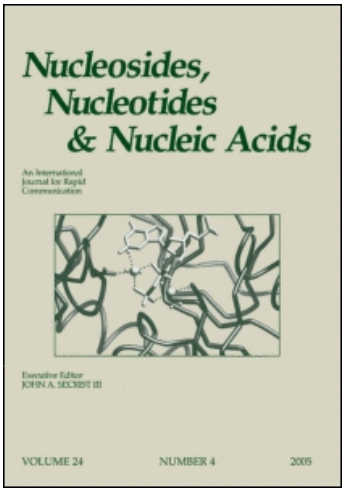

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Pharmacological Inhibition of AMP-Deaminase in Rat Cardiac Myocytes

T. Borkowski[a]; C. Orlewska[b]; E. M. Slominska[a]; A. Yuen[c]; M. Lipinski[d]; I. Rybakowska[e]; H. Foks[b]; K. K. Kaletha[e]; M. H. Yacoub[c]; R. T. Smolenski[ac]
[a] Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland [b] Department of Organic Chemistry, Medical University of Gdansk, Gdansk, Poland [c] Heart Science Centre, Imperial College London, London, United Kingdom [d] Department of Pharmaceutical Biochemistry, Medical University of Gdansk, Gdansk, Poland [e] Department of Clinical Biochemistry and Physiology, Medical University of Gdansk, Gdansk, Poland

To cite this Article Borkowski, T. , Orlewska, C. , Slominska, E. M. , Yuen, A. , Lipinski, M. , Rybakowska, I. , Foks, H. , Kaletha, K. K. , Yacoub, M. H. and Smolenski, R. T.(2008) 'Pharmacological Inhibition of AMP-Deaminase in Rat Cardiac Myocytes', Nucleosides, Nucleotides and Nucleic Acids, 27: 6, 867 — 871
To link to this Article: DOI: 10.1080/15257770802146536
URL: http://dx.doi.org/10.1080/15257770802146536

Nucleosides, Nucleotides, and Nucleic Acids, 27:867–871, 2008

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802146536

Taylor & Francis
Taylor & Francis Group

PHARMACOLOGICAL INHIBITION OF AMP-DEAMINASE IN RAT CARDIAC MYOCYTES

T. Borkowski,[1] C. Orlewska,[4] E. M. Slominska,[1] A. Yuen,[5] M. Lipinski,[2] I. Rybakowska,[3] H. Foks,[4] K. K. Kaletha,[3] M. H. Yacoub,[5] and R. T. Smolenski[1,5]

[1]*Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland*
[2]*Department of Pharmaceutical Biochemistry, Medical University of Gdansk, Gdansk, Poland*
[3]*Department of Clinical Biochemistry and Physiology, Medical University of Gdansk, Gdansk, Poland*
[4]*Department of Organic Chemistry, Medical University of Gdansk, Gdansk, Poland*
[5]*Heart Science Centre, Imperial College London, London, United Kingdom*

□ *Because mutation of AMP deaminase 1 gene leading to reduced AMP deaminase activity may result in protection of cardiac function in patients with heart disease, inhibitors of AMP deaminase (AMPD) may have therapeutic applications. This study evaluated the effect of a specific inhibitor of AMP deaminase 3-[2-(3-carboxy-4-bromo-5,6,7,8-tetrahydronaphthyl)ethyl]-3,6,7,8-tetrahydroimidazo [4,5-d][1,3]diazepin-8-ol (AMPDI) on the isolated human enzyme and on nucleotide catabolism in rat cardiomyocytes. AMPDI effectively inhibited isolated human AMPD with an $IC_{50} = 0.5\ \mu M$. AMPDI was much less effective with isolated cardiomyocytes ($IC_{50} = 0.5$ mM). AMPDI is a very effective inhibitor of AMPD that despite lower efficiency in the cell system examined could be useful for in vivo studies.*

Keywords AMP deaminase; AMP deaminase inhibitor; cardiomyocytes; rat

INTRODUCTION

A number of clinical studies published in the last decade have highlighted an association of the C34T mutant AMPD1 allele with decreased cardiac AMP deaminase (AMPD) activity and improved mechanical function.[1,2] Although other authors found different results[3] there is a potential pathway leading to protection of heart function that may include activation of AMP regulated protein kinase and increased production

This study was supported by the Ministry of Science of Poland (N401 101 31/2201, W-511, W-172) and the Magdi Yacoub Institute.

Address correspondence to R. T. Smolenski, Heart Science Centre, Imperial College at Harefield Hospital, Harefield UB9 6JH, UK. E-mail: r.smolenski@imperial.ac.uk

of adenosine. One way to prove this concept and potentially establish new treatment of heart failure is pharmacological inhibition of AMPD. Unfortunately, adequate inhibitors are not readily available. Chemical synthesis of such inhibitors was described by Kasibhatla et al.[4] However, only very preliminary evaluation of these inhibitors in a biological system has been presented. In our study we tested the most effective molecule among those described with regard to its effect on purified human AMPD, rat heart homogenate AMPD, and apparent AMPD enzyme activity in rat cardiac myocytes.

MATERIALS AND METHODS

An inhibitor of AMP deaminase (AMPDI): 3-[2-(3-carboxy-4-bromo-5,6,7,8 tetrahydronaphthyl)ethyl]-3,6,7,8-tetrahydroimidazo[4,5-d][1,3] diazepin-8-ol was chemically synthesized according to the procedure established by Kasibhatla et al.[4] Its identity was confirmed by NMR and mass spectrometry. AMP deaminase was isolated using two step phosphocellulose column chromatography from human skeletal muscle collected during autopsy according to our previously established procedures.[5] Purified enzyme was incubated with different concentrations of the inhibitor and activity was measured by conversion of AMP into IMP by HPLC. Rat hearts were homogenized at 4°C in a ratio of 1 g of wet weight of tissue per 9 ml buffer (150 mM KCl, 20 mM TRIS, 1 mM EDTA, 1 mM dithiothreitol, pH 7.0) as we previously described.[6] Rat cardiomyocytes were isolated as we have described previously.[7] Isolated myocytes suspended in HEPES-buffered Krebs solution with 2% bovine albumin were preincubated with 0.1–3 mM AMPDI for 30 minutes to 5 hours. Following preincubation, oligomycin and deoxyglucose were added for 45 minutes to stimulate adenine nucleotide catabolism in the presence of selective adenosine kinase (5′-iodotubercidine [ITU]) and adenosine deaminase (erythro-9-(2-hydroxy-3-nonyl)adenine [EHNA]) inhibitors to discriminate between alternative AMP catabolic pathways. At the end of the experiment, perchloric acid extracts were prepared and analysed by HPLC as we have described previously.[8] The ratio of adenosine to the sum of IMP and inosine (Ado/Ino+IMP) was used to estimate catabolic flow through 5′-nucleotidase relative to that through AMP deaminase.

RESULTS

Figure 1 shows that IMP concentration markedly decreased in cardiac myocytes treated with AMPDI and then subjected to stimulation of adenine nucleotide catabolism with deoxyglucose/oligomycin. This was accompanied by increasing concentrations of adenosine (Figure 2). Con-

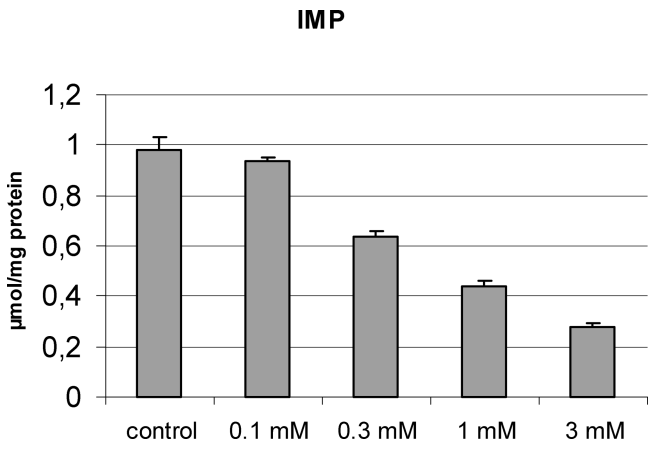

FIGURE 1 IMP concentration in rat cardiomyocytes in control conditions and with 0.1, 0.3, 1, and 3 mM AMPDI following stimulation of catabolism with oligomycin and deoxyglucose. EHNA and ITU were present during incubation Values are mean ± SEM, n = 3.

sequently, the ratio of Ado/(Ino+IMP) was increased several times, indicating relatively greater adenine nucleotide catabolism in the direction of 5′nucleotidase. Notably, the total amount of adenine nucleotide degradation products ($\sum$ Ado + IMP + Ino + Hx) does not change after the addition of AMPDI (data not shown). Figure 3 presents inhibition of purified human skeletal muscle AMPD and rat heart homogenate AMPD by AMPDI compared with its effect in isolated cardiomyocytes. These data indicate very effective inhibition of purified and homogenate AMPD, but highlights that a much higher concentration is necessary for in vivo inhibition of the enzyme.

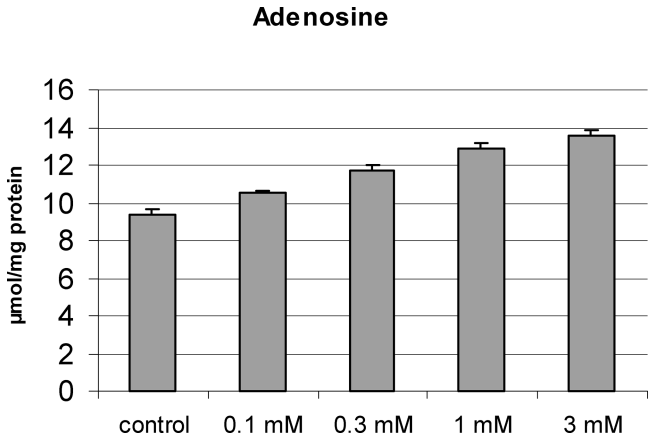

FIGURE 2 Adenosine concentration in cardiomyocytes incubated as described in Figure 1. Values are mean ± SEM, n = 3.

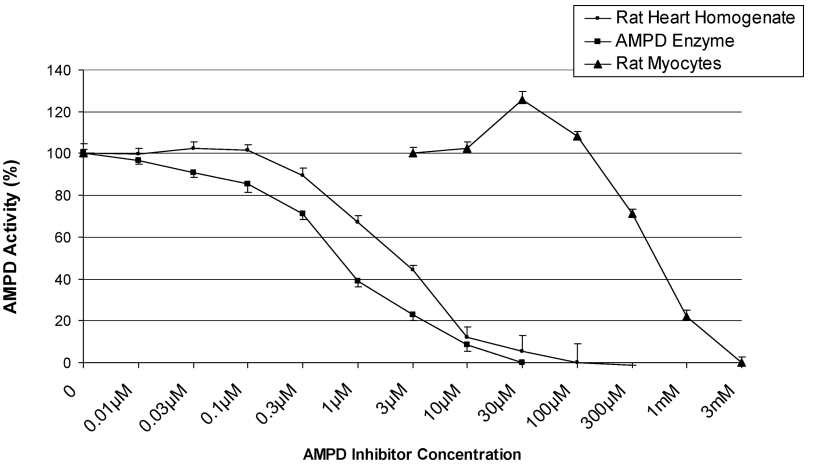

FIGURE 3 Effect of AMP deaminase (AMPD) inhibitor on AMPD activity in rat heart homogenate, rat cardiomyocytes and with isolated human skeletal AMPD. Values are expressed as percentage of activity without inhibitor and represent mean ± SEM, n = 3.

DISCUSSION

The main finding of this study demonstrates that AMPDI effectively inhibits AMPD in rat cardiomyocytes. The Ado/(Ino+IMP) ratio that was used to discriminate whether AMP degradation proceeded via deamination by AMPD or dephosphorylation by 5′nucleotidase was increased several fold. Therefore, the pharmacological inhibition of AMPD shifts AMP degradation towards elevated adenosine production.

Unfortunately, about 1,000 times more AMPDI is needed to achieve this effect in isolated cardiomyocytes compared to the effective concentration observed with isolated human AMPD and rat heart homogenate AMPD. Nevertheless, the high concentration of AMPDI required for AMPD inhibition in vivo does not appear to increase toxicity, as the rate of total catabolite formation was not changed. This study provides essential information for further evaluation of metabolic and functional consequences of AMPD inhibition and shows that AMPDI could be used in pharmacological and mechanistic studies that require specific and effective inhibition of this enzyme.

REFERENCES

1. Loh, E.; Rebbeck, T.R.; Mahoney, P.D.; DeNofrio, D.; Swain, J.L.; Holmes, E.W. Common variant in AMPD1 gene predicts improved clinical outcome in patients with heart failure. *Circulation* **1999**, 99, 1422–1425.
2. Anderson, J.L.; King, G.J.; Bair, T.L.; Elmer, S.P.; Muhlestein, J.B.; Habashi, J.; Mixson, L.; Carlquist, J.F. Associations between a polymorphism in the gene encoding glycoprotein IIIa and myocardial infarction or coronary artery disease. *J. Am. Coll. Cardiol.* **1999**, 33, 727–733.

3. de Groote P.; Lamblin, N.; Helbecque, N.; Mouquet, F.; Hermant, X.; Amouyel, P.; Dallongeville, J.; Bauters, C. The impact of the AMPD1 gene polymorphism on exercise capacity, other prognostic parameters, and survival in patients with stable congestive heart failure: a study in 686 consecutive patients. *Am. Heart J.* **2006**, 152, 736–741.
4. Kasibhatla, S.R.; Bookser, B.C.; Xiao, W.; Erion, M.D. AMP deaminase inhibitors. 5. Design, synthesis, and SAR of a highly potent inhibitor series. *J. Med. Chem.* **2001**, 44, 613–618.
5. Kaletha, K.; Spychala, J.; Nowak, G. Developmental forms of human skeletal muscle AMP-deaminase. *Experientia.* **1987**, 43, 440–443.
6. Kochan, Z.; Smolenski, R.T.; Yacoub, M.H.; Seymour, A.M.L. Nucleotide and adenosine metabolism in different cell types of human and rat heart. J. *Mol. Cell Cardiol.* **1994**, 26, 1497–1503.
7. Smolenski, R.T. The elevation of adenylate pool in rat cardiomyocytes by S-adenosyl-L-methionine. *Acta Biochim. Pol.* **2000**, 47, 1171–1178.
8. Smolenski, R.T.; Lachno, D.R.; Ledingham, S.J.M.; Yacoub, M.H. Determination of sixteen nucleotides, nucleosides and bases using high-performance liquid chromatography and its application to the study of purine metabolism in hearts for transplantation. *J. Chromatogr.* **1990**, 527, 414–420.