

Sodium Fusidate Inhibits rCYP3A4 *in vitro*-A Possible Mechanism Defining the Interaction with Statins

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Abstract

Sodium fusidate (fusidic acid) is an antimicrobial agent used to treat methicillin-resistant *Staphylococcus aureus* infections. Several case reports have described drug interactions between sodium fusidate and CYP3A4 metabolised statins, leading to statin myotoxicity, including fatal myotoxicity. The mechanism of this interaction is unknown. We investigated the effects of sodium fusidate on recombinant CYP3A4 *in vitro* and found it to be a time-dependent inhibitor of this enzyme at concentrations likely to be achieved with clinical dosing regimens. This finding may help to explain the mechanism of the apparent statin-fusidate interaction.

Introduction

Sodium fusidate (fusidic acid) is a naturally occurring antibiotic derived from the fungus *Fusidium coccineum*. It has a narrow antimicrobial spectrum, exhibiting particular activity against *Staphylococcus aureus* [1]. Sodium fusidate exhibits its primarily bacteriostatic action by inhibition of elongation factor G at a bacterial ribosomal level, which in turn inhibits protein synthesis [2]. As this mode of action is unrelated to that of beta lactams, sodium fusidate retains activity against methicillin-resistant *Staphylococcus aureus* (MRSA)[3]. Increasing rates of MRSA infection has led to renewed interest in and usage of sodium fusidate [4].

Cardiovascular diseases are the leading cause of disability and death in the world [5]. Hydroxymethyl glutaryl coenzyme A reductase inhibitors (statins) are established therapies in primary and secondary prevention of cardiovascular disease [6,7]. Consequently, the use of statins has increased worldwide over the last two decades, for example, statin use (measured in defined daily doses per 1000 population per day) increased in Europe by 35 per cent annually over the period 1997 to 2003 [8]. Usage in Australia was even higher during this period [9].

Statins are generally well tolerated, although some toxicity is associated with their use. Significant concentration-dependent adverse effects with potentially lethal consequences include muscle toxicity (such as rhabdomyolysis) and hepatotoxicity [10,11]. As these adverse effects are concentration-dependent they may be potentiated by drug-drug interactions which lead to decreased statin clearance. Cytochrome P450 3A4 (CYP3A4) is an important enzyme in the elimination of numerous statins, including atorvastatin and simvastatin, and inhibition of this enzyme is known to increase the likelihood and significance of statin toxicity [11].

Sodium fusidate and statins may be co-prescribed for commonly occurring co-conditions. One example is diabetic foot infection. MRSA is an important pathogen in diabetic foot infection and worsens the outcome of this infection, compared with infections with

methicillin-sensitive strains, with severe or deep seated infections requiring protracted antibiotic administration [12]. Patients with diabetes have higher rates of cardiovascular disease and hence are more likely to require treatment with statins [13]. Hence, it is feasible that statins and sodium fusidate may be commonly co-prescribed and may become more so in future.

Since 2003 there have been numerous reports of suspected interactions between sodium fusidate and statins leading to statin myotoxicity, including cases of fatal toxicity [14-25]. The majority of the cases involved simvastatin or atorvastatin, which are metabolised by CYP3A4 [26]. Consequently, some authors have suggested that sodium fusidate increases statin concentrations by inhibition of CYP3A4 [14,19] although no data exist to support this claim. Conversely, one case study explicitly states that sodium fusidate does not inhibit CYP3A4 [20]. In addition, there have been recent case reports of an interaction between sodium fusidate and rosuvastatin, however this statin is not metabolised by CYP3A4 [19,25]. These reported fusidate-statin interactions have prompted warnings from regulatory bodies, including in United Kingdom and Ireland, with specific advice to avoid the co-prescription of statins and sodium fusidate[27,28]. Some infections for which sodium fusidate may be prescribed, such as MRSA osteomyelitis, require weeks or months of antibiotic therapy [29]. Withholding cardiovascular medications such as statin for this length of time may not be feasible in many patients and hence a greater understanding the mechanism of this proposed interaction may permit more appropriate recommendations regarding clinical management. On this basis we investigated the effect of sodium fusidate on the metabolic activity of recombinant CYP3A4 (rCYP3A4) *in vitro* as a possible mechanism for the fusidate-statin interaction.

Materials and methods

Reagents

P450-Glo™ CYP3A4 Screening System with Luciferin-IPA was purchased from Promega (Melbourne, Australia). The system includes rCYP3A4 with reductase, control membranes with no enzymatic activity, luciferin-IPA, NADPH regeneration system, luciferin detection reagent and potassium phosphate buffer. All other reagents and chemicals were purchased from Sigma Aldrich (Sydney, Australia). Water used throughout the experiments was either milli-Q previously determined to be luciferin free or luciferin-free water supplied by Promega Australia. All luminescence readings were performed using a FlexStation 3™ bench top multi-mode microplate reader with a 500 ms integration time.

Half maximal inhibitory concentration (IC50) determination

The reactions were conducted according to the manufacturer's instructions. Briefly, activity of rCYP3A4 was determined using a luminescent assay with luciferin-IPA. All reactions were performed in opaque Costar™ 96 well flat-bottomed plates at room temperature. 0.1 pmol of control and rCYP3A4 membranes were incubated with the luciferin-IPA probe and sodium fusidate at a range of concentrations (0.1 μM, 1 μM, 10 μM, 100 μM, 1000 μM and 10000 μM). Ketoconazole 5 μM, a known potent inhibitor of CYP3A4, was included as a positive control [30]. Baseline luminescence of the system was established by using control membranes with similar protein content but no enzymatic activity. Each experiment was performed in triplicate at room temperature.

The luciferin-IPA probe and sodium fusidate reactions were pre-incubated for 10 minutes with the microsomes prior to initiation of the reaction by the addition of 25 μl NADPH regeneration system to each well. Reactions were terminated after 10 minutes with the addition of ice-cold Luciferin Detection Reagent (50 μl). The luminescent signal was allowed to stabilize for 20 minutes prior to measurement. The degree of rCYP3A4 inhibition was normalised to the mean of the background control membranes luminescence, and expressed as the percentage enzymatic activity relative to the untreated control rCYP3A4. The concentration needed to produce 50% inhibition (IC50) was calculated using Graph Pad Prism software.

Time-dependent inhibition determination

Time-dependent inhibition of rCYP3A4 was determined as described above, with some modifications to the experimental design. Two strengths of sodium fusidate were investigated (100 μM and 500 μM). Ketoconazole was used as a positive control inhibitor with no time-dependent activity at a concentration of 0.03 μM, which we previously determined to be near the IC50 for this system (data not shown). Verapamil 10 μM was included as a positive control with known time dependent inhibition[30]. Baseline control membranes and untreated rCYP3A4 were prepared in a similar fashion. Unlike the above system, the rCYP3A4 was preincubated with sodium fusidate or positive control inhibitors and NADPH regenerating system for 0 minutes, 10 minutes, 20 minutes and 30 minutes prior to the addition of the luciferin-IPA probe. The luciferin-IPA was added and the experiments terminated with Luciferin Detection Reagent at the following times: 0 minutes, 10 minutes, 20 minutes and 30 minutes. The experiment was performed with all controls six times at each of these time points at 37°C. Percent inhibition was calculated as above.

Results

Half maximal inhibitory concentration (IC50) determination

Sodium fusidate was found to inhibit rCYP3A4 with an IC50 of 461.4 μM (369.7-575.8) at room temperature, with no pre-incubation in the presence of active enzyme (Figure 1). Ketoconazole 5 μM completely inhibited the system (not shown). The control membrane system produced no appreciable luminescence in any of the experiments.

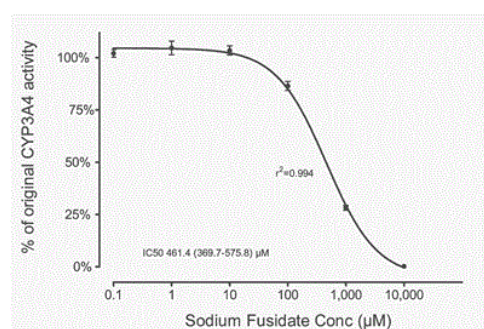


Figure 1: Inhibition of rCYP3A4 by sodium fusidate at room temperature. Data are expressed as mean ± SEM (N=3 experiments).

Time-dependent inhibition determination

Sodium fusidate was observed to be a time-dependent inhibitor of rCYP3A4 at both 100 μM and 500 μM at all-time points (Figure 2). As expected verapamil (10 μM) displayed time-dependent inhibition of rCYP3A4, whilst ketoconazole did not.

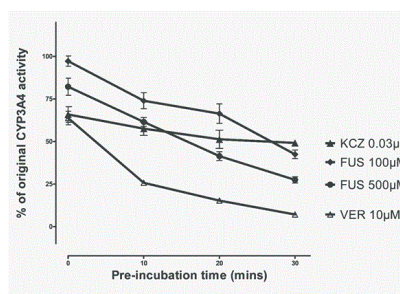


Figure 2: Investigation of time-dependent inhibition of rCYP3A4 at 37°C. Sodium fusidate (FUS) inhibited rCYP3A4 in a time-dependent manner at both 100 μM and 500 μM. Verapamil (VER) also inhibited rCYP3A4 in a time-dependent manner. Ketoconazole (KCZ) inhibited rCYP3A4 without time-dependence. Data are presented as the mean ± SEM (N=6 experiments).

Of the 4 compounds, verapamil demonstrated the most potent inhibitory activity compared with ketoconazole and both concentrations of sodium fusidate (Figure 2). The control membrane system produced no appreciable luminescence in any of the systems.

Discussion

These results of this investigation indicate that sodium fusidate is a time-dependent inhibitor of rCYP3A4 *in vitro*. Sodium fusidate demonstrated appreciable time dependence in its inhibitory activity with 30 minute preincubation approximately doubling the inhibitory potency. This finding may in part explain the observed clinical interaction of sodium fusidate with atorvastatin and simvastatin.

Time dependent inhibition of drug metabolising enzymes generally occur via either the formation of an inhibitory metabolite or via mechanism-based inactivation (MBI) of the enzyme. MBI generally involves conversion of the drug to reactive intermediate which subsequently covalently binds to the enzyme or associates with the heme moiety, rendering it persistently inactive. Enzymatic activity generally only returns with new protein synthesis [31,32]. This delayed and potentially extended period of inhibition may therefore have significant clinical consequences, which are likely to be greater than those caused by competitive inhibition of drug metabolising enzymes.

Plasma concentrations of sodium fusidate required to produce therapeutic efficacy are not well characterised. Whilst the IC₅₀ obtained in this study for non-time dependent inhibition of rCYP3A4 at room temperature is likely to be higher than clinically obtained pharmacokinetic parameters, it has been recently demonstrated that standard dosing regimens of sodium fusidate produce observed or predicted steady state plasma concentrations of approximately 100-200 mg/L, approximating those found to inhibit rCYP3A4 in a time-dependent fashion in this study [33]. Although a high IC₅₀ does indicate a less potent inhibition, time-dependent inhibition may represent a more serious form of drug interaction as the drug metabolising enzymes can be rendered permanently inactivated by this process [34].

Sodium fusidate monotherapy is known to carry the risk of the development of resistance in MRSA, and as such recommendations for its use as an antimicrobial often recommend combining it with another agent, frequently a rifamycin, which are known to be potent inducers of CYP3A4 *in vivo* [35,36]. This clinical scenario may explain why the interaction has not been more widely reported, and further studies in animals or in whole human hepatocytes may yield further information about the effects of the combination of antimicrobials on drug metabolizing enzymes.

This study has several limitations. Time-dependent inhibition demonstrated *in vitro* does not necessarily imply clinically significant inhibition will occur in humans or animals [37]. Caution must be used when applying these results to clinical practice. The reporting of a potential sodium fusidate-rosuvastatin interaction [19,25] may suggest another or an alternative pathway is also involved. There is overlap in the range of compounds which interact with both CYP3A4 and transporters important in drug handling such as P-glycoprotein and organic anion transporting polypeptides [38]. However, results from this study indicate a direct inhibitory effect of sodium fusidate on rCYP3A4 and further work is required to characterise the interaction between sodium fusidate and other drug transporters, if any.

In summary, sodium fusidate is a time-dependent inhibitor of rCYP3A4 *in vitro* at concentrations likely to be achieved with clinical dosing regimens. These findings may help to explain the reported drug interaction of sodium fusidate with CYP3A4-metabolised statins. Further investigation is warranted to confirm the inhibition and elucidate the mechanism of inhibition.

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