

Commentary

Cytisine for smoking cessation: A research agenda

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Abstract

Cytisine has a molecular structure somewhat similar to that of nicotine and varenicline. The concept for the new smoking cessation drug varenicline was based partly on cytisine. Like varenicline, cytisine is a partial agonist of nicotinic acetylcholine receptors, with high affinity for $\alpha 4\beta 2$ receptors. Cytisine has been used since the 1960s as a smoking cessation drug in Eastern and Central Europe, but has remained largely unnoticed elsewhere. Three placebo-controlled trials, conducted in East and West Germany in the 1960s and 1970s, suggest that cytisine, even with minimal behavioural support, may be effective in aiding smoking cessation. Cytisine tablets are very inexpensive to produce and could be a more affordable treatment than nicotine replacement, bupropion and varenicline. There is however a dearth of scientific research on the properties of cytisine, including safety, abuse liability and efficacy. This paper seeks to identify research priorities for molecular, animal and clinical studies. In particular, new studies are necessary to define the nicotinic receptor interaction profile of cytisine, to establish its pharmacokinetics and pharmacodynamics in humans, to determine whether animals self-administer cytisine, and to ascertain whether cytisine is safe and effective as a smoking cessation drug. Potentially, this research effort, contributing to wider use of an inexpensive drug, could save many lives.

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1. Introduction

Nicotine replacement therapy and bupropion only help some 5–15% of those who use them to remain long-term abstinent from smoking, depending on the context (Etter et al., 2006; Lancaster et al., 2006). These two treatments have about equal efficacy (Mills et al., 2007). New treatments are needed that are either more effective, can be more widely applied, or treat individuals that are not helped by existing treatments. A new medication, varenicline, has been found to be more effective than placebo (pooled odds ratio from meta-analysis = 2.80) and than bupropion (pooled odds ratio = 1.59), in clinical trials funded by the manufacturer (Gonzales et al., 2006; Jorenby et al., 2006; Nides et al., 2006; Oncken et al., 2006; Tonstad et al., 2006; Mills et al., 2007). Varenicline is a new chemical entity devel-

oped partly as an analog of cytisine, a natural insecticide present in several plants, e.g. in *Cytisus laburnum* (Coe et al., 2005). Cytisine has a molecular structure somewhat similar to that of nicotine, and it is a partial agonist of nicotinic acetylcholine receptors (nAChRs) with a high affinity for $\alpha 4\beta 2$ -nAChRs (Coe et al., 2005; Papke and Heinemann, 1994; Chandler and Stolerman, 1997). Cytisine has been used since the 1960s as a smoking cessation drug in East and Central European countries, where it is marketed as Tabex, registered for this purpose in 20 countries (Sopharma, Sofia, Bulgaria, www.tabex.net) (Etter, 2006). Despite its widespread use, cytisine has not been available for clinical use outside Eastern and Central Europe. This may in part be explained by limited access to the clinical studies of cytisine, which were conducted in East and Central European countries and were not published in English (Etter, 2006).

Cytisine can be manufactured at a very low cost. For instance in Russia, Poland and Bulgaria, a 25-day course of Tabex is currently 5–15 times cheaper than a 25-day course of the nicotine patch or gum. The use of cytisine for smoking cessation is out

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of patent, so this drug could have an important public health impact in countries and subpopulations where other smoking cessation drugs are too expensive or are otherwise unavailable. Because the clinical pharmacology of cytosine is incompletely characterized, there is a need for state-of-the-art research in this field.

The aim of this paper is to identify priorities for research on cytosine, focusing on its potential use as a tobacco dependence treatment. This paper contains expert opinion and is not a systematic literature review. The authors of this paper cover a broad range of scientific expertise, from basic science to public health.

2. In vitro studies

Cytosine has been used as a template for the design and development of novel nicotinic ligands (Bencherif et al., 1998; Cassels et al., 2005; Coe et al., 2005; Fitch et al., 2005; Slater et al., 2003). This work has been informed by preclinical studies characterizing interactions of cytosine and related compounds with nAChRs, which exist as a diverse family of subtypes (Lukas, 2006). Each subtype is defined by its unique subunit composition and is distinguished by its drug interaction profile. For example, $\alpha 4\beta 2$ -nAChRs, composed of $\alpha 4$ and $\beta 2$ subunits, are the most abundant, high affinity sites for nicotine binding in the brain.

Cytosine, like varenicline, has been classified as a selective, partial agonist at human $\alpha 4\beta 2$ -nAChRs (Lukas, 2006). *Selective* means that cytosine activates function of human $\alpha 4\beta 2$ -nAChRs at a lower concentration than required to activate some other human nAChR subtypes (cytosine is selective for $\alpha 4\beta 2$ -nAChRs, as is nicotine, relative to many nAChR subtypes). *Partial agonist* means that, in some test systems, the magnitude of the maximal functional responses of $\alpha 4\beta 2$ -nAChRs to cytosine is lower than that for the responses to nicotine or acetylcholine, the presumptive native ligand. Cytosine has submaximal functional efficacy, or acts as a partial agonist, at $\alpha 4\beta 2$ -nAChRs in in vitro studies, although such action in vivo has not been definitely demonstrated (Chandler et al., 1997).

However, numerous studies suggest that cytosine has a more complex pharmacological profile. Cytosine's affinity for other nAChR subtypes, such as $\alpha 4\beta 4$ - or nAChRs-containing $\alpha 6$ subunits is or may be greater than or equal to its affinity for $\alpha 4\beta 2$ -nAChRs (Lukas, 2006; Marks et al., 2006; Salminen et al., 2005). Cytosine may interact with more than one form of $\alpha 4\beta 2$ -nAChR (i.e., receptors with the stoichiometry $(\alpha 4)_2(\beta 2)_3$ or $(\alpha 4)_3(\beta 2)_2$), or more than one class of functional sites on $\alpha 4\beta 2$ -nAChRs (Slater et al., 2003). Cytosine's efficacy can vary across studies of the same nAChR subtype from different species and perhaps depending on the experimental system and approach used (Chavez-Noriega et al., 1997; Eaton et al., 2000, 2003; Stauderman et al., 1998). Simple modifications, such as halogenation, markedly affect binding affinities of nAChRs for cytosine analogues (Chellappan et al., 2006; Fitch et al., 2005; Slater et al., 2003). Reciprocally, modest changes in the presumed agonist-binding pocket of nAChRs can alter cytosine's functional potency (Papke et al., 2005). Further studies of cytosine-based compounds are likely to be informative about

the ligand-binding pockets on nAChR and molecular mechanisms involved in nicotine dependence. Moreover, these studies are necessary to define the nAChR subtype interaction profile of cytosine, especially in comparison to the in vitro profiles for receptor interactions with bupropion, varenicline, and nicotine itself. Also needed are studies of the effects of longer-term exposure to cytosine on the numbers and function of different nAChR subtypes, as well as on the activity of neurons or other cells expressing nAChRs. Use of cytosine as a smoking cessation medication may involve chronic dosing and will be best informed by an understanding of its long-term effects.

3. Behavioural pharmacology of cytosine: animal studies

Behavioural pharmacological studies have been generally consistent in reporting that the behavioural effects of cytosine in animals are somewhat similar to those of nicotine (Brioni et al., 1994; Chandler and Stolerman, 1997; Craft and Howard, 1988; Pratt et al., 1983; Reavill et al., 1990; Stolerman et al., 1984). However, cytosine does not produce the same degree of behavioural activation as nicotine (measured as ambulatory activity) in animals chronically treated with nicotine (Stolerman et al., 1995). There are few studies of cytosine in direct or indirect measures of reinforcement and reward. Rasmussen and Swedberg (1998) have reported that drug-naïve mice would self-administer cytosine intravenously, which suggests that cytosine has reinforcing effects. However, the acute self-administration model used by Rasmussen et al. may not reflect the chronic reinforcing properties of the drug, and the tail-vein procedure used in this study induces stress, a potential confounding factor. In addition, cytosine can condition a preference for the environment in which it is administered, which is indirect evidence that it has rewarding effects (Museo and Wise, 1994).

The paucity of studies of reinforcement and associated animal behavioural measures that are believed to model drug use in humans suggest the need for experiments to examine the extent to which cytosine (i) will maintain self-administration behaviour compared with nicotine, (ii) will antagonize or otherwise shift/reduce the dose-effect curve for nicotine self-administration, (iii) will reduce the extent of relapse to self-administration behaviour caused by nicotine or re-exposure to the drug-taking environment (or in fact precipitate relapse behaviour), and (iv) will attenuate withdrawal symptoms (or, again, precipitate some withdrawal). Given the recent introduction of varenicline onto the market, the expectation of emerging clinical reports as use of this medication expands, and the limited number of animal studies that have been reported with varenicline, conjoint behavioural pharmacological experiments with varenicline might provide a valuable comparison. These proposed studies are, however, not a necessary precondition to the use of cytosine in humans.

4. Pharmacokinetic and pharmacodynamic studies

The human pharmacology of cytosine buccal films was reported in one Russian study of 78 patients (Ostrovskaja,

1994a,b). To our knowledge, there are otherwise no published studies of the pharmacokinetics (PK) or pharmacodynamics (PD) of cytisine in humans. Phase 1-type PK and PD studies are recommended so as to optimize the dose of cytisine and to better understand individual differences in efficacy and toxicity of this drug.

Studies in rats find that cytisine crosses the blood–brain barrier less readily than nicotine (Reavill et al., 1990), which suggests that higher blood levels (and therefore higher doses) compared to nicotine may be required to achieve adequate brain concentrations. However, this has not been tested in humans, and research is needed to quantify this parameter in humans.

Pharmacodynamic measures should include assessments of nicotine-related effects, including subjective and toxic events, as well as effects on blood pressure and heart rate. Measurement of urine catecholamine excretion is also useful to determine if cytisine has nicotine-like cardiovascular and endocrine effects (Gourlay and Benowitz, 1997). Such studies are important in predicting the likelihood of adverse effects of cytisine in smokers with cardiovascular disease.

The usual starting dose of cytisine is 1.5 mg administered six times per day (Zatonski et al., 2006). It is possible that a higher dose may be safe and even more effective than the standard dose; this could be tested in a dose escalation study. As an alternative to dose escalation, the effects of administering the drug more frequently than six times per day might be examined.

A multiple administration study would also be useful for learning whether there is accumulation of the drug over time and whether there are time-dependent and dose-dependent changes in pharmacokinetics. A PK–PD study as described above should be performed after the first administration (single dose) and also following the standard daily dosing (multiple dose). Periodic measurement of peak and trough levels would further clarify the issue of accumulation.

5. Clinical studies

5.1. Safety and adverse effects

Tabex is widely used in Eastern and Central European countries. For instance, there were 51,324 courses of Tabex sold in Bulgaria in 2004, 70,962 in 2005 and 78,846 in 2006. The numbers of units of nicotine replacement products sold in Bulgaria (boxes of 30 gums or 7 patches) were 7205 in 2004, 8989 in 2005 and 21,187 in 2006, respectively (PharmMIS, 2007). Decades of use in hundreds of thousands of users suggest that cytisine is safe at the dose currently recommended for smoking cessation, but most of the safety data were collected before current standards of adverse event reporting were put into place. Therefore, more safety data are required. There are at least 10 published clinical studies of cytisine for smoking cessation (Etter, 2006). The safety data within these studies suggest that cytisine is well tolerated (Etter, 2006; Tutka et al., 2007). The side effects that were slightly more frequent for cytisine than for placebo were weight gain (Benndorf et al., 1968, 1969), headache and heartburn (Schmidt, 1974). Nausea, vertigo, diarrhea and digestive problems were reported in some studies, with

no comparison with placebo (Zatonski, 2006), or at the same rate as placebo (Schmidt, 1974). Data on the effect of cytisine on blood pressure and heart rate are contradictory (Etter, 2006). Interestingly, the side effects reported in published trials differ from those described in the manufacturer's leaflet. The manufacturer of Tabex lists the following side effects: "changes in both taste and appetite, dryness in the mouth, headache, irritability, nausea, constipation, tachycardia, slight elevation of the arterial pressure". Tabex is contraindicated for people with arterial hypertension and advanced atherosclerosis (Tabex website, 2007).

The side effects that were more frequent for varenicline than for placebo were nausea and abnormal dreams (Jorenby et al., 2006; Gonzales et al., 2006). Thus, as might be expected for a partial nicotinic agonist, the adverse events from using cytisine for a smoking cessation aid are somewhat similar to nicotine replacement therapy (NRT) and varenicline used for the same purpose.

5.2. Overdose

There are numerous reports of people poisoned from seeds of *Cytisus laburnum*, which contain cytisine (Forrester, 1979), including one fatal case (Richards and Stephens, 1970). The lethal dose in humans is, however, unknown. One report described two suicide attempts by the same patient, a pharmacist who swallowed 40–50 Tabex tablets (60–75 mg cytisine) on her first suicide attempt and 90 tablets (135 mg cytisine) on her second attempt (Stoyanov and Yanachkova, 1972). These suicide attempts were not fatal. Acute adverse effects observed in this patient included clonic spasms, headache, vertigo, profuse sweating and trembling, and residual symptoms included reduction in visual acuity and renal pain, but no objective organ lesions (Stoyanov and Yanachkova, 1972). Similar symptoms are seen in people suffering from a toxic dose of nicotine (Ballard et al., 1995). In animals, there are reports of fatal accidental poisoning with seeds of *Cytisus laburnum* (Clarke et al., 1971). In the rat, the lethal dose of cytisine (dose at which half the animals die) is 1.7 mg/kg i.v. and 101 mg/kg per os (Barlow and McLeod, 1969).

5.3. Abuse liability

When NRT products were reviewed for regulatory approval in the USA, abuse liability trials were required to confirm that children or adolescents would not abuse these products, and that these products did not have addiction potential in adults (Houtsmuller et al., 2002, 2003). However, similar trials have not yet been published for bupropion, varenicline or cytisine. The fact that cytisine has reinforcing and rewarding effects in the animal suggests that abuse liability trials should be conducted in human (Museo and Wise, 1994; Rasmussen et al., 1998). Slow release forms (e.g. transdermal patch) might minimize the addictive potential of the substance, improve compliance, and reduce variability in blood levels. Studies of the addictive potential were not a requirement for varenicline, and arguably, they should not be one for cytisine.

5.4. Efficacy

There are currently three published, placebo-controlled trials of cytosine for smoking cessation. All were conducted in East and West Germany in the 1960s and 1970s (Benndorf et al., 1969; Paun and Franze, 1968; Schmidt, 1974). A meta-analysis of these trials showed that cytosine doubled the odds of quitting smoking, compared with placebo (pooled odds ratio = 1.83 after 3–6 months, 95% confidence interval = 1.12–2.99) (Etter, 2006). However, these trials were not conducted to current methodological standards and would therefore not satisfy some regulatory authorities. One randomized, controlled trial is currently under way in Poland, but results may not be known before 2 years (Witold Zatonski, pers. commun.). One randomized trial has been recently completed in Bulgaria, but its results are difficult to interpret because of methodological limitations (Monova and Nikolov, 2006).

5.5. New trials

Data from published clinical trials suggest, but do not prove, that at the current dosing regimen, cytosine is safe and effective. The situation with regard to clinical studies is made more complex by the fact that cytosine is already in widespread use in several countries, in spite of the scarcity of phase I and II trials. If new trials were conducted, the first priority would be to determine whether the drug in its current form (Tabex) and with its current dosing regimen is safe and effective, and to estimate the size of its effect. This will require at least two appropriately designed clinical trials that would satisfy regulatory authorities. The reason for using the current dosing regimen is that if the drug is effective, then it can be used sooner in countries where cytosine is not currently approved, and more lives will be saved. The risk attached to this strategy is that the current dosing regimen may not be the most effective. The evidence to date suggests, however, that this risk is low and worth taking.

5.6. Dosing regimen

All but one of the published trials of cytosine for smoking cessation used the Bulgarian drug Tabex (Etter, 2006; Tutka and Zatonski, 2006). Each tablet of Tabex contains 1.5 mg of cytosine. These tablets are swallowed and the manufacturer recommends using six tablets per day (total 9 mg of cytosine) during the first 3 days after smoking cessation, then to decrease dosage gradually to two tablets per day until the 25th day, when the treatment is stopped. With a 25-day treatment period, Tabex has the shortest treatment duration of any of the currently approved smoking cessation medications. However, in most smokers undergoing treatment, tobacco dependence has been maintained for many years, thus a longer duration of cytosine treatment could improve efficacy. It will be important to know whether better success rates can be obtained by giving cytosine for 12 weeks or longer. It should be noted, however, that most people do not use smoking cessation medications as long as recommended, particularly nicotine replacement therapy (Wiggers et al., 2006). Furthermore, it is possible that starting with the

highest dose may result in more severe adverse effects. It could be tested whether starting with a lower dose 1 week prior to cessation and incrementally increasing the dose produces fewer side effects, with possible improvement of compliance and efficacy. However, side effects are reportedly already low. In addition, having to take six doses per day in the first 3 days, when relapse is more likely, may reduce compliance and, therefore, efficacy. It should be tested whether a less frequent administration schedule results in better efficacy. The efficacy of various doses of cytosine should also be compared. Comparisons should also be made of the acceptability, side effects and efficacy of various administration forms: tablets, lozenges, buccal films, chewing gums, transdermal patches, inhalers or nasal sprays. However, only cytosine tablets are currently commercially available. Given that cytosine has reinforcing and rewarding effects in the animal, slow release forms will probably have the lowest abuse liability. A sustained release form may also decrease side effects and improve compliance, compared to taking six pills a day. Testing new dosing regimens or administration forms is, however, not a priority, compared with conducting placebo-controlled trials with the current regimen.

5.7. Active-control trials

Ideally, active-control trials should be conducted to compare cytosine with NRT, bupropion and varenicline. Even if cytosine proved less effective than a more expensive drug like varenicline (but more effective than placebo), it may still have a larger public health impact because of its lower price, and thus, its potentially wider use. Given the low therapeutic efficacy of NRT (Etter and Stapleton, 2006), cytosine should be at least as effective as NRT to be recommended. However, active-control trials and non-inferiority trials require large samples and, even though they would be informative, they are not required for most regulatory agencies.

Subsequent to demonstrate the safety and efficacy of cytosine, the effects of cytosine on urges to smoke and tobacco withdrawal symptoms could be studied. It should also be tested whether cytosine is effective in subgroups of people who failed with the other medications. Finally, participants in the three published placebo-controlled trials received no behavioural support (Benndorf et al., 1969; Paun and Franze, 1968; Schmidt, 1974). Addition of behavioural therapy could improve the efficacy of cytosine, as it does for NRT (Silagy et al., 2004). Thus, cytosine should be studied both with and without behavioural support. However, behavioural support cannot be offered to all users in all contexts. Thus, as mentioned previously, initial trials of cytosine should not include amounts of behavioural support that would be prohibitive, as then behavioural support would be required for approval.

6. Discussion

Research conducted over the past 40 years in Central and Eastern Europe suggests, but does not prove, that cytosine is effective and safe for smoking cessation. However, many questions remain unanswered. In the animal, additional studies

should assess whether cytosine will antagonize the effects of nicotine, attenuate nicotine withdrawal effects and relapse to nicotine. Further research should also test whether cytosine will maintain self-administration behaviour compared with nicotine. In humans, additional PK and PD studies should be conducted, with exploration of effects at different doses. As for safety, the side effects of cytosine in humans have been studied, but not using methods that would allow us to be confident that it is fully described. As for clinical research, the priority is to test the possible efficacy of cytosine in appropriately designed clinical trials. For registration and practical purposes, new trials should probably use the current dosing regimen, even though the optimal dosing regimen has not been explored thus far. New trials should not use amounts of behavioural support that would be prohibitive.

In a second step, studies could investigate the additional effect of behavioural support on efficacy, the efficacy of cytosine compared with existing treatments, and the relative efficacy of the medication in different types of smokers (e.g. those who have failed when using other medications). Studies are also necessary to establish the optimal dosage and route of administration. Progress needs to be made on all these fronts, but the biggest problem at this stage is how to invest in such a research programme when the current data are suggestive that cytosine is safe and effective, but there is little effort internationally to explore this drug. We know the tremendous toll that tobacco has taken to date, and that smoking cessation substantially decreases smoking-related deaths (Anthonisen et al., 2005). Therefore, the public health community must work to develop resources to explore the safety and efficacy of cytosine. Potentially, this research effort, contributing to a larger dissemination of an inexpensive smoking cessation drug, could save many lives.

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