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# STUDIES ON THE ACTIVITY OF BEPRIDIL AS A SCAVENGER OF FREE RADICALS

MARCELLE CONSTANTIN,* CHRISTINE BROMONT, RENÉ FICKAT and ROY MASSINGHAM
Department of Pharmacology, RL-CERM, Route de Marsat, 63203 Riom Cedex, France

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**Abstract**—Bepridil, a calcium antagonist with anti-anginal, anti-ischemic, and anti-arrhythmic properties was assessed for its ability to scavenge free radicals. Bepridil reduced the stable free radical 1,1-diphenyl-2-picrylhydrazil (DPPH) in the molar ratio 2:1 and, in this respect, was as active as the reference anti-oxidants hydroquinone and α-tocopherol. Allopurinol and SOD inhibited cytochrome *c* reduction in a hypoxanthine–xanthine oxidase superoxide generating system, whereas bepridil was ineffective. Deoxyribose degradation induced by the ·OH radical was prevented by bepridil ($IC_{50}$ = 0.050 mM). This ability to scavenge ·OH was similar to that of dimethyl sulfoxide (DMSO) ($IC_{50}$ = 0.056 mM) and more potent than that observed with mannitol and allopurinol ($IC_{50}$ values of 0.74 mM and 0.92 mM, respectively). The powerful ·OH scavenging activity of bepridil was confirmed *in vivo* on alloxan induced diabetes in mice. Bepridil exerted a marked protective effect at 0.150 mmol/kg whilst, ethanol and DMSO were active at the doses of 90 and 94 mmol/kg, respectively. These results demonstrate that bepridil is a potent ·OH radical scavenger. This property may contribute to the therapeutic activity of this drug in myocardial ischaemia.

Reactive oxygen species have been proposed to play a role in the production of ischemic myocardial injury [1–3] particularly in reperfusion associated injury [4, 5], arrhythmias [6–8] and in depression of contractility in the so-called stunned myocardium phenomenon [9].

It has been suggested that oxygen free radicals exert their cytotoxic effect by causing peroxidation of membrane phospholipids, resulting in increases in membrane fluidity and permeability with an eventual loss of structure [10–12]. Consequently interest has been focused recently on the possibility of reducing myocardial ischemic injury [13] and post-ischemic dysfunction [14–18] with free radical scavengers or with substances able to interfere with oxygen metabolism.

Bepridil is a calcium antagonist [19–21] with potent anti-anginal [22] and anti-ischemic properties [23]. This drug has proven efficacy in unstable [24] and chronic stable angina [25–28] and acute ischemia following myocardial infarction [23, 29]. Moreover bepridil has anti-arrhythmic properties [30–35]. Experimental studies in animal have shown the efficacy of bepridil in preventing ischemia and reperfusion-induced arrhythmias [36, 37].

The mechanism of action of bepridil as an anti-ischemic drug probably involves more than just the inhibition of the slow inward calcium current in myocardial [19, 38] and vascular smooth cells [20, 39] since it is avidly taken up by both myocardial and smooth muscle cells [40–42] and has been claimed to exert effects upon processes involved in intracellular calcium ion translocations [21].

It is not known if oxygen free radical scavenging activity is implicated in the anti-ischemic and anti-arrhythmic properties of bepridil. Consequently the present study was performed to investigate the ability of bepridil to scavenge active oxygen species.

Potential anti-oxidant properties of the molecule were examined by using the stable free radical DPPH. The efficacy of bepridil to inhibit xanthine oxidase or to scavenge superoxide anion was assessed using inhibition of ferricytochrome *c* reduction, whilst its reactivity with ·OH was examined on ·OH radicals, generated by $Fe^{3+}$, ascorbate, $H_2O_2$ system, detected by their ability to degrade deoxyribose. Reference compounds for these different systems were also studied.

Finally, since numerous studies *in vivo* have implicated oxygen-derived free radicals (mainly ·OH radicals) in the diabetogenic action of alloxan we have investigated the ability of bepridil to prevent alloxan induced diabetes.

Results clearly demonstrate that bepridil is a potent ·OH radical scavenger. This ability of bepridil to scavenge free radicals may contribute to the anti-ischemic and anti-arrhythmic properties of the molecule.

## MATERIALS AND METHODS

### *Materials*

1,1-Diphenyl-2-picrylhydrazil (DPPH), cysteine, hydroquinone, α-tocopherol, allopurinol, 2-deoxy-D-ribose, ethylenediamine tetraacetic acid (EDTA), hydrogen peroxide ($H_2O_2$), L-ascorbic acid, ferric chloride ($FeCl_3.6H_2O$), 2-thiobarbituric acid (TBA), dimethyl sulfoxide (DMSO), alloxan, hypoxanthine cytochrome *c* (type III analytical preparation from horse heart), catalase ($H_2O_2$:$H_2O_2$ oxidoreductase; EC 1.11.1.6; analytical preparation from bovine liver; 14.000 Units/mg) and superoxide dismutase (SOD) (superoxide:superoxide oxidoreductase EC 1.15.1.1; analytical preparation from bovine erythrocytes; 3000 units/mg) were from the Sigma Chemical Co. (St Louis, U.S.A.). Xanthine oxidase (XOD;

* To whom correspondence should be addressed.

xanthine: oxygen oxidoreductase; EC 1.1.3.2.2; analytical preparation from cows milk; 1 unit/mg) was purchased from Boehringer Mannheim (Indianapolis, U.S.A.). 1,1,3,3 Tetramethoxypropan malonaldehyde bis (MDA) was obtained from Aldrich (Milwaukee, U.S.A.), D (−) Mannitol from Merck (Darmstadt, F.R.G.) and all other chemicals and solvents were of analytical grade (Merck). Bepridil was synthetized as the hydrochloride, monohydrate salt by the Chemistry Department RL-CERM.

*Methods*

*Reactivity with DPPH.* The reactivity of bepridil with DPPH and anti-oxidant compounds such as cysteine, hydroquinone and α-tocopherol was carried out using a modification of an earlier method [43]. DPPH was dissolved in 95% ethanol at a concentration of 58.3 μM. To 3 mL of the ethanolic solution of DPPH was added 0.5 mL of test substances, dissolved in ethanol to give a final concentration of 50 μM. Immediately after the addition of test substance, the absorbance of the DPPH was read continuously with a Kontron spectrophotometer, until the obtention of constant absorbance. Tangents were drawn to the curve obtained to determine an initial rate. For stochiometric determinations of the reaction, test substances were dissolved at different concentrations in ethanol. A control solvent obtained with 3 mL of ethanolic solution of DPPH and 0.5 mL of ethanol was performed in each experiment. For control and each concentration of test substance, absorbance was evaluated when the reaction was completed. Reaction values of $\Delta A$ 517 nm in respect to concentration ($c$) were analysed by computer. From the curve $\Delta A = f(c)$, the molar ratio calculated at the equivalent point, was equal to [reduced DPPH]: [oxidized antioxidant]. For each compound five separate experiments were performed. Concentrations in a final volume of 3.5 mL were: cysteine and hydroquinone (0–90 μM), α-tocopherol (0–50 μM) and bepridil (0–100 μM).

*Effects on cytochrome* c *reduction.* Cytochrome $c$ reduction by a hypoxanthine–xanthine oxidase superoxide generating system was quantified at pH 6.8 according to a previous method [44], modified as follows: xanthine oxidase was introduced into the assay before incubation with the appropriate concentrations of each compound under test. The reaction was initiated with hypoxanthine (120 μM) after 3 min preincubation. The superoxide dependent reduction of cytochrome $c$ at 37° was followed by measuring the increase in absorbance at 546 nm for 15 min. The rate of reaction was evaluated from the slope of the absorption time function curve. The effects of bepridil and reference compounds such as SOD and allopurinol on the reduction rate were investigated. Percentage inhibition was calculated by computer analysis comparatively to the assay using solvent only.

SOD was solubilized in water, bepridil in ethanol, the final concentration of ethanol in the reaction being 1.5%. Allopurinol was dissolved in a small volume of M NaOH (10 mg/mL) and the solution made up to desired concentrations using water.

*Degradation of deoxyribose.* Hydroxyl radicals were generated by a mixture of $Fe^{3+}$, EDTA, ascorbic acid and $H_2O_2$ [45]. Degradation of deoxyribose by $^{\cdot}OH$ radical was carried out in: 200 mM phosphate buffer, pH 7.4 or 6.2. Reactants were added in the following order 0.104 mM EDTA; 0.1 mM freshly prepared $FeCl_3$; test substance or solvent; 1 mM $H_2O_2$; 0.336 mM deoxyribose; 0.1 mM ascorbate. Tube contents (1 mL) were incubated at 37° for 30 min instead of 1 hr as described previously [45], then TBA reagent (0.33% w/v in M HCl) was added, followed by heating at 100° for 5 min.

The absorbance was evaluated at 532 nm comparatively to MDA-TBA standards performed as described previously.

Bepridil was dissolved in water and was compared with the known hydroxyl radical scavengers: mannitol [46], DMSO and allopurinol [45, 47]. Mannitol and DMSO were dissolved in water and allopurinol as described above. The effects of compounds on the deoxyribose degradation were used to construct regression lines and to obtain their respective $IC_{50}$ values (the concentration of compound required for 50% reduction in deoxyribose degradation).

*Effects on alloxan induced diabetes.* Diabetes was induced in male OF1 Swiss mice (22 ± 3 g) as described previously [48]. Alloxan monohydrate, dissolved in normal saline was injected into the tail vein (60 mg/kg, 0.2 mL per animal). All compounds or solvent were given as an intraperitoneal injection 0.5 hr before alloxan administration.

Two hydroxyl radical scavengers, ethanol and DMSO reported to have protective effects on alloxan diabetes, were used as reference drugs. Ethanol and DMSO were diluted in isotonic saline. Bepridil was given in a sterile glucose solution (bepridil 4 mg/mL, glucose 48.4 mg/mL). Control animals received solvent and alloxan. Additional groups of animals also received test compounds to ensure that they did not affect basal blood glucose values.

Blood glucose was measured 72 hr after alloxan administration by the glucose oxidase method (Boehringer Mannheim kits).

## RESULTS

*Reactivity with DPPH*

Antioxidants react with the free radical DPPH and convert it to 1,1-diphenyl-2-picrylhydrazine. The change in absorbance produced by this reaction was used to test the ability of bepridil to act as a free radical scavenger. Known reference antioxidants (50 μM) as cysteine, hydroquinone, α-tocopherol reacted with DPPH (50 μM) at initial rate ($A_{517/min}$) respectively of 0.532, 0.550 and 0.420. The equilibration of reaction was reached in 4 min for cysteine, 3 min for hydroquinone and 6 min for α-tocopherol. These three anti-oxidants had different reduction capacities (see Table 1). Decrease in absorbance of DPPH measured with various concentrations of anti-oxidants had shown that 1 mole of cysteine was able to neutralize 1 mole of DDPH and hydroquinone and α-tocopherol 2 moles of DPPH. Molar ratios were, respectively, 1 for cysteine and 2 for α-tocopherol, which agrees with previous studies [49] and 2 for hydroquinone. Ahnfelt-Ronne and Haagen-Nielsen [50] however have

Table 1. Stochiometry of the reduction of DPPH

| Compound | Equivalent point: Reduced DPPH (μM) | Equivalent point: Oxidized compound (μM) | MR |
|---|---|---|---|
| Cysteine | 45 | 49 | 0.9 |
| α-Tocopherol | 40 | 24 | 1.7 |
| Hydroquinone | 42.3 | 23.5 | 1.8 |
| Bepridil | 42 | 21 | 2 |

The equivalent point was determined by the titration of DPPH as shown in Fig. 1. MR, molar ratio = [reduced DPPH] : [oxidized antioxidant] at the equivalent point.

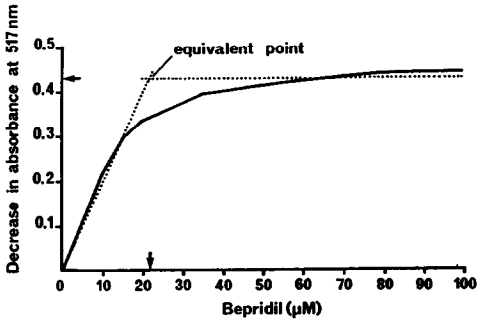


Fig. 1. Titration of DPPH with bepridil. To 3 mL of ethanolic DPPH (58.3 μM) was added 0.5 mL of bepridil in ethanol at concentrations of 0–100 μM in final volume (3.5 mL). Absorbance of DPPH was measured at 517 nm. Reduced DPPH was evaluated from the decrease in absorbance at the equivalent point and compared to absorbance of a DPPH solution (50 μM) without bepridil.

reported a molar ratio of 1 for α-tocopherol. The facilities of one exchangeable hydrogen from free sulfhydryl in cysteine and of two exchangeable hydrogens from hydroquinone and α-tocopherylhydroquinone produced by opening of the side ring in the α-tocopherol [51] may explain the high rate of DPPH reduction by these compounds.

Following the addition of bepridil, the reduction of DPPH with an initial rate $A_{517}$/min of 0.002 was very slow, compared to cysteine, hydroquinone or α-tocopherol. However 80% of the DPPH was reduced at 24 hr with the end of reduction at 96 hr. The influence of the concentration of bepridil on the decrease in absorbance of DPPH at 517 nm is shown in Fig. 1. From the titration of DPPH with bepridil it was estimated that 1 mole of bepridil reduced 2 moles of DPPH, this indicating a transfer of 2 hydrogen atoms per molecule of bepridil. As described for tinoridine [43] these two hydrogen atoms might be abstracted of pyrrolidine ring (Fig. 2). However, the presence of non-substituted phenyl ring [52] may be also involved in the DPPH reduction.

*Cytochrome c reduction*

The rate of cytochrome *c* reduction was linear with respect to time between 0 and 5 min. In the presence of SOD, which specifically dismutases superoxide anion, the cytochrome *c* reduction was inhibited by 66% for an activity of 9.15 units/mL. An activity of 45.75 units/mL was required to obtain an almost complete inhibition (see Table 2). Allopurinol, a xanthine oxidase inhibitor, starting at a concentration of 20 μM produced a marked decrease of cytochrome *c* reduction. This reduction of cytochrome *c* was not affected by bepridil even at the high concentration of 100 μM. This finding indicates that bepridil has no activity either as an $^{\cdot}O_2$ scavenger or as an inhibitor of xanthine oxidase.

*Degradation of deoxyribose*

Results are shown in Table 3. Hydroxyl radical scavengers inhibit deoxyribose degradation presumably by preventing $^{\cdot}OH$ generation in the reaction mixture. In agreement with published results, the mannitol concentration producing 50% inhibition of deoxyribose degradation ($IC_{50}$) was 0.742 mM. However, with allopurinol ($IC_{50}$ = 0.925 mM) our results are slightly higher than those reported by Moorhouse *et al.* [45]. This author reports a deoxyribose degradation inhibition of 57% at 0.5 mM. The reference compound DMSO was active from a concentration of 0.01 mM where an inhibition of 24.5% was obtained. The hydroxyl radical scavenger DMSO was over 10 times more potent than either mannitol or allopurinol. A marked inhibition was obtained with bepridil (27.2%), starting at the concentration of 0.01 mM. $IC_{50}$ values were of the same order for bepridil as DMSO. Bepridil is therefore a powerful scavenger of $^{\cdot}OH$ radicals.

*Alloxan-induced diabetes in mice*

Alloxan (60 mg/kg i.v.), produced an increase in blood glucose levels in mice of between 135–172%, compared to the control animals, receiving saline. Pretreatment with the hydroxyl radical scavengers, ethanol and DMSO [53], prevented this hyperglycemic effect of alloxan. The protective action of DMSO was not significant at 37 mmol/kg, but at 94 mmol/kg, inhibition was of the order of 86%. The diabetogenic action of alloxan was also partially prevented by ethanol at the dose of 87 mmol/kg where the glucose increase was inhibited by 79% (see Table 4). These results are in agreement with those reported previously [54]. The protective effect of DMSO and ethanol is certainly dependent on the dose of alloxan and, in fact, a complete protection by DMSO against the diabetogenic action of alloxan

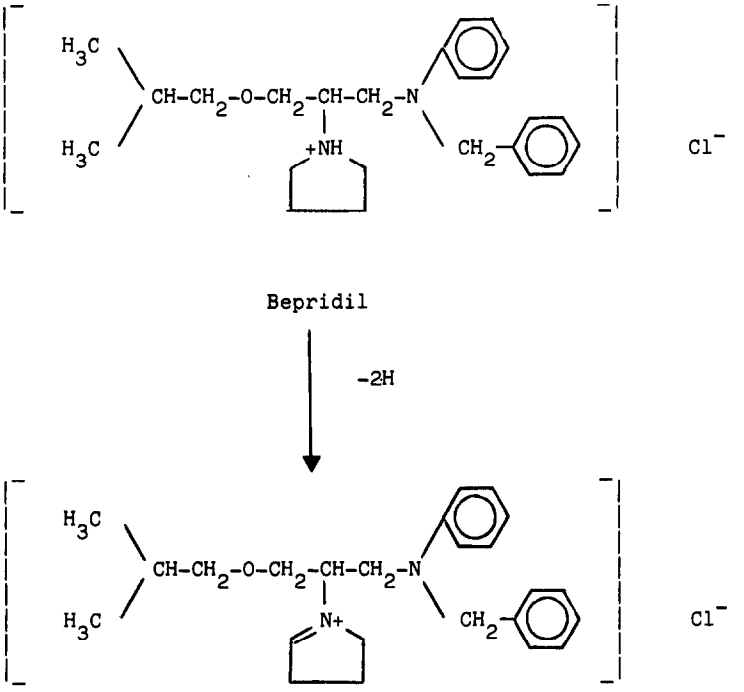


Fig. 2. Possible reaction for bepridil and 1,1-diphenyl-2-picrylhydrazyl.

Table 2. Effects of bepridil, SOD and allopurinol on the reduction of cytochrome *c* by a hypoxanthine–xanthine oxidase system

| Addition | *b* | N | *r* | Inhibition (%) |
|---|---|---|---|---|
| Control solvent ($H_2O$) | 0.041 | 3 | 0.967 | |
| SOD 9.15 units/mL | 0.014 | 3 | 0.977 | 66 |
| 45,75 units/mL | 0.007 | 3 | 0.973 | 83 |
| Control ($NaOH/H_2O$) | 0.041 | 7 | 0.969 | |
| Allopurinol 20 μM | 0.020 | 7 | 0.886 | 51 |
| 40 μM | 0.004 | 7 | 0.850 | 90 |
| Control (ethanol) | 0.044 | 4 | 0.961 | no action |
| Bepridil 40 μM | 0.043 | 4 | 0.981 | no action |
| 80 μM | 0.046 | 4 | 0.992 | no action |

Samples containing EDTA (0.1 mM), cytochrome *c* (0.08 mM), catalase (200 units/mL) and XO (0.03 unit/mL) were incubated with solvent or test compounds in 50 mM phosphate buffer (pH 6.8 at 37°). Cytochrome *c* reduction was initiated by addition of hypoxanthine (120 μM) and followed at 546 nm. From absorbance values obtained 5 min following hypoxanthine addition, regression lines were calculated (*r*, regression coefficient; *b*, the slope of the regression line; N, number of experiments. The percentage inhibition was calculated from the slopes of the regression lines obtained with control solvent and test compounds.

Table 3. Inhibition of deoxyribose degradation by mannitol, DMSO, allopurinol and bepridil

| Compound | $IC_{50}$ value (mM) | N | *r* |
|---|---|---|---|
| Mannitol | 0.742 (0.704–0.779) | 5 | 0.98 |
| DMSO | 0.056 (0.045–0.066) | 6 | 0.86 |
| Allopurinol | 0.925 (0.800–1.049) | 6 | 0.89 |
| Bepridil | 0.050 (0.045–0.062) | 6 | 0.84 |

Reaction mixtures (1 mL) containing EDTA (104 μM), FeCl3 (100 μM), test substance or solvent, $H_2O_2$ (1 mM), deoxyribose (336 μM), and ascorbate (100 μM), were incubated in phosphate buffer (200 mM, pH 7.4 or 6.2 at 37°) for 30 min. Samples were then boiled (for 5 min with TBA and the TBA–MDA complex measured at 532 nm. The absorbance of samples containing test compounds were compared with absorbance of their respective controls with solvent, and the per cent inhibition of MDA formation calculated. From results obtained for various concentrations of test compounds, regression lines were constructed and $IC_{50}$ calculated (N = number of experiments; *r*, regression coefficient).

at 50 mg/kg has been reported, while protection was only partial after 75 mg/kg alloxan [54].

Pretreatment with bepridil at the dose of 0.150 mmol/kg elicited a small decrease of 19% in basal blood glucose levels. It seems unlikely that the small reduction in basal blood glucose levels is responsible for the antagonism of alloxan-induced hyperglycaemia. On the contrary it has been found that compounds which transiently elevate blood glucose levels are able to protect against the diabetogenic action of alloxan [55, 56], although for some other workers this fact was less clear [48]. When given at the dose of 0.150 mmol/kg, 30 min before alloxan, bepridil largely protected against the induced hyperglycaemia, the alloxan-induced glucose increase being inhibited by 80%. There was also a significant inhibition (34%) at 0.075 mmol/

Table 4. Protective effect of ethanol and DMSO on the induction of hyperglycemia by alloxan

| Treatment | Dose mmol/kg | Blood glucose mM ± SE |
|---|---|---|
| Saline | | 9.76 ± 0.25 (12) |
| DMSO | 37 | 9.33 ± 0.38 (6)* |
| | 94 | 10.01 ± 1.10 (12)* |
| Saline + alloxan | | 23.03 ± 1.64 (34) |
| DMSO + alloxan | 37 | 18.27 ± 2.10 (20) |
| | 94 | 11.82 ± 0.75 (29)† |
| Saline | | 9.65 ± 0.18 (24) |
| Ethanol | 87 | 10.06 ± 0.24 (24)* |
| Saline + alloxan | | 26.20 ± 1.67 (42) |
| Ethanol + alloxan | 87 | 13.54 ± 1.02 (39)† |

The number of animals in each group is shown in parentheses.

Control animals received only i.p. saline, DMSO (37 and 94 mmol/kg) or ethanol (87 mmol/kg).

* Not significant when compared to saline control.

† $P < 0.05$ when compared to saline + alloxan (Student's *t*-test).

diabetes has previously been shown with series of compounds having structural similarities. The differences in effectiveness of the ·OH scavengers DMSO and bepridil *in vivo* cannot be explained solely on their abilities to react with ·OH radicals. Seeing that bepridil possess in addition marked antioxidant properties, two mobile hydrogen atoms may augment the scavenging activity on ·OH radicals. On the other hand, bepridil is highly lipophilic and is bound to a high degree (> 99%) to serum proteins [58, 59]. A high value ($> 10^7$) for the octanol/water partition coefficient confirms the lipophilic characteristics of bepridil. This could facilitate the uptake of bepridil into $\beta$ pancreatic cells where, as suggested by Oberley [60], alloxan induces cytotoxicity by producing active oxygen species.

Whilst the dose of bepridil shown to be active in mice in the alloxan study appears to be high (0.075 mmol/kg, equivalent to 32 mg/kg), it should be remembered that accumulation of bepridil occurs in certain tissues (see e.g. Ref. 41). Furthermore,

Table 5. Protective effect of bepridil on the induction of hyperglycemia by alloxan

| Treatment | Dose bepridil mmol/kg | Blood glucose mM ± SE |
|---|---|---|
| Saline | | 9.12 ± 0.25 (20) |
| (4.84%) Glucose | | 9.71 ± 0.19 (20)* |
| Bepridil | 0.075 | 9.45 ± 0.25 (20) |
| Bepridil | 0.150 | 7.85 ± 0.23 (20)† |
| (4.84%) Glucose + Alloxan | | 22.84 ± 1.44 (40) |
| Bepridil + Alloxan | 0.075 | 18.18 ± 1.40 (40)‡ |
| Bepridil + Alloxan | 0.150 | 10.46 ± 1.16 (40)‡ |

Control animals received only i.p. saline, 4.84% glucose or bepridil (0.075 and 0.150 mmol/kg).

The number of animals in each group is shown in parentheses.

Data are means ± SE.

* Not significant when compared to saline control.

† $P < 0.01$ when compared to (4.84%) glucose control.

‡ $P < 0.05$ when compared to (4.84%) glucose + alloxan (Student's *t*-test).

kg bepridil (see Table 5). Compared to DMSO, a powerful ·OH scavenger, the protective effect of bepridil on the diabetogenic action of alloxan was apparent at a dose of bepridil about 100 times lower than that of DMSO. These findings therefore confirm the powerful ·OH activity of this molecule.

## DISCUSSION

In the deoxyribose degradation model, the potency of DMSO and bepridil as ·OH radical scavengers was similar ($IC_{50} = 0.05$ mM). As previously reported (e.g. see Ref. 57), the compounds order of reactivity with ·OH *in vitro* was the same as their order of protection against alloxan diabetes in the *in vivo* model. A positive correlation has been reported in these two models for an homologous series of alcohols [48] and for various urea derivatives [57]. It must be mentioned, however, that this correlation between molecules having reactivity with ·OH radicals and the degree of protection in alloxan-induced

biopsy of myocardial tissue in patients undergoing coronary artery bypass operation, stabilised on 300 mg a day bepridil, gave mean tissue levels some 17 times higher (= 12.4 $\mu$g/g tissue) than plasma levels of bepridil in these subjects (RL-CERM, unpublished internal data). Extrapolating therefore to the situation *in vivo*, it would seem quite possible that levels of the drug capable of scavenging free radicals could be obtained following administration of doses of bepridil in the therapeutic dose range.

Among oxygen derived free radicals which are implicated in ischemia and reperfusion myocardial injury, the hydroxyl radical is reported to be one of the most cytotoxic [14, 61]. This highly reactive radical oxidizes membrane lipids and thereby initiates a cascade of lipid peroxidation [62, 63] resulting in membrane disruption. Due to its ·OH radical scavenging activity, in addition to the hydrophobic nature of the molecule, it could be argued that bepridil possesses anti-lipoperoxidant properties, which might partially explain the cardioprotective effects of this drug observed during ischemic heart disease

[64]. Indeed a recent study indicated that bepridil very effectively attenuated ($IC_{50} = 55\ \mu M$) $^{\cdot}O_2$ dependent iron-promoted peroxidation of phospholipids obtained from rat myocardium [52]. This antiperoxidant protective activity of bepridil being similar to the potency of bepridil as $^{\cdot}OH$ radical scavenger on the deoxyribose degradation: ($IC_{50} = 50\ \mu M$).

Moreover free radical-induced membrane damage may be involved in the genesis of reperfusion induced rhythm disturbances [65]. In fact oxygen radical species may compromise membrane ion pump activity and lead to local electrophysiological derangements that trigger ventricular arrhythmias [66]. The involvement of free radicals in reperfusion arrhythmias and the protective effects of anti-free radical interventions have been demonstrated in several animal models [6, 7, 67, 68]. Thus the efficacy of bepridil in preventing ischemia and reperfusion induced arrhythmias [30, 36, 37] could be partially explained by $^{\cdot}OH$ scavenging properties.

Finally, in recent years, a number of studies have suggested that oxygen metabolites, in particular $^{\cdot}OH$ radicals, are implicated in the genesis of myocardial stunning [9]. Thus, it has been shown that the recovery of post-ischemic cardiac dysfunction is enhanced by free radical scavengers [15, 17, 18, 69]. Transient ischemia associated with myocardial stunning occurs in numerous clinical settings, thus following both brief (fully reversible) and prolonged (partially irreversible) ischemia there is only a slow recovery of myocardial function which can take hours or days to fully reverse [70]. It could be that hydroxyl radical scavengers like bepridil would be active in preventing such myocardial reperfusion injury as in the so called stunned myocardium phenomenon. In this respect, it may be of relevance that bepridil, unlike certain other calcium antagonists, is capable of reversing ischaemia-induced contractile dysfunction in the dog heart following partial, permanent ligation of the left circumflex coronary artery [71].

In conclusion, these studies have demonstrated that bepridil is a potent $^{\cdot}OH$ radical scavenger. This ability of bepridil to scavenge free radicals may contribute to the anti-ischemic and anti-arrhythmic properties of the molecule.

## REFERENCES


1. Rao PS, Cohen MY and Mueller HS, Production of free radicals and lipid peroxides in early experimental myocardial ischemia. *J Mol Cell Cardiol* **15**: 713–716, 1983.
2. Gardner TJ, Stewart JR, Casale AS, Downey JM and Chambers DE, Reduction of myocardial ischemic injury with oxygen-derived free radical scavengers. *Surgery* **94**: 423–427, 1983.
3. Simpson PJ, Michelson JK and Lucchesi BR, Free radical scavengers in myocardial ischemia. *Fed Proc* **46**: 2413–2417, 1987.
4. Hess ML and Manson NH, The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemic/reperfusion injury. *J Mol Cell Cardiol* **16**: 969–985, 1984.
5. Werns SW, Shea MJ and Lucchesi BR, Free radicals and myocardial injury: pharmacologic implications. *Circulation* **74**: 1–4, 1986.
6. Manning AS, Coltart DJ and Hearse DJ, Ischemia and reperfusion-induced arrhythmias in the rat. Effects of xanthine oxidase inhibition with allopurinol. *Circ Res* **55**: 545–548, 1984.
7. Woodward B and Zakaria MNM, Effects of some free radical scavengers on reperfusion-induced arrhythmias in the isolated rat heart. *J Mol Cell Cardiol* **17**: 485–493, 1985.
8. Hearse DJ and Tosaki A, Free radicals and reperfusion-induced arrhythmias: protection by Spin Trap agent PBN in the rat heart. *Circ Res* **60**: 375–383, 1987.
9. Bolli R, Oxygen-derived free radicals and postischemic myocardial dysfunction (stunned myocardium). *J Am Coll Cardiol* **12**: 239–249, 1988.
10. Meerson FZ, Kagan VE, Kozlov YP, Belkina LM and Arkhipenko YV, The role of lipid peroxidation in pathogenesis of ischemic damage and anti-oxidant protection of the heart. *Basic Res Cardiol* **77**: 465–485, 1982.
11. Freeman BA and Crapo JP, Biology of disease, free radicals and tissue injury. *Lab Invest* **47**: 412–426, 1982.
12. Shlafer M, Kane PF, Wiggins VY and Kirsh MM, Possible role for cytotoxic oxygen metabolites in the pathogenesis of cardiac ischemic injury. *Circulation* **66**: I-85-I-92, 1982.
13. Steward JR, Blackwell WH, Crute SL, Loughlin V, Greenfield LJ and Hess ML, Inhibition of surgically induced ischemia/reperfusion injury by oxygen free radical scavengers. *J Thorac Cardiovasc Surg* **86**: 262–272, 1983.
14. Myers ML, Bolli R, Lekich RF, Hartley CJ and Roberts R, Enhancement of recovery of myocardial function by oxygen free radical scavengers after reversible regional ischemia. *Circulation* **72**: 915–921, 1985.
15. Myers ML, Bolli R, Lekich RF, Hartley CJ and Roberts R, *N*-2 Mercaptopropionylglycine improves recovery of myocardial function after reversible regional ischemia. *J Am Coll Cardiol* **8**: 1161–1168, 1986.
16. Gross GJ, Farber NE, Hardman HF and Warltier DC, Beneficial actions of superoxide dismutase and catalase in stunned myocardium of dogs. *Am J Physiol* **250**: H372–H377, 1986.
17. Bolli R, Wei-Xi-Zho, Hartley CJ, Michael LH, Repine JE, Hess ML, Kukreja RC and Roberts R, Attenuation of dysfunction in the postischemic stunned myocardium by dimethylthiourea. *Circulation* **76**: 458–468, 1987.
18. Farber NE, Vercellotti GM, Jacob HS, Pieper GM and Gross GJ, Evidence for a role of iron-catalyzed oxidants in functional and metabolic stunning in the canine heart. *Circ Res* **63**: 351–360, 1988.
19. Labrid C, Grosset A, Dureng G, Mironneau J and Duchene-Marullaz P, Some membrane interactions with bepridil, a new antianginal agent. *J Pharmacol Exp Ther* **211**: 546–554, 1979.
20. Harder DR and Sperelakis N, Bepridil blockade of $Ca^{++}$-dependent action potentials in vascular smooth muscle of dog coronary artery. *J Cardiovasc Pharmacol* **2**: 906–914, 1981.
21. Flaim SF, Ratz PH, Swigart SC, Gleason ME, Bepridil hydrochloride alters potential-dependent and receptor-operated calcium channels in vascular smooth muscle of rabbit aorta. *J Pharmacol Exp Ther* **234**: 63–71, 1985.
22. Cohn JN, Symposium on bepridil: a new antianginal agent. Introduction. *Am J Cardiol* **55**: 1C–2C, 1985.
23. Remme WJ, Kruyssen D, Van Hoogenhuyze D, Hofman B, Krauss XH and Storm CJ, Acute hemodynamic and antiischemic properties of intravenous bepridil in coronary artery disease. *Am J Cardiol* **63**: 670–675, 1989.
24. Amat G, Boch C, Curnier JM and Colonna D, Etude

clinique de l'efficacité et de la tolérance du bépridil dans l'angor instable. *Sem Hop Paris* **58**: 2660–2663, 1982.
25. Narahara KA, Shapiro W, Weliky I and Park J, Hemodynamic actions of bepridil during treatment of stable angina pectoris. *Am J Cardiol* **55**: 55C–58C, 1985.
26. Di Bianco R, Katz RJ, Chesler E, Alpert JS and Spann JF, Long-term efficacy of bepridil in patients with chronic stable angina pectoris: results of a multicenter placebo-controlled study of extended bepridil use. *Am J Cardiol* **55**: 50C–54C, 1985.
27. Hill JA, O'Brien JT, Alpert JS, Gore JM, Zusman RM, Christensen D, Boucher ChA, Vetrovec G, Borer JS, Friedman C, Mack R, Conti CR and Pepine CJ, Effect of bepridil in patients with chronic stable angina: results of a multicenter trial. *Circulation* **71**: 98–103, 1985.
28. Pflugfelder PW, Humen DP, O'Brien PA, Purves PD, Jablonsky G and Kostuk WJ, Comparison of bepridil with nadolol for angina pectoris. *Am J Cardiol* **59**: 1283–1288, 1987.
29. Flammang D, Waynberger M and Paillet R, Beneficial effects of bepridil (BEP). A new calcium antagonist in acute myocardial infarction. *Eur Heart J* **5** (Suppl I): 220, 1984.
30. Prystowski E, Electrophysiologic and antiarrhythmic properties of bepridil. *Am J Cardiol* **55**: 59C–62C, 1985.
31. Roy D, Montigny M, Klein GJ, Sharma AD, Cassidy D, Electrophysiologic effects and long term efficacy of bepridil for recurrent supraventricular tachycardias. *Am J Cardiol* **59**: 89–92, 1987.
32. Levy S, Cointe R, Metge M, Faugere G, Valeix B and Gerard R, Bepridil for recurrent sustained ventricular tachycardias: assessment using electrophysiologic testing. *Am J Cardiol* **54**: 579–581, 1984.
33. Davy JM, Laine JF, Sebag C and Motte G, Bepridil a new calcium antagonist for the treatment of ventricular tachycardia. *Circulation* **68** (Suppl III): 310, 1983.
34. Kane KA and Winslow E, Antidysrrythmic and electrophysiological effects of a new antianginal agent, bepridil. *J Cardiovasc Pharmacol* **2**: 193–203, 1980.
35. Alpert JS, Coumel P, Greeff K, Krikler DM, Remme WJ and Schoenbaum E, Bepridil: a review of its pharmacology and clinical efficacy as an anti-anginal agent with anti-arrhythmic properties. *Pharmatherap* **4**: 195–222, 1985.
36. Lynch JL, Montgomery DG, Ventura A and Lucchesi BR, Antiarrhythmic and electrophysiologic effects of bepridil in chronically infarcted conscious dogs. *J. Pharmacol Exp Ther* **234**: 72–80, 1985.
37. Marshall RJ and Winslow E, Prevention by bepridil of ischemia and reperfusion induced arrhythmias in experimental animals. *International Cordium Symposium (bepridil), organized by RIOM Laboratories-CERM, Athenes, October 21*, 1988.
38. Vogel S, Crampton R and Sperelakis N, Blockade of myocardial slow channels by bepridil (CERM 1978). *J Pharmacol Exp Ther* **210**: 378–385, 1979.
39. Mras S and Sperelakis N, Bepridil (CERM 1978) blockade of action potentials in cultured rat aortic smooth muscle cells. *Eur J Pharmacol* **71**: 13–19, 1981.
40. Mras S and Sperelakis N, Comparison of ($^3$H] bepridil and ($^3$H) verapamil uptake into rabbit aortic rings. *J Cardiovasc Pharmacol* **4**: 777–783, 1982.
41. Pang DC and Sperelakis N, Nifedipine, Diltiazem, Bepridil and Verapamil uptakes into cardiac and smooth muscles. *Eur J Pharmacol* **87**: 199–207, 1983.
42. Cramb G and Dow JW, Uptake of bepridil into isolated ventricular myocytes. Retention by actin. *Biochem Pharmacol* **32**: 227, 1983.
43. Shimada O and Yasuda H, Hydroxyl radical scavenging action of tinoridine. *Agents Actions* **19**: 208–214, 1986.
44. Spectror T, Oxypurinol as an inhibitor of xanthine oxidase catalyzed production of superoxide radical. *Biochem Pharmacol* **37**: 349–352, 1988.
45. Moorhouse Ph, Grootveld M, Halliwell B, Quinlan G and Gutteridge JMC, Allopurinol and oxypurinol are hydroxyl radical scavengers. *FEBS Lett* **213**: 23–28, 1987.
46. McCord JM and Fridovich I, Production of $^1O_2$ in photolysed water demonstrated through the use of superoxide dismutase. *Photochem Photobiol* **17**: 115–121, 1973.
47. Parks DA and Granger DN, Ischemia-induced vascular changes, role of xanthine oxidase and hydroxyl radicals. *Am J Physiol* **245**: G285–G289, 1983.
48. Heikkila RE, Winston B and Cohen G, Alloxan-induced diabetes. Evidence for hydroxyl radical as a cytotoxic intermediate. *Biochem Pharmacol* **25**: 1085–1092, 1976.
49. Banda PW, Sherry AE and Blois MS, The reaction of diphenylpicryl hydrazil with physiological compounds. *Anal Lett* **7**: 41–52, 1974.
50. Ahnfelt-Ronne and Haagen-Nielsen O, The anti-inflammatory moiety of sulfasalazine, 5-amino salicylic acid, a radical scavenger. *Agents Actions* **21**: 191–194, 1987.
51. Blois MS, Antioxidant determinations by the use of a stable free radical. *Nature* **181**: 1199–1200, 1958.
52. Janero DR, Burghardt B and Lopez R, Protection of cardiac membrane phospholipid against oxidative injury by calcium antagonists. *Biochem Pharmacol* **37**: 4197–4203, 1988.
53. Ashwood-Smith MJ, Current concepts concerning radioprotective and cytoprotective properties of dimethyl sulfoxide in cellular systems. *Ann NY Acad Sci* **243**: 246–256, 1975.
54. Heikkila RE, The prevention of alloxan-induced diabetes in mice by dimethylsulfoxide. *Eur J Pharmacol* **44**: 191–193, 1977.
55. Zawalich WS and Beidler LM, Glucose and alloxan interactions in the pancreatic islets. *Am J Physiol* **224**: 963–966, 1973.
56. Schauberger CW, Thies RL and Fischer LJ, Mechanisms of protection from alloxan diabetes provided by *n*-butanol. *J Pharmacol Exp Ther* **201**: 450–455, 1977.
57. Tibaldi J, Benjamin J, Cabbat FS and Heikkila RE, Protection against alloxan-induced diabetes by various urea derivatives: relationship between protective effects and reactivity with the hydroxyl radical. *J Pharmacol Exp Ther* **211**: 415–418, 1979.
58. Albengres E, Urien S, D'Athis P, Pognat JF and Tillement JP, Etude de la fixation du bépridil sur les protéines du sérum et sur les éléments figurés du sang. *J Pharmacol* **13**: 81–82, 1982.
59. Pritchard JF, Mckown LA, Dvorchik BH and O'Neill PJ, Plasma protein binding of bepridil. *J Clin Pharmacol* **25**: 347–353, 1985.
60. Oberley LW, Free radicals and diabetes. *J Free Rad Biol [Med]* **5**: 113–124, 1988.
61. Burton KP, McCord JM and Ghai G, Myocardial alterations due to free-radical generation. *Am J Physiol* **246**: H776–H783, 1984.
62. Halliwell B and Gutteridge JMC, Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* **219**: 1–14, 1984.
63. Thompson JA and Hess ML, The oxygen free radical system: a fundamental mechanism in the production of myocardial necrosis. *Prog Cardiovasc Dis* **28**: 449–462, 1986.
64. Jap TJW, Hugtenburg JG, Boddeke HWGM and Van Zwieten PA, The cardioprotective effect of different calcium antagonists during normothermic global ischemia. *Br J Pharmacol* **96** (Suppl I): 196P, 1989.

65. Manning AS, Reperfusion-induced arrhythmias: do free radicals play a critical role? *J Free Rad Biol Med* **4**: 305–316, 1988.
66. Mjos OD, Possible role of lipids and of free radicals in arrhythmogenesis. In: *Handbook of Experimental Pharmacology, Antiarrhythmic Drugs* (Eds. Vaughan Williams EM, Campbell TJ), p. 591–600. Springer, Berlin, 1989.
67. Bernier M, Hearse DJ and Manning AS, Reperfusion-induced arrhythmias and oxygen-derived free radicals. Studies with "anti-free radical" interventions and a free radical-generating system in the isolated perfused rat heart. *Circ Res* **58**: 331–340, 1986.
68. Riva E, Manning AS and Hearse DJ, Superoxide dismutase and the reduction of reperfusion-induced arrhythmias: *in vivo* dose–response studies in the rat. *Cardiovasc Drugs Ther* **1**: 133–139, 1987.
69. Przyklenk K and Kloner RA, Superoxide dismutase plus catalase improve contractile function in the canine model of the "stunned" myocardium. *Circ Res* **58**: 148–156, 1986.
70. Greenfield RA and Swain JL, Disruption of myofibrillar energy use: dual mechanisms that may contribute to postischemic dysfunction in stunned myocardium. *Circ Res* **60**: 283–289, 1987.
71. Beaughard M, Lamar JC, Piris P and Tisné-Versailles J, Effects of bepridil and nifedipine on regional myocardial contractility during ischaemia in anesthetized dogs. *Arch Int Pharmacodyn Ther* **279**: 83–102, 1986.