sup>b,c</sup>, Natalie Walker<sup>b,c</sup> and Malcolm Tingle<sup>b,d</sup>

*<sup>a</sup>School of Pharmacy, Faculty of Medical and Health Sciences, the University of Auckland, Auckland, New Zealand; <sup>b</sup>Centre for Addiction Research, Faculty of Medical and Health Sciences, the University of Auckland; <sup>c</sup>School of Population Health, Faculty of Medical and Health Sciences, the University of Auckland, Auckland, New Zealand; <sup>d</sup>Department of Pharmacology & Clinical Pharmacology, School of Medical Sciences, Faculty of Medical and Health Sciences, the University of Auckland, Auckland, New Zealand*

\*Author for Correspondence.

School of Pharmacy, Faculty of Medical and Health Sciences, the University of Auckland, Private Bag, 92019, Auckland, 1142, New Zealand. Email: s.jeong@auckland.ac.nz Tel: +64 9 923 6023

# **Ascending single dose pharmacokinetics of cytisine in healthy adult smokers**

## **Abstract**

1. Cytisine, a partial agonist for the  $\alpha_4\beta_2$ -nAChR, is used as a smoking cessation medication. Cytisine's current dosing is complex and involves taking 1.5 mg several times a day. The aim of this study was to explore the effect of dose on the pharmacokinetics and safety of cytisine after a single dose in healthy adult smokers.

2. Participants were assigned to one of three groups (n=6 in each group) to receive a single oral dose of 1.5, 3 or 4.5 mg of cytisine. Blood samples were collected up to 24 hours post dose. Pulse, blood pressure and respiratory rate were measured. Adverse effects were recorded.
3. Cytisine reached peak plasma concentration 1 – 2 hours post dose in all participants irrespective of dose, with no dose-dependent changes in the elimination phase. Mean (SD) cytisine exposure ( $AUC_{0-24h}$ ) were 81.9 (15.8), 181.9 (40.8) and 254.5 (48.1) ng.h/mL following 1.5, 3 and 4.5 mg respectively.
4. Cytisine appears to have predictable pharmacokinetics following a single dose of up to 4.5 mg and may be safe given as a single 4.5 mg dose, which is 3-fold greater than the recommended dose taken at one time. This study is registered in ClinicalTrials.gov (ID:NCT02585024).

Keywords: Cytisine; plant alkaloid; plasma concentration; pharmacokinetics; dose escalation; safety; smoking cessation therapy

## Introduction

Cytisine, a plant-derived alkaloid, is a partial agonist for the alpha-4-beta-2-nicotinic acetylcholine receptor (Tutka and Zatonski, 2006). It has been shown to be more effective than placebo and superior to nicotine replacement therapy in achieving long-term abstinence in smokers (West *et al.*, 2011, Cahill *et al.*, 2012, Walker *et al.*, 2014).

Cytisine is available as a smoking cessation medication in Eastern and Central Europe (marketed as Tabex<sup>®</sup> and Desmoxan<sup>®</sup>) (Prochaska *et al.*, 2013) and has become available in Canada (Cravv<sup>®</sup>) since mid-2017. The main advantage of cytisine therapy

compared to other smoking cessation pharmacotherapies is its current low cost, and there are calls for cytisine to become licensed world-wide (Walker *et al.*, 2016).

Cytisine is commercially available in different formulations including an oral tablet and an oral capsule (each tablet/capsule contains 1.5 mg of cytisine). The current standard dosing for both formulations is identical and involves a tapering dose over 25 days: Days 1 – 3: one tablet/capsule (1.5 mg) 6 times a day (each dose 2 hours apart), days 4 – 12: one tablet/capsule 5 times a day (each dose 2.5 hours apart), days 13 – 16: one tablet/capsule 4 times a day (each dose 3 hours apart), days 17 – 20: one tablet/capsule 3 times a day (each dose 5 hours apart) and days 21 – 25: one tablet/capsule twice a day (each dose 6 hours apart). Smokers are recommended to quit smoking on Day 5 of the treatment. However, this complex dosing regimen lacks pharmacokinetic basis. As well as being complex, the cytisine dosing regimen is shorter than other smoking cessation medications (e.g. nicotine replacement therapy: 8 weeks (West *et al.*, 2011) or varenicline: 12 weeks (Ebbert *et al.*, 2010)), but it is unknown whether this is an optimal treatment regimen for smokers.

Previously it has been shown that cytisine has a relatively short plasma half-life (4.8 hours) (Jeong *et al.*, 2014) and with the current recommended dosing regimen, does not reach steady state throughout the duration of the treatment (Jeong *et al.*, 2017). There have been no reported attempts to establish optimal or even therapeutic concentrations of cytisine in smokers. Despite the lack of pharmacological evidence to support the current recommended dosing regimen, studies to date have used this dosing regimen to test the effectiveness and safety of cytisine (Vinnikov *et al.*, 2008, West *et al.*, 2011, Walker *et al.*, 2014).

As well as pharmacokinetic considerations, a complex dosing regimen may be a barrier to adherence to cytisine therapy and a disadvantage over other smoking

cessation pharmacotherapies that have a simpler dosing regimen; for example, varenicline therapy involves taking one or two tablets once or twice daily for the whole treatment period of 12 weeks (Ebbert *et al.*, 2010). In a previous study that involved smokers following the current recommended standard dosing regimen for cytisine, only 53% of the participants self-reported taking at least 80 tablets during the treatment period when 100 tablets should have been taken (Walker *et al.*, 2014). In that same study, the dosing regimen and having to remember to take the tablets were in the top three reasons why participants disliked taking cytisine. It would thus be worthwhile to explore new dosing regimens that involve fewer cytisine doses per day, especially during the first few days of the treatment when smokers are required to take cytisine as often as every 2 hours for 6 times a day.

In order to explore whether different dosing schedules are possible, it is necessary to first, investigate whether there are dose-dependent changes in the pharmacokinetics and safety of cytisine. The aim of the current study was to investigate the pharmacokinetics of cytisine following one of three single oral doses (1.5, 3 and 4.5 mg) in healthy adult smokers. Heart rate, blood pressure and respiratory rate were measured and adverse effects recorded throughout the study to determine whether there are dose-dependent changes to these measures. Although this is a small pilot study, findings from this study will inform the design of larger, randomised and controlled trials that investigate alternative dosing regimen of cytisine for smoking cessation.

## **Materials and methods**

### ***Ethical approval and registration of clinical trial***

This study was approved by the Northern A Health and Disability Ethics Committee (Reference: 15/NTA/174), Auckland, New Zealand. All participants gave written

informed consent before entering the study. This study is registered in ClinicalTrials.gov (ID: NCT02585024).

### ***Study design***

This was a pilot, single-centre, open-label dose-escalation study. To be included in the study, participants had to be at least 18 years of age, able to provide written consent, have no significant medical or psychiatric disorder (detailed in exclusion criteria) and smoke at least 10 cigarettes a day. Smokers were excluded from the study if they: were pregnant or breastfeeding, were current users of nicotine replacement therapy (NRT) products, were current users of non-NRT smoking cessation medications (e.g. bupropion, clonidine, nortriptyline or varenicline), were enrolled in another smoking cessation programme or another cessation study, had suffered a heart attack, stroke or severe angina within the past three months, had uncontrolled high blood pressure (>150 mmHg systolic, >100 mmHg diastolic), severe renal impairment (creatinine clearance <30 mL/min), phaeochromocytoma, epilepsy, or had a history of suffering from significant mental health problems or were currently taking medications that are significantly affected by smoking cessation (e.g. warfarin, olanzapine, clozapine, theophylline).

All participants were screened in two steps: 1. Telephone screening: the person was asked to give verbal consent before the researcher asked a series of questions to screen for inclusion and exclusion criteria (above). If the person was deemed eligible, they were asked to come to the clinic for an onsite screening. 2. Onsite screening: the consent form was signed by the person and questions on medication and medical history were asked. Fagerström Test for Nicotine Dependence (FTND) (Heatherton *et al.*, 1991) was administered. NicAlert<sup>®</sup> (<https://nymox.com/nicalert-product-insert-urine->

samples/) was used to confirm the person's smoking status (score must be greater than 0 to be eligible. A level 0 indicates no exposure to nicotine). Heart rate, blood pressure and breathing rate were measured. Blood and urine samples were collected to test for kidney function. The blood sample was analysed at Labtests, Auckland for eGFR and creatinine clearance. Urine samples were tested using a dipstick test for leukocytes, nitrite, protein, glucose and blood (Bayer Multistix®). The Medical Officer of the study checked all information obtained at screening and confirmed the eligibility of the person.

Participants received a single oral dose of cytisine (Desmoxan<sup>®</sup>, Aflofarm, Poland; one Desmoxan capsule contains 1.5 mg of cytisine), either 1.5 mg (one capsule), 3 mg cytisine (two capsules) or 4.5 mg cytisine (three capsules). There were 6 participants per group, but participants could take part more than once to receive a different dose, with a wash-out period of at least 48 hours in between doses if a person wished to take part in the study more than once (three participants took part in the study more than once). Allocation to dose group was determined sequentially by order of entry into the study. The lowest dose was studied first (1.5 mg) followed by 3 mg and 4.5 mg.

During visit 1, blood samples were collected at 0 (just before dosing), 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10 hours post dose. Participants then left the clinics to return the following day (visit 2- 24 hours post dose) to provide a single blood sample. Blood pressure, heart rate and breathing rate were measured at 0, 2, 4, 8, 10 and 24 hours. Participants were instructed to collect all urine during the study period (24 hours). Urine collected during visit 1 was handed to the investigator at the end of the visit and a new bottle was provided to the participant to take home. Participants were instructed to continue collecting urine until their subsequent visit (Visit 2: 24 hours post dose)..

Participants were also asked to report any adverse events throughout the study period. There were no restrictions on food or smoking during the study. Height and weight was measured to calculate body mass index (BMI). Participants were instructed to record any adverse events during the study in a study diary provided. Participants were offered \$100 in petrol vouchers as compensation for their time and \$10 petrol vouchers for each visit to the clinic to cover their travel expenses.

### ***Pharmacokinetic analyses***

Blood samples were centrifuged at 3000 g for 10 minutes to separate the plasma and red blood cell fractions. Plasma samples were kept at -80°C until analysis. Urine samples were stored at -20°C until analysis.

Plasma and urine cytosine concentrations were determined by high performance liquid chromatography with mass spectrometry using electrospray ionisation (ESI) using a validated method that has been previously published (Jeong *et al.*, 2014). The previously validated method was adapted as follows. Plasma samples (200 µL) were mixed with 6-aminoquinoline (internal standard) and then deproteinised with ice-cold methanol (440 µL), vortex mixed and left overnight at -20°C. Samples were then centrifuged at 10 000 g for 15 minutes and the clear supernatant was removed and evaporated to dryness (SC210A SpeedVac® Plus). The dry extract was then reconstituted in acetonitrile: ammonium formate buffer (10:90, v/v; 100 µL) and vortex mixed for 5 minutes. Samples were centrifuged at 10 000 g for 5 minutes before they were injected onto the column. Cytosine was detected in urine samples after being diluted 1:10 in LC-grade water (Millipore®, Milli-Q system). Detection by selective ion monitoring (SIM; positive ion mode) for each mass ion was used: m/z 191.2 (cytosine) and 145.1 (6-aminoquinoline). These compounds were resolved using a

Gemini C18 (4 mm x 100 mm, 5  $\mu$ m) column with a guard column (C18, 4.6 x 10 mm, 5 $\mu$ m) and eluted with a mobile phase of 10 mM ammonium formate buffer, pH 10 (solvent A) and acetonitrile (solvent B) with a phase gradient 10% (B) from 0 to 1 minute, 80% from 1 to 6 minutes, 80% from 6 to 7 minutes and 10% from 8 to 11 minutes. Drying gas flow was 12.0 L/min and nebuliser pressure was 35 psig. The total run time was 11 minutes plus 1 minute post injection time with a flow rate of 0.5 mL/min. Calibration curves were prepared to assess linearity, precision and accuracy. Linearity was verified for this method by visual inspection and coefficients of determination ( $R^2$ ) of calibration curves were above 0.99. Precision and accuracy were within 15% of the nominal values ( $\pm$  20% at the lowest limit of quantification). The lowest limit of quantification was 0.5 ng/mL.

The primary pharmacokinetic variables assessed were: area under the plasma concentration-time curve ( $AUC_{0-24h}$ ),  $AUC_{0-24h}$  normalised for dose, peak plasma concentration ( $C_{max}$ ) and  $C_{max}$  normalised for dose. Area under curve ( $AUC_{0-24h}$ ) values were calculated using built-in analysis in Prism, version 7.03. Other variables assessed including elimination half-life ( $t_{1/2}$ ), time to peak plasma concentration ( $t_{max}$ ), apparent volume of distribution ( $V_d/f$ ) and apparent total plasma clearance ( $CL/f$ ) were calculated based on the terminal slope. Percentage of dose recovered unchanged in urine from 0 to 24 hours post dose was also calculated.

## Results

### *Participants*

Participants in the study were smokers between 18 and 62 years of age. Participant characteristics in the three groups are summarised in Table 1.

### ***Pharmacokinetics***

Cytisine reached its plasma  $C_{\max}$  1 – 2 hours post dose in all participants in all three groups. In group 1 and 2, the mean plasma concentrations were highest at 2 hours post dose (11.4 ng/mL and 24.8 ng/mL respectively). In group 3, the mean plasma concentration was highest at 1 hour post dose (41.6 ng/mL) (Figure 1). No dose-dependent changes were observed in the elimination phase.

To assess dose proportionality, dose-normalised plasma concentrations were compared between the three groups (Figures 2 and 3). There were no statistically significant differences in the dose-normalised  $C_{\max}$  and dose-normalised  $AUC_{0-24h}$ .

The mean  $AUC_{0-24h}$  following a 1.5 mg dose was 81.9 ng.h/mL, 181.9 ng.h/mL following a 3 mg dose and 254.5 ng.h/mL following a 4.5 mg dose (Table 2). Therefore, the increase in  $AUC_{0-24h}$  for 3 mg and 4.5 mg doses were 2.2 and 3.1-folds higher than the 1.5 mg dose respectively. Mean elimination half-life was similar for all doses studied (1.5 – 4.5 mg) although it appeared to be shorter in group 3 by approximately 11%. Table 2 shows a summary of the pharmacokinetic results.

Volume of 24 hour urine collected by participants was highly variable, ranging from 386 mL to 1836 mL. Recovery of cytisine (% of dose) in urine was also highly variable ranging from 4.4% to 93.9%, 32.1% to 98.2% and 51.4% to 99.4% following administration of 1.5, 3 and 4.5 mg of cytisine respectively.

Overall, there were no clinically significant differences between male and female participants in this study for pharmacokinetic variables, although it was not possible to formally compare the pharmacokinetics between females and males in all

three groups as the groups did not have equal ratios of females to males (e.g. group 1 and group 3 had only one male participant).

### ***Safety and tolerability***

Self-reported adverse effects in group 1 (1.5 mg) were headache (one participant explained it may be due to their toothache) (1/6) and feeling tired/exhausted (1/6). In group 3 (4.5 mg), two participants (out of 6) reported feeling a little dizzy and one participant reported having a swollen calf in an old injury area. Thus dizziness was the most commonly reported adverse effect in the study. The onset of dizziness did not however align to cytosine peak plasma concentration. There were no severe/serious adverse events. There were no dose-dependent changes in heart rate, blood pressure or respiratory rate.

### **Discussion**

This is the first study to report the pharmacokinetics and safety of escalating single oral doses (1.5, 3, 4.5 mg) of cytosine in healthy adult smokers. There were no significant differences in the dose-normalised mean  $AUC_{0-24h}$  or mean  $C_{max}$  values in the three groups studied and dose-normalised mean  $AUC_{0-24h}$  and  $C_{max}$  values for the 3 dose groups were within 17.3 and 12.9% of each other respectively. As there can be >2-fold differences in the concentrations measured between participants receiving the same dose of cytosine (i.e. these differences are less than the between-subject variability), these results suggest that there are no dose-dependent changes in the pharmacokinetics up to 4.5 mg. Overall, no clinically relevant differences between male and female participants were found in the pharmacokinetics in this study, although small numbers mean this finding should be treated with caution. Although group 3 (4.5 mg group) had

a shorter mean elimination half-life than the other two dose groups, the difference (11%) may be an effect of small study number.

No concentration-dependent changes were observed in heart rate, blood pressure and respiratory rate and single dose administration of 3 mg and 4.5 mg of cytosine were well tolerated by participants. No severe adverse events were reported in the study and all adverse events were resolved by the end of the study. Most adverse effects reported in this study (headache, tiredness and dizziness) were symptoms that have been reported in previous cytosine trials (Tutka and Zatonski, 2006, West *et al.*, 2011). Interestingly, gastrointestinal adverse effects are one of the most commonly reported adverse effects in the literature but was not reported in the study. This may be because only a single dose of cytosine was given, and future studies that look at different doses of cytosine multiple times should pay attention to these effects.

The fraction of cytosine recovered in urine was highly variable (4.4% to 99.4%) in this study. However, urine collection volume, was also highly variable. The volume of urine collected for 24 hours in 10 of the 18 participants was less than 1 L and 8 participants collected less than 800 mL over 24 hours. It seems that the most likely explanation for the variability in % fraction recovered may be incomplete 24 hours collection by the participants. Thus, from these findings, it can only be reported that up to 99.4% excretion of cytosine unchanged is possible. This fits with previous findings which reported no metabolites of cytosine (Jeong *et al.*, 2014) and thus high variability may in fact reflect incomplete urine collection.

Although the two commercial formulations of cytosine (oral capsule and oral tablet) have the same recommended dosing regimen, it has not been shown that they are bioequivalent. Ideally, a direct comparison of tablets and capsules in the same individuals (i.e. a cross over study) would be able to confirm these findings. However,

as an initial assessment, comparing the pharmacokinetic profiles from this study to the results from a previous study on cytosine tablets (Jeong *et al.*, 2014) revealed that there were no differences in the absorption profiles between capsules and tablets in terms of  $t_{\max}$ . Mean (SD)  $C_{\max}$  was, however, higher with the tablets than the capsules by 10% (27.8 ng/mL vs 24.8 ng/mL). When the, the same time points were used for both tablets and capsules to calculate the exposure ( $AUC_{0-24h}$ ) in each group (a time point at 10 hours was removed in the capsules group). The  $AUC_{0-24h}$  in the capsules group was 190.9 ng.h/mL which was 20% less than the  $AUC_{0-24h}$  in the tablets group. Since a difference of >20% is also observed between subjects receiving the same dose, there is no evidence to state that there is a difference between the bioavailability of cytosine tablet and cytosine capsule. The reason for these differences may in fact be due to inconsistencies in between-subject variability or an artefact of the relatively small sample size so will need to be confirmed in future studies.

A number of limitations have been identified in the study. Firstly, this study was conducted in healthy adult smokers (i.e. normal renal function) and thus the results may not be applicable to a wider population of smokers who may have underlying co-morbidities. For example, impaired renal function would be predicted to have an impact on pharmacokinetics and will need to be studied in the future. Secondly, it was not possible to keep 24-hour surveillance of participants in the study and thus it is unclear whether 24-hour collection of urine was complete for all participants which was crucial for the determination of 24-hour urinary fractional clearance. Thirdly, this study only evaluated the linearity of pharmacokinetics following single doses up to 4.5 mg (3-fold higher than the recommended single dose taken) and pharmacokinetics and safety of doses beyond this dose may be different.

Findings from this study suggest that dose modifications to the current standard dosing regimen of cytisine may be worth investigating. The current recommended daily dose is 9 mg for the first three days of the treatment with split dosing (1.5 mg taken six times a day with each dose 2 hours apart). If giving up to 4.5 mg as a single dose is safe in smokers, it may be possible to simplify the current dosing regimen by reducing the dosing frequency and increasing the dosing interval (e.g. 4.5 mg twice a day). In order to investigate whether an alternative (i.e. simple) dosing regimen of cytisine is possible, a larger, randomized, placebo-controlled study that includes pharmacodynamics endpoints and safety outcomes should be carried out. A pharmaceutical company has announced that a trial is underway (November 2018) to evaluate 1.5 mg and 3 mg doses of cytisine on a titrating dosing schedule over 25 days as well as the effectiveness of three times daily dosing (<https://www.prnewswire.com/news-releases/achieve-life-sciences-announces-initiation-of-phase-2b-orca-1-trial-evaluating-cytisinicline-cytisine-for-smoking-cessation-300739825.html>). It is expected that a simpler dosing regimen would result in improved treatment adherence which may ultimately lead to better treatment outcomes in smokers. However, it is unknown at this stage whether dosing frequency of cytisine has an impact on cigarette craving and whether this would influence the treatment outcome. Studies to explore the effect of dose, dosing frequency and dosing interval are warranted to further establish cytisine safety and its effect on cigarette craving and treatment effectiveness.

In conclusion, cytisine appears to have predictable pharmacokinetics in healthy adult smokers after a single dose administration of up to 4.5 mg. The results from this study suggests that it is safe to give a higher single dose of cytisine (4.5 mg) in healthy smokers than the current recommended dose taken at one time (1.5 mg). Further studies

to investigate the pharmacokinetics and safety of different treatment schedules of cytisine are warranted.

Acknowledgments: This work was supported by the Auckland Medical Research Foundation (AMRF), New Zealand [Grant 1 1 15 011]. Cytisine capsules (Desmoxan) were supplied free of charge, from Aflofarm who had no involvement in the design, conduct or write-up of the study.

Disclosure of interest: The authors report no conflicts of interest.

## References

- Cahill, K., Stead, L.F. & Lancaster, T., 2012. Nicotine receptor partial agonists for smoking cessation. *The Cochrane database of systematic reviews*, 4, CD006103.
- Ebbert, J.O., Wyatt, K.D., Hays, J.T., Klee, E.W. & Hurt, R.D., 2010. Varenicline for smoking cessation: efficacy, safety, and treatment recommendations. *Patient Preference and Adherence*, 4, 355-362.
- Heatherton, T.F., Kozlowski, L.T., Frecker, R.C. & Fagerstrom, K.O., 1991. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *British Journal of Addiction*, 86, 1119-27.
- Jeong, S.H., Newcombe, D., Sheridan, J. & Tingle, M., 2014. Pharmacokinetics of cytisine, an alpha beta nicotinic receptor partial agonist, in healthy smokers following a single dose. *Drug testing and analysis*.

- Jeong, S.H., Sheridan, J., Newcombe, D. & Tingle, M., 2017. Plasma concentrations of cytosine, a commercially available plant-based alkaloid, in healthy adult smokers taking recommended doses for smoking cessation. *Xenobiotica*, 1-4.
- Prochaska, J.J., Das, S. & Benowitz, N.L., 2013. Cytisine, the world's oldest smoking cessation aid. *BMJ*, 347, f5198.
- Tutka, P. & Zatonski, W., 2006. Cytisine for the treatment of nicotine addiction: from a molecule to therapeutic efficacy. *Pharmacological Reports* 58, 777-98.
- Vinnikov, D., Brimkulov, N. & Burjubaeva, A., 2008. A Double-Blind, Randomised, Placebo-Controlled Trial of Cytisine for Smoking Cessation in Medium-Dependent Workers. *Journal of Smoking Cessation*, 3, 57-62.
- Walker, N., Bullen, C., Barnes, J., McRobbie, H., Tutka, P., Raw, M., Etter, J.-F., Siddiqi, K., Courtney, R.J., Castaldelli-Maia, J.M., Selby, P., Sheridan, J. & Rigotti, N.A., 2016. Getting cytosine licensed for use world-wide: a call to action: Editorial. *Addiction*.
- Walker, N., Howe, C., Glover, M., McRobbie, H., Barnes, J., Nosa, V., Parag, V., Bassett, B. & Bullen, C., 2014. Cytisine versus nicotine for smoking cessation. *The New England Journal of Medicine*, 371, 2353-62.
- West, R., Zatonski, W., Cedzynska, M., Lewandowska, D., Pazik, J., Aveyard, P. & Stapleton, J., 2011. Placebo-Controlled Trial of Cytisine for Smoking Cessation. *The New England Journal of Medicine*, 365, 1193-1200.

Table 1. Participant characteristics.

Characteristic	Group 1 (1.5 mg)	Group 2 (3 mg)	Group 3 (4.5 mg)
Group size	6	6	6
Age, years (Median , range)	26 (25 – 39)	22.5 (18 – 62)	27.5 (21 – 48)
Female : Male	5:1	2:4	5:1
FTND <sup>a</sup> score (Median, range)	3.5 (3 – 6)	3 (1 – 6)	3.5 (1 – 6)
Weight, kg (Median, range)	100.5 (51.3 – 122)	82.7 (63.2 – 102)	95.2 (63 – 104.2)
Height, cm (Median, range)	174.0 (165 – 190.5)	176.5 (168.6 – 189.2)	170.7 (155 – 196)
BMI, kg/m <sup>2</sup> (Median, range)	33.1 (16.4 – 37.1)	28.0 (19.9 – 33.8)	30.3 (26.2 – 33.8)
Creatinine	68.5 (60 – 95)	81.5 (60 – 94)	68 (60 – 86)

<sup>a</sup> FTND= Fagerström Test for Nicotine Dependence (Heatherton et al. 1991).

Table 2. Summary of pharmacokinetic results (mean  $\pm$  SD) after single-dose administration of cytosine in healthy adult smokers.

Variable	Group 1 1.5 mg (n=6)	Group 2 3 mg (n=6)	Group 3 4.5 mg (n=6)
AUC <sub>0-24h</sub> (ng.h/mL) <sup>a</sup>	81.9 $\pm$ 15.8	181.9 $\pm$ 40.8	254.5 $\pm$ 48.1
AUC <sub>0-24h</sub> /Dose (ng.h/mL)/mg)	52.1 $\pm$ 10.5	58.8 $\pm$ 13.6	56.4 $\pm$ 10.7
C <sub>max</sub> (ng/mL) <sup>b</sup>	12.1 $\pm$ 2.2	27.8 $\pm$ 9.3	43.1 $\pm$ 9.1
C <sub>max</sub> /Dose ((ng/mL)/mg)	8.1 $\pm$ 1.5	9.2 $\pm$ 3.1	9.5 $\pm$ 2.0
t <sub>1/2</sub> (h) <sup>c</sup>	4.4 $\pm$ 0.5	4.4 $\pm$ 1.0	3.9 $\pm$ 0.3
V <sub>D</sub> /f (L) <sup>d</sup>	110.1 $\pm$ 19.0	97.0 $\pm$ 26.8	95.8 $\pm$ 10.8
CL/f (L/h) <sup>e</sup>	17.5 $\pm$ 3.7	15.6 $\pm$ 2.8	17.3 $\pm$ 2.6

<sup>a</sup>AUC, area under the plasma concentration-time curve; <sup>b</sup>C<sub>max</sub>, maximum observed plasma concentration; <sup>c</sup>t<sub>1/2</sub>, half-life; <sup>d</sup>VD/f, volume of distribution following oral administration; <sup>e</sup>CL/f, oral clearance.

Figure 1. Mean plasma concentration profile of cytisine following a single dose administration in healthy adult smokers. Values are shown as mean  $\pm$  SEM.

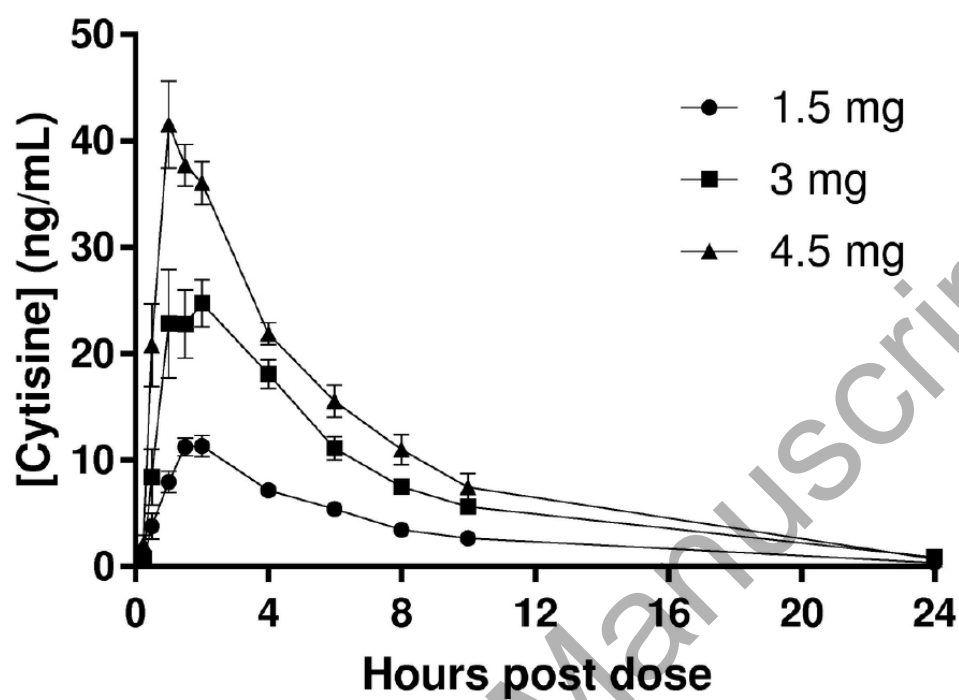


Figure 2. Individual peak plasma concentration ( $C_{\max}$ ) values following administration of single doses of cytosine in healthy smokers ( $n=6$  per group) (left). Dose-normalised  $C_{\max}$  values following a single dose of cytosine (right).

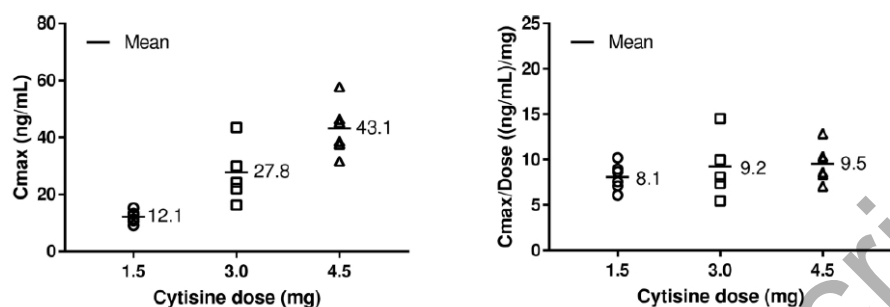


Figure 3. Individual area under the concentration-time curve (AUC) values following administration of single doses of cytosine in healthy smokers ( $N=6$  per dose) (left). Dose-normalised  $AUC_{0-24h}$  values following a single dose of cytosine (right).

