ELSEVIER

European Journal of Pharmacology 435 (2002) 73–77

www.elsevier.com/locate/ejphar

Bimoclomol elevates heat shock protein 70 and cytoprotects rat neonatal cardiomyocytes

James S. Polakowski [a,*], Craig D. Wegner [b], Bryan F. Cox [a]

[a] *Department of Integrative Pharmacology, Abbott Laboratories, Abbott Park, IL, USA*
[b] *Discovery Biology, Pharmacia Corporation, Skokie, IL, USA*

Received 13 August 2001; received in revised form 12 November 2001; accepted 23 November 2001

Abstract

Bimoclomol is a new compound that improves cell survival under experimental stress conditions partly by increasing intracellular heat shock proteins (HSPs). HSPs, especially HSP70, play a cytoprotective role in the rat heart. Rat neonatal cardiomyocytes were used to determine the ability of bimoclomol to induce HSP70 and affect cell survival across a broad concentration range (0.01–100 μM). Bimoclomol significantly elevated HSP70 levels at concentrations ranging from 0.01 to 10 μM, depending on the time of exposure. Pretreatment with bimoclomol for 24 h significantly increased survival of cells. There was a significant correlation between the increased levels of HSP70 and the increase in cell survival as a result of the treatment with bimoclomol. In conclusion, bimoclomol improved cell survival in rat neonatal cardiomyocytes, in part, by increasing the levels of HSP70. This cytoprotection began at the relatively low concentration of 0.1 μM, which is a concentration that can be achieved clinically. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Bimoclomol; Heat shock protein; Cardiomyocyte; Cytoprotection

1. Introduction

Bimoclomol (Fig. 1) was discovered by the Hungarian pharmaceutical firm Biorex. The compound has been shown to dose-dependently increase nerve conduction velocities in a rat model of diabetes (Bíró et al., 1997) and improve early electrophysiological signs of diabetic retinopathy (Bíró et al., 1998). Treatment with bimoclomol also protects against vascular consequences in experimental subarachnoid hemorrhage (Erdö and Erdö, 1998) and preserves endothelial function in spontaneously hypertensive rats (Jednákovits et al., 2000b). While the mechanism of action is unknown, bimoclomol is hypothesized to act via up-regulation of heat shock proteins (HSPs) (Vígh et al., 1997).

The ability to pharmacologically induce HSPs at clinically relevant concentrations would have therapeutic value in a variety of disease states. Initially, HSPs were identified as a cellular response to hyperthermia (Lindquist, 1986), but have since been shown to be up-regulated under a variety of conditions. These proteins, also called molecular chaperones, are important to the survival of cells under different experimental/physiological stress conditions (Morimoto, 1993). They are under active investigation for their role(s) as markers for cell injury associated with ischemia/reperfusion, various aspects of the immune response, and as a screening tool for toxicology (Welch, 1992). Experimental induction of HSPs via a sub-lethal heat shock improves cell survival upon subsequent exposure to various stresses in different cell culture preparations (Chen et al., 1999; Heads et al., 1995; Rokutan, 2000; Takuma et al., 1996). The important cytoprotective role of HSPs in the heart, especially HSP70, is well established (Currie et al., 1988; Morris et al., 1996). In a rat model of myocardial ischemia/reperfusion injury, in vivo hyperthermia results in induction of HSP70 in rat myocardium, which correlates with a reduction in infarct size (Hutter et al., 1994).

Bimoclomol elevates HSPs, especially HSP70, synergistically with high temperature stress in rat heart myogenic cells and improves survival in HeLa cells (Vígh et al., 1997). Our objectives were to determine if bimoclomol could increase HSP70 levels at clinically relevant concentrations. Further, was the increase in HSP70 associated with an increase in cell survival? Our results demonstrate that in rat neonatal cardiomyocytes treated with bimoclomol at concentrations ranging from 0.1 to 10 μM, there are significant increases in HSP70 levels and cell survival. Additionally, there is a significant linear relationship between the increased levels of HSP70 after 6 h and the increase in cell survival after 24 h

* Corresponding author. Tel.: +1-847-937-9519; fax: +1-847-938-5286.
E-mail address: jim.polakowski@abbott.com (J.S. Polakowski).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(01)01551-5

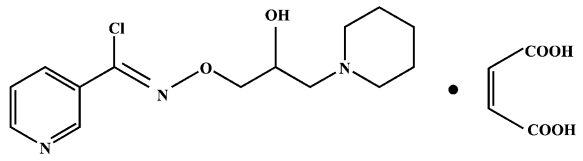

Fig. 1. Chemical structure of bimoclomol maleate.

of treatment with bimoclomol. Therefore, the compound exerts its cytoprotection in rat neonatal cardiomyocytes, at least in part, by increasing the levels of HSP70. This effect began at 0.1 μM, an exposure that can be achieved clinically.

2. Materials and methods

All experiments were conducted in accordance with the National Institutes of Health guidelines for the use of experimental animals and approved by the Abbott Laboratories Internal Animal Care and Use Committee.

2.1. Isolation procedure: rat neonatal cardiomyocytes

Rat neonatal cardiomyocytes were prepared from hearts collected from 1- to 2-day-old rat pups using an established kit (Worthington Biochemical Corporation, Freehold, NJ). Cardiomyocytes were isolated by incubating minced heart tissue with trypsin (50 μg/ml) in a 10-cm sterile Petri dish and refrigerated (4–8 °C) overnight. The following morning trypsin inhibitor (2 mg/ml) was added, followed by a collagenase (300 U/ml) digestion for 30–45 min in a waterbath set at 37 °C. The tissue was dispersed via trituration, allowed to settle and the supernatant filtered through a cell strainer. The cells were spun at 100 × *g* for 5 min, and the pellet resuspended in the final culture medium (either Leibovitz-15 or Dulbecco's Modified Eagle Medium) containing 5% fetal bovine serum. The cardiomyocytes were counted using a hemocytometer and viability determined using trypan blue exclusion (usually >90%). Cells were plated in 6-well culture plates (2 ml/well) that were precoated with laminin in culture medium at 20 μg/ml, to promote the adherence of cells in a monolayer. Final plating densities for the evaluation of HSP70 levels were at 1.5–2 million cells/ml.

2.2. Effects of bimoclomol on HSP70 levels

Plated cardiomyocytes were placed in a 37 °C incubator with a 5% CO_2 environment for 24 h. Media were then changed to serum free. Separate sets of cells were either heat shocked at 42 °C for 1 h or treated as sham (no heat shock). Heat shock was accomplished by sealing the 6-well plate with parafilm and floating it in a waterbath set at 42 °C. After the heat shock or sham procedure, bimoclomol was added to individual wells at 0, 0.01, 0.1, 1, 10 and 100 μM and the plates were placed back in the 37 °C incubator for either 6 or 24 h. The plates were then removed from the incubator after the above-mentioned treatment times. The culture medium was decanted and the adhered cells washed two times with 1 ml of cold 10 mM phosphate-buffered saline (PBS). Solubilizing buffer (200 μl) was added to each well and a scraped cell sample collected. The solubilizing buffer consisted of the following: 50 mM Tris (pH 7.7), 2 mM of ethylenediaminetetraacetic acid (EDTA), 100 mM sodium chloride, 1 mM sodium-vanadate, 1% nonylphenoxy polyethoxy ethanol (Tergitol NP 40), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 5 μg/ml pepstatin A, and 0.025% Bacitracin. The sample was kept on ice until being exposed to a sonic membrane disruptor, twice for 2 s each time, to liberate cytosolic HSP70. These final samples were then frozen at −80 °C until undergoing Western blot analysis.

Protein concentrations were determined based on the Bradford method (Biorad, Hercules, CA). Approximately equal amounts of total protein were loaded onto polyacrylamide gels for electrophoretic separation and subsequent blotting. Gel electrophoresis (Ready Gel Cell System, Biorad) was performed using precast 15-well Tris-glycine for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels with a 10–20% linear gradient and 4% stacking gel. Two gels were run simultaneously, one for a total protein profile, the other for the transfer of proteins to polyvinylidene difluoride (PVDF) membranes. The electrophoretic transfer of proteins was accomplished using the Mini Trans-Blot Cell System (Biorad). After the transfer of proteins, the PVDF membrane was soaked in blocking buffer (Tris buffered saline + 0.1% Tween-20 + 5–10% non-fat dry milk) overnight at 4–8 °C. The next morning, the membranes were probed with HSP70 (K-20) goat polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA). This antibody is specific for HSP70 (inducible form), is non-cross-reactive with HSC70 (constitutive form) and is reactive with mouse, rat and human forms of the protein. After an hour, the membranes were washed and then probed with anti-goat immunoglobulin gamma (IgG) fragment crystallizable (Fc) (rabbit) peroxidase conjugate (Calbiochem, San Diego, CA), incubated for 1 h, washed and developed using the Enhanced Chemi Luminescence Western Blotting System (Amersham, Piscataway, NJ). The developed membrane was wrapped in cellophane placed in a film cassette and developed for 5–10 min. The optimized image was developed using a Kodak film processor. The levels of HSP70 were then quantitated from the bands on the developed film using a PC-based Molecular Dynamics Personal Densitometer SI. Final HSP70 levels were expressed as pixel densities.

2.3. Effects of bimoclomol on cell survival

Using the same cell preparation, a cytoprotection (cell survival) assay was configured to assess the ability of bimoclomol to protect cells exposed to a lethal stress. To optimize the cell survival determinations, final plating densities for this protocol were reduced to approximately 0.5 million cells/ml. Plated cardiomyocytes were placed in an

incubator (37 °C, 5% CO_2) for 24 h. The plates were removed from the incubator and the media changed to serum free. Separate sets of cells were either heat shocked at 42 °C for 1 h or treated as sham (no heat shock). Bimoclomol was then added to individual wells at 0, 0.01, 0.1, 1, 10 and 100 μM and the plates were placed back in the 37 °C incubator for 24 h. The plates were removed from the incubator and after another media change, (serum free) all plates were exposed to a lethal heat stress for 2 h in a waterbath set at 47 °C. The plates were then placed back in the 37 °C incubator overnight (16–18 h). The following morning, cell survival was determined using trypan blue exclusion. Equal volumes of culture medium and trypan blue solution (0.4% Sigma) were mixed. After removing the spent media from the wells, the above mixture was added to the wells for 10 min. The cells were then washed three times with cold PBS and counted with an inverted light microscope (10 ×). The final survival values from this protocol were expressed as the percentage of viable cells per treatment using the formula [(stained cells − total cells) ÷ total cells] × 100.

2.4. Statistical analysis

Results were analyzed by one-way analysis of variance. The Bonferroni method was used to adjust for the five bimoclomol-treatment groups compared to the appropriate vehicle control. All values expressed are mean ± S.E.M.

3. Results

3.1. Effects of bimoclomol on heat shock protein 70 levels

Heat shock significantly ($P<0.05$) elevated HSP70 (Fig. 2). Treatment with bimoclomol alone for 6 h also significantly ($P<0.01$) elevated HSP70 at concentrations ranging from 0.1 through 10 μM. These concentrations achieved HSP70 levels comparable to that achieved by heat shock alone. Twenty-four-hour treatment with bimoclomol significantly elevated ($P<0.01$) HSP70 levels at concentrations ranging from 0.01 through 1 μM. Again, the amplitude of these responses was comparable to heat shock (Table 1).

Table 1
Effect of bimoclomol on HSP70 levels after 24-h incubation

Treatment	Pixel density
(−)HS	35.0 ± 2.62
(+)HS	50.8 ± 6.27 *
0.01 μM	56.3 ± 4.54 **
0.1 μM	51.8 ± 4.91 **
1 μM	49.4 ± 1.67 **
10 μM	44.3 ± 3.16
100 μM	40.4 ± 4.43

Values are mean ± S.E.M.
(−)HS = vehicle-treated cells without sub-lethal heat shock.
(+)HS = vehicle-treated cells with sub-lethal heat shock.
Bimoclomol-treated cells are without heat shock.
Triplicate cell isolations for each treatment.
* $P<0.05$ vs. vehicle, no heat shock.
** $P<0.01$ vs. vehicle, no heat shock.

There were no significant synergistic effects of heat shock plus bimoclomol treatment on HSP70 levels. After the heat shock and 6 h of treatment with bimoclomol at 0.01 through 100 μM, HSP70 pixel densities were 41.1 ± 1.67, 49.8 ± 4.65, 55.5 ± 7.04, 53.5 ± 3.69 and 46.1 ± 6.87, respectively. The value for vehicle-treated heat-shocked cells was 46.6 ± 2.46. After 24 h, bimoclomol at 0.01 through 100 μM resulted in HSP70 levels of 55.6 ± 3.83, 57.8 ± 3.09, 42.3 ± 6.07, 39.5 ± 3.86 and 34.4 ± 6.22, respectively. The value for vehicle-treated heat-shocked cells was 50.8 ± 6.27. In

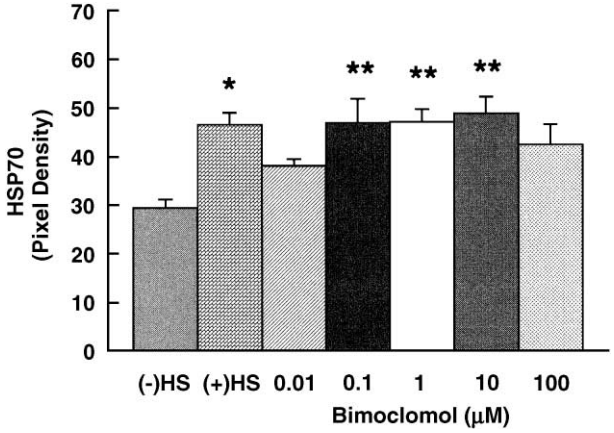

Fig. 2. Effect of bimoclomol on heat shock protein 70 levels in rat neonatal cardiomyocytes after a 6-h incubation. Bimoclomol-treated cells are without sub-lethal heat shock. Triplicate cell isolations for each treatment. Values are mean ± S.E.M. * = $P<0.05$ vs. vehicle-treated cells without sub-lethal heat shock, (−)HS. ** = $P<0.01$ vs. (−)HS. (+)HS = vehicle-treated cells with sub-lethal heat shock.

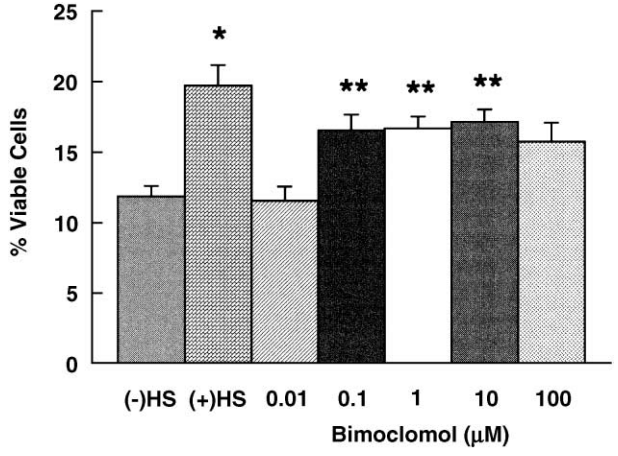

Fig. 3. Effect of bimoclomol on cell survival (expressed as percent of viable cells remaining after a lethal heat stress) in rat neonatal cardiomyocytes after a 24-h incubation. Bimoclomol-treated cells are without heat shock. Triplicate cell isolations for each treatment. Values are mean ± S.E.M. * = $P<0.05$ vs. vehicle-treated cells without sub-lethal heat shock, (−)HS. ** = $P<0.01$ vs. (−)HS. (+)HS = vehicle-treated cells with sub-lethal heat shock.

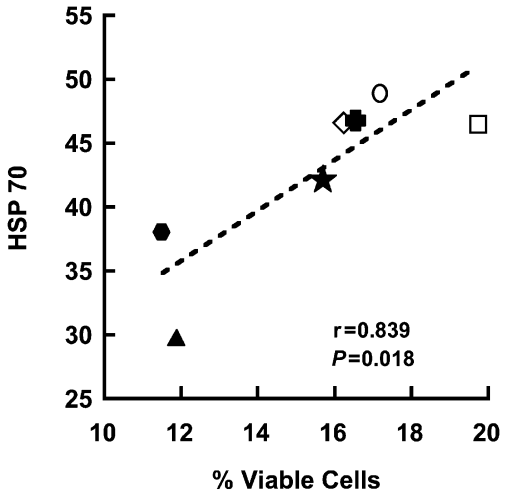

Fig. 4. Correlation between HSP70 levels (6-h incubation) and cell survival (24-h incubation) after treatment with bimoclomol in rat neonatal cardiomyocytes. ▲ = vehicle, no heat shock; □ = vehicle, heat shock; ⬣ = bimoclomol at 0.01 μM; ◊ = bimoclomol at 0.1 μM; ⬟ = bimoclomol at 1 μM; ○ = bimoclomol at 10 μM; ★ = bimoclomol at 100 μM.

both the 6- and 24-h incubation experiments, heat shock plus bimoclomol treatment did not significantly increase HSP70 above either treatment alone for the same incubation times.

3.2. Effects of bimoclomol on cell survival

Cell survival was significantly ($P<0.05$) increased in cells exposed to the sub-lethal heat shock, 42 °C for 1 h, before subjected to the lethal heat stress, 47 °C for 2 h, (Fig. 3). Bimoclomol treatment at 0.1, 1 and 10 μM significantly ($P<0.01$) improved cell survival compared to vehicle-treated cells (Fig. 3).

Heat shock plus bimoclomol did not synergistically improve cell survival after the lethal stress at any concentration compared to sham plus bimoclomol. The percentages of viable cells after sub-lethal heat shock plus bimoclomol at 0.01 through 100 μM were 21.9 ± 1.90, 21.5 ± 1.35, 23.6 ± 1.45, 23.3 ± 1.39 and 21.4 ± 1.51, respectively.

3.3. Correlation between HSP70 and cell survival

Fig. 4 depicts the linear correlation between the amount of HSP70 induced after incubation with bimoclomol for 6 h and cell survival when tested over the same concentration range ($r=0.839$ and $P=0.018$). When bimoclomol was present for 24 h, there was no correlation between HSP70 and cell survival ($r=0.191$ and $P=0.682$, data not shown).

4. Discussion

Our objectives were to determine, across a broad concentration range, if treatment with bimoclomol could increase HSP70. Further, could the compound improve cell survival? Lastly, is there a correlation between elevation of HSP70 and cell survival? The minimal concentration that significantly elevated HSP70 and improved cell survival was 0.1 μM. To our knowledge, this is the first time "efficacy" has been demonstrated at this relatively low concentration. This appears to be a plasma concentration that can be reached clinically. The correlation between elevated HSP70 levels and increased cell survival supports the description of bimoclomol as an "HSP-coinducer" (Jednákovits et al., 2000a). This relationship was not maintained with a longer treatment of bimoclomol. A 24-h incubation shifted the concentration–response to the left for HSP70 levels and therefore resulted in no correlation with cell survival. We in-terpret that distinction to be based on the importance of the early elevation of HSP70, and possibly other HSPs, to initiate a cascade of intracellular events that results in improved cell survival after treatment with bimoclomol.

In the absence of a stress, bimoclomol does not affect HSP levels, but does act synergistically with heat stress to elevate them (Vígh et al., 1997). Our results did not demonstrate this synergistic property. We reached a ceiling-effect response that prevented bimoclomol plus heat shock to significantly increase HSP70 or cell survival above that of either treatment alone. We think that our results may not conflict with those of the earlier study and that a potential explanation may be in methodological differences. Our study utilized primary cell culture in which the cells were switched over to a serum-free media after the first 24 h. This was done to minimize potential fibroblast contamination. The earlier study (Vígh et al., 1997) utilized the immortal cell lines, H9c2 and HeLa, and maintained them in serum-containing medium throughout the duration of the protocols. Changing over to a serum-free medium itself is a "stress" to the cells. This could result in an induction of HSP70 levels above what would occur if cells were allowed to stay in serum-containing medium. In fact, our results show that pixel densities start at approximately 30–35 (6- and 24-h time course, respectively) before any bimoclomol or heat shock intervention. Therefore, our methodology by itself results in some activation of HSP70. Whether this is above what would occur if cells remained in serum-containing medium was not addressed in this study. If a serum-free media environment elevates HSP70 to a relatively high level, this may contribute to the ceiling-effect and subsequent lack of a synergistic enhancement of HSP70 levels when heat shock is combined with bimoclomol. Also, having bimoclomol present before and during the heat shock, which was the case in the earlier study, may enhance the compound's efficacy compared to afterwards, which was the case in our study.

Heat shock proteins, especially HSP70, play an important cytoprotective role in the heart particularly in the condition of myocardial ischemia/reperfusion injury (Knowlton, 1995; Mestril and Dillmann, 1995; Yellon and Latchman, 1992; Plumier and Currie, 1996). This contributed in part to our decision to use rat neonatal cardiomyocytes, to stay within a tissue type where cytoprotection has been shown to involve

HSP70 and has an established use in the literature (Bluhm et al., 1998; Brar et al., 1999; Cumming et al., 1996). Since the compound was effective in improving the survival of cardiomyocytes, bimoclomol may have utility in the treatment of cardiomyopathy and/or congestive heart failure.

It is not clear what structural features of bimoclomol contribute to the cytoprotection. A structurally similar amidoxime has been shown to be cytoprotective in the rat heart (Szabados et al., 2000). This compound modulates poly(-ADP-ribose) polymerase (PARP) and results in mono-ADP-ribosylation of the glucose-regulated protein GRP78, a member of the HSP70 family. Thus, the amidoximes may act on multiple proteins and/or pathways leading to cytoprotection.

In conclusion, bimoclomol can enhance the induction of HSP70 and improve cell survival in rat neonatal cardiomyocytes. A significant correlation exists between HSP70 levels in cells incubated with bimoclomol for 6 h and cell survival, suggesting that the early increase in HSP70 can impact the survival of cells exposed to an otherwise lethal stress. Under the current conditions, these effects start at 0.1 μM, which may be a concentration that is readily achieved in the clinical setting. The therapeutic utility of this agent is currently under clinical investigation.

Acknowledgements

The authors thank the scientists at Biorex Research and Development for supplying bimoclomol used in these studies. To Deborah Kramer and Glenn Reinhart, PhD, for providing the protein level results of the cell culture samples, also to Drs. Gary Gintant and Glenn Reinhart for the helpful suggestions.

References

Bíró, K., Jednákovits, A., Kukorelli, T., Hegedüs, E., Korányi, L., 1997. Bimoclomol (BRLP-42) ameliorates peripheral neuropathy in streptozotocin-induced diabetic rats. Brain Res. Bull. 44, 259–263.

Bíró, K., Pálhalmi, J., Tóth, A.J., Kukorelli, T., Juhász, G., 1998. Bimoclomol improves early electrophysiological signs of retinopathy in diabetic rats. NeuroReport 9, 2029–2033.

Bluhm, W.F., Martin, J.L., Mestril, R., Dillman, W.H., 1998. Specific heat shock proteins protect microtubules during stimulated ischemia in cardiac myocytes. Am. J. Physiol. 275, H2243–H2249.

Brar, B.K., Stephanou, A., Wagstaff, M.J.D., Coffin, R.S., Marber, M.S., Engelmann, G., Latchman, D.S., 1999. Heat shock proteins delivered with a virus vector can protect cardiac cells against apoptosis as well as against thermal or hypoxic stress. J. Mol. Cell. Cardiol. 31, 135–146.

Chen, H.C., Guh, J.Y., Tsai, J.H., Lai, Y.H., 1999. Induction of heat shock protein 70 protects mesangial cells against oxidative injury. Kidney Int. 56, 1270–1273.

Cumming, D.V.E., Heads, R.J., Watson, A., Latchman, D.S., Yellon, D.M., 1996. Differential protection of primary rat cardiocytes by transfection of specific heat stress proteins. J. Mol. Cell. Cardiol. 28, 2343–2349.

Currie, R.W., Karmazyn, M., Kloc, M., Mailer, K., 1988. Heat-shock response is associated with enhanced postischemic ventricular recovery. Circ. Res. 63, 543–549.

Erdö, F., Erdö, S.L., 1998. Bimoclomol protects against vascular consequences of experimental subarachnoid hemorrhage in rats. Brain Res. Bull. 45, 163–166.

Heads, R.J., Yellon, D.M., Latchman, D.S., 1995. Differential cytoprotection against heat stress or hypoxia following expression of specific stress protein genes in myogenic cells. J. Mol. Cell. Cardiol. 27, 1669–1678.

Hutter, M.M., Sievers, R.E., Barbosa, V., Wolfe, C.L., 1994. Heat-shock protein induction in rat hearts: a direct correlation between the amount of heat-shock protein induced and the degree of myocardial protection. Circulation 89, 355–360.

Jednákovits, A., Ferdinándy, P., Jaszlits, L., Bányász, T., Magyar, J., Szigligeti, P., Körtvély, Á., Szentmiklósi, J.A., Nánási, P.P., 2000a. In vivo and in vitro acute cardiovascular effects of bimoclomol. Gen. Pharmacol. 34, 363–369.

Jednákovits, A., Kurucz, I., Nánási, P.P., 2000b. Effect of subchronic bimoclomol treatment on vascular responsiveness and heat shock protein production in spontaneously hypertensive rats. Life Sci. 67, 1791–1797.

Knowlton, A.A., 1995. The role of heat shock proteins in the heart. J. Mol. Cell. Cardiol. 27, 121–131.

Lindquist, S., 1986. The heat shock response. Annu. Rev. Biochem. 55, 1151–1191.

Mestril, R., Dillmann, W.H., 1995. Heat shock proteins and protection against myocardial ischemia. J. Mol. Cell. Cardiol. 27, 45–52.

Morimoto, R.I., 1993. Cells in stress: transcriptional activation of heat shock genes. Science 259, 1409–1410.

Morris, S.D., Yellon, D.M., Marber, M.S., 1996. Stress proteins: a future role in cardioprotection? Heart 76, 97–98.

Plumier, J.C., Currie, R.W., 1996. Heat shock-induced myocardial protection against ischemic injury: a role for HSP70? Cell Stress Chaperones 1, 13–17.

Rokutan, K., 2000. Role of heat shock proteins in gastric mucosal protection. J. Gastroenterol. Hepatol. 15, D12–D19.

Szabados, E., Literáti, P.N., Farkas, B., Sumegi, B., 2000. BGP-15, a nicotinic amidoxime derivate protecting heart from ischemia reperfusion injury through modulation of poly(ADP-ribose) polymerase. Biochem. Pharmacol. 59, 937–945.

Takuma, K., Matsuda, T., Kishida, Y., Asano, S., Seong, Y.H., Baba, A., 1996. Heat shock protects cultured rat astrocytes in a model of reperfusion injury. Brain Res. 735, 265–270.

Vígh, L., Literáti, P.N., Horváth, I., Török, Z., Balogh, G., Glatz, A., Kovács, E., Boros, I., Ferdinándy, P., Farkas, B., Jaslits, L., Jednákovits, A., Korányi, L., Maresca, B., 1997. Bimoclomol: a nontoxic, hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. Nature Med. 3, 1150–1154.

Welch, W.J., 1992. Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol. Rev. 72, 1063–1081.

Yellon, D.M., Latchman, D.S., 1992. Stress proteins and myocardial protection. J. Mol. Cell. Cardiol. 24, 113–124.