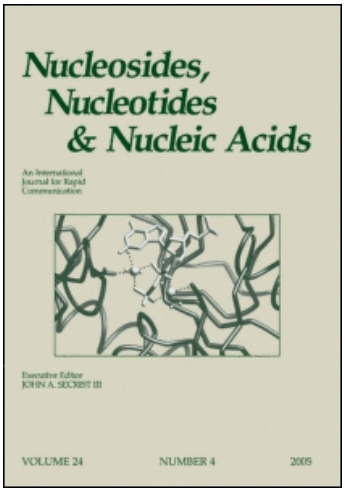

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PROTECTION OF MOUSE HEART AGAINST HYPOXIC DAMAGE BY AMP DEAMINASE INHIBITION

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□ *Clinical observation in patients with heart disease indicates that reduced activity of AMP deaminase could be protective in heart failure and ischemic heart disease. This study evaluated the effect of 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, an AMP deaminase inhibitor (AMPDI) in the mouse heart subjected to hypoxia. ApoE/LDLR knock-out mice were subjected to reduced oxygen tension in breathing air. AMPDI was infused before hypoxia in the treated group. We observed amelioration of elcetrocardiographic changes during hypoxia in the treated group that are consistent with a protective effect.*

Keywords AMP deaminase; hypoxia; heart; mouse

INTRODUCTION

Several recent papers indicate that mutation in AMP deaminase (AMPD) resulting in its reduced cardiac activity is protective in heart failure, ischemic heart disease and in organ donors.[1,2] These observations highlight a potential for development of new drugs to treat heart dysfunction. We recently characterised the biological effects of an AMP deaminase inhibitor (AMPDI) chemically synthesised according to a procedure described by Kasibhatla et al.[3] We have demonstrated effective inhibition of AMP deaminase using AMPDI in heart homogenates, with isolated enzyme and with isolated cardiomyocytes.[4] Our parallel study in this issue shows inhibition of cardiac

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AMP deaminase using AMPDI in perfused heart.[5] We conducted also extensive analysis of the in vivo application of AMPDI based on analysis of IMP accumulation in hearts subjected to global ischemia that demonstrated 70% inhibition of AMP deaminase. This study is currently in preparation for publication. The current study was designed to evaluate whether application of AMPDI in vivo protects hearts during experimental hypoxia.

MATERIALS AND METHODS

An AMP deaminase inhibitor (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.[3] Its identity was confirmed by NMR and mass spectrometry. The experimental model used was hypoxia in ApoE/LDLR-/- knockout mice. This strain is prone to develop atherosclerosis in coronary arteries that makes it vulnerable to reduction of oxygen tension in breathing air.[6] Consequently, heart infarction develops with typical electrocardiographic changes. The ApoE/LDLR-/- mice were anesthetized with pentobarbital and intubated. Ventilation was carried out with a rodent ventilator at fixed volume. Reduction of oxygen tension in breathing air was achieved by mixing air with nitrogen. A rapid response oxygen sensor was connected in line to achieve the desired oxygen tension. Preliminary experiments established that reduction of oxygen tension in breathing air to 5% for 5 minutes was sufficient to induce electrocardiographic changes typical for cardiac ischemia with minimum mortality during the procedure. AMPDI at 100mg/kg/30 minutes or saline in controls was administered intravenously starting 30 minutes before hypoxia. This dose has been established to reduce IMP accumulation during ischemia to less than 30% of control in a preliminary experiments. ECG was monitored during hypoxia using the electrodes attached to the chest.

RESULTS AND DISCUSSION

As illustrated in Figure 1, administration of AMPDI resulted in attenuation of ischemia in treated animals, according to electrocardiographic changes. After 5 minutes of hypoxia, ST segment decreased by 0.15 ± 0.01 mV in controls and by 0.05 ± 0.01 mV in AMPDI treated animals ($p < 0.05$). These results indicate that inhibition of AMP deaminase could be cardioprotective in this in vivo experimental model of heart disease. Electrocardiographic changes are an indication of altered myocardial cell potentials that is a consequence of impaired energy metabolism in cardiomyocytes. Prior infusion of AMPDI markedly reduced these changes. We have also established in a preliminary experiments that the AMPDI concentration used in this study is specific and effective in terms of inhibition of AMP deaminase in the heart

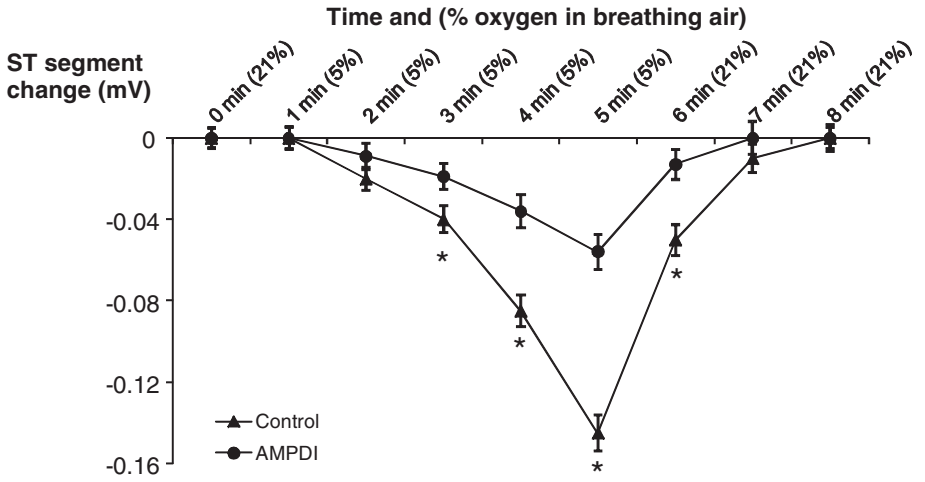

FIGURE 1 Change of electrocardiographic ST segment in C57BL ApoALDLR-/- mouse during hypoxia induced by reduction of oxygen content in breathing air to 5% (A). A dose of 5 mg of AMPDI was intravenously infused for 30 minutes immediately before hypoxia. Values represent mean ± SEM, n = 5. *P < 0.05 versus control.

and causes a 70% reduction of the activity with no effect on other enzymes or cell viability. One potential mechanism for the cardioprotective effect of AMP deaminase inhibition may involve an elevated AMP concentration that could enhance AMP regulated protein kinase activity and trigger a protective pathway in the heart.[7] Alternatively, elevated AMP concentration may lead to increased production of adenosine via the 5′-nucleotidase pathway. Increased adenosine concentration is reportedly protective in a number of experimental models of heart disease.[8] Establishing the exact mechanism will require further studies. There is increasing demand for new therapies of heart failure and ischemic heart disease and the results of our study indicate that AMP deaminase could be a therapeutic target.

REFERENCES

1. Loh, E.; Rebbeck, T.R.; Mahoney, P.D.; DeNofrio, D.; Swain, J.L.H.; Holmes, E.W. Common variant in AMPD1 gene predicts improved clinical outcome in patients with heart failure. *Circ.* **1999**, 99, 1422–1425.
2. Anderson, J.L.; Habashi, J.; Carlquist, J.F.; Muhlestein, J.B.; Horne, B.D.; Bair, T.L.; Pearson, R.R.H.; Hart, N. A common variant of the AMPD1 gene predicts improved cardiovascular survival in patients with coronary artery disease. *J. Am. Coll. Cardiol.* **2000**, 36, 1248–1252.
3. Kasibhatla, S.R.; Bookser, B.C.; Xiao, W.H.; Erion, M.D. AMP deaminase inhibitors. 5. Design, synthesis, and SAR of a highly potent inhibitor series. *J. Med. Chem.* **2001**, 44, 613–618.
4. Borkowski, T.; Orlewska, C.; Slominska, E.M.; Yuen, A.; Lipinski, M.; Rybakowska, I.; Foks, H.; Kaletha, K.K.; Yacoub, M.H.H.; Smolenski, R.T. Pharmacological inhibition of AMP-deaminase in rat cardiac myocytes. *Nucleosides Nucleotides Nucleic Acids* **2008**, 27, 867–871.

5. Borkowski, T.; Slominska, E.M.; Orlewska, C.; Yuen, A.H.Y.; Al-Ayoubi, S.; Siondalski, P.; Yacoub, M.H.H.; Smolenski, R.T. Biological efficiency of AMP deaminase inhibitor: 3-[2-(3-carboxy-4-bromo-5,6,7,8-tetrahydronaphthyl)ethyl]-3,6,7,8-tetrahydroimidazo[4,5]-[1,3]diazepin-8-ol. *Nucleosides Nucleotides Nucleic Acids* **2010** (this issue).
6. Caligiuri, G.; Levy, B.; Pernow, J.; Thoren, P.H.; Hansson, G.K. Myocardial infarction mediated by endothelin receptor signaling in hypercholesterolemic mice. *Proc. Natl. Acad. Sci. U. S. A* **1999**, 96, 6920–6924.
7. Hardie, D.G. AMP-activated protein kinase as a drug target. *Annu. Rev. Pharmacol. Toxicol.* **2007**, 47, 185–210.
8. Smolenski, R.T.; Raisky, O.; Slominska, E.M.; Abunasra, H.; Kalsi, K.K.; Jayakumar, J.; Suzuki, K.H.; Yacoub, M.H. Protection from reperfusion injury after cardiac transplantation by inhibition of adenosine metabolism and nucleotide precursor supply. *Circ.* **2001**, 104, I246–I252.