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Hemodynamic effects of MF 10058, a new cardioselective muscarinic M_2 receptor antagonist, in conscious dogs

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Abstract

The 5-{4-[4-(diethylamino)butyl]-1-piperidinyl}acetyl-5*H*-dibenz[*b*,*f*]-azepine (MF 10058) is a new potent and selective muscarinic M_2 receptor antagonist. The hemodynamic effects of MF 10058 were investigated in conscious freely moving dogs. Placebo and three doses of MF 10058 (2, 4 and 8 mg/kg) were orally administered according to a randomised four-way crossover design. Heart rate, cardiac conduction times, systolic and diastolic blood pressure were telemetrically recorded for 12–24 h after dosing. After placebo administration, a consistent reduction over time in heart rate was observed during the night-time period (-15%, $P = 0.019$). MF 10058 administration antagonised the nocturnal bradycardia and shortened QT interval. The effect of the drug reached statistically significance, compared to placebo, with the highest dose of 8 mg/kg ($+19\%$ on heart rate, $P = 0.013$; -4% on QT interval, $P = 0.049$). The effect on heart rate lasted for the entire 24-h observation period ($+16\%$, $P = 0.030$). Nocturnal systolic and diastolic blood pressure were not significantly affected by MF 10058. No other signs of peripheral or central cholinergic block were observed at any dose. The results of this study demonstrated that oral administration of MF 10058 produces long-lasting hemodynamic effects in the conscious dog. The drug has a therapeutic potential for the treatment of bradycardic disorders.

Keywords: MF 10058; Muscarinic M_2 receptor antagonist; Cardioselective muscarinic receptor antagonist; Antibradycardic agent; Sick sinus syndrome; Bradycardia

1. Introduction

Muscarinic receptors regulate nerve-to-nerve transmission, smooth-muscle contraction, and exocrine and endocrine secretion mediated by acetylcholine (Goyal, 1989). Five molecular subtypes of muscarinic receptors (m_1, m_2, m_3, m_4 and m_5) have been cloned (Hulme et al., 1990). Four of them, designated as M_1, M_2, M_3 and M_4, have been characterised pharmacologically (Mutschler et al., 1989). The muscarinic M_2 receptor subtype is widely distributed in peripheral effector organs, such as exocrine glands, intestinal smooth muscle and heart (Caulfield, 1993). The stimulation of muscarinic M_2 receptors in cardiac sinoatrial and atrioventricular nodes and cardiac muscle induces bradycardia and decreases cardiac contractility (DiFrancesco et al., 1989). The over-activation of muscarinic M_2 receptors is thought to be a significant factor in sick sinus syndrome and atrioventricular block (Santinelli et al., 1984). This suggests that muscarinic M_2 receptor antagonists may be useful in the treatment of functional sinus disorders caused by hypervagotonia. Atropine, a prototype of non-selective muscarinic antagonist, has been employed in the treatment of sinus nodal dysfunction (Hlucky et al., 1991). However, its use is limited by the short duration of action and the occurrence of untoward side effects (dry mouth, mydriasis, decreased sweating, constipation and urinary retention) caused by antagonism of other muscarinic receptor subtypes. Thus, a selective M_2 receptor antagonist, devoid of side effects, could be used in the treatment of cardiac dysfunction associated to an increased parasympathetic tone.

5-{4-[4-(Diethylamino)butyl]-1-piperidinyl}acetyl-5*H*-dibenz[*b*,*f*]-azepine (MF 10058) is a novel aminoacyl

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derivative of 5*H*-dibenzazepine (Mandelli et al., 2000). In receptor binding studies, MF 10058 has showed a high affinity to the human recombinant muscarinic M_2 receptors ($K_i = 2.6$ nmol/l), a low affinity for the muscarinic M_4 receptors (40-fold less than for muscarinic M_2 receptors), and a very low affinity for muscarinic M_1 and M_3 receptors (110-fold less than for muscarinic M_2 receptors). Functional experiments have indicated MF 10058 as a competitive antagonist with high affinity to the cardiac muscarinic receptors ($pA_2 = 7.1$) and low affinity for intestinal muscarinic receptors ($ED_{50} = 0.54$ μM). In rats, MF 10058 dose-dependently antagonises acetylcholine-induced bradycardia and hypotension. Other cholinergic-mediated functions, like salivary secretion, pupil diameter, gastric emptying and intestinal transit were not affected by oral administration of MF 10058 in rats. Multiple of antibradycardic doses were used (15–50–150 mg/kg). Studies were carried out in comparison with atropine and 11-{2-[(diethylamino)methyl]-1-piperidinyl}acetyl-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (AF-DX 116). As expected, atropine significantly delayed the rate of gastric emptying (−84%) and intestinal transit time (−40%), inhibited oxotremorine-stimulated salivary secretion (−54%) and increased pupil diameter (+855%). At the lower dose of 15 mg/kg, MF 10058 and AF-DX 116 had no effects on any of these functions. At the medium dose (50 mg/kg), AF-DX 116 significantly affected gastric emptying (−74%), intestinal transit time (−25%) and pupil diameter (+176%), while MF 10058 only influenced the rate of gastric emptying (−48%). At the highest dose of 150 mg/kg, both compounds delayed gastric emptying and intestinal transit time. At this dose, AF-DX 116 also influenced pupil diameter (+631%), while MF 10058 had no effect (Mandelli et al., 2000).

In the present study, we investigated the effects of oral administration of MF 10058 on nocturnal bradycardia in conscious dogs. The effects of this compound on cardiac conduction times, as well as on systolic and diastolic blood pressure, were also studied.

2. Materials and methods

2.1. Animals

Experiments were performed on eight beagle dogs, four males and four females, weighing between 11 and 13 kg. Animals were housed in individual stainless steel boxes (1.2 m^2) with non-recycled filtered air at 17–21°C and 45–65% relative humidity. Air was changed approximately 10 times per hour. The artificial day/night cycle entailed 12 h of light and 12 h of darkness with lights on at 7:30 a.m. Food was distributed daily between 2:00 and 5:00 p.m. Water was available ad libitum. Animals were identified by tattoo inside the ear.

2.2. Apparatus and procedure

Hemodynamic variables and electrocardiograms (ECG) were recorded using a surgically implanted telemetric transmitter (model TL2M10-D70, Data Science, St. Paul, MN, USA) in freely moving animals. Implantation was performed at least 10 days before the drug administration under general anaesthesia (20 mg/kg i.v. thiopental followed by 1–1.5% i.v. halothane). The telemetric transmitter was implanted in the left flank and the sensor catheter was introduced into the femoral artery of the animals. The ECG leads of transmitters were placed in lead II, with one electrode on the right forelimb and one electrode on the left hindlimb. Measurements were done in the animal room. Data were transmitted to an on-line-data acquisition system by means of RLA2000 radio receivers (Data Science). A sampling rate of 500 Hz and an ART 1.0 telemetry data acquisition system (Data Science) were used. Heart rate, systolic and diastolic blood pressure were measured for 15 s every 5 min and averaged over 1-h periods. ECG (lead II) was recorded for 15 s every 5 min and values of conduction times were determined at each time point. Measurements began at least 1 h before drug administration and lasted for 12 (for QT and PR and QRS intervals) or 24 h (for heart rate, systolic and diastolic blood pressure) after dosing. Any gross behavioural or autonomic changes, observed during the experiment, were recorded.

Placebo and MF 10058 (2, 4 and 8 mg/kg) were orally administered according to a randomised four-way, crossover design with a washout period of at least 1 week.

2.3. Drugs

MF 10058 was synthesised by Mediolanum Farmaceutici (Milan, Italy). The drug was administered as a maleate salt and formulated in gelatine capsules. Administrations were done approximately at 10:00 p.m. by oral gavage.

2.4. Statistics

Statistical comparisons were performed with an analysis of covariance (ANCOVA) model with the hemodynamic variable as dependent variable, the last pre-treatment value of the hemodynamic variable as covariate and the dose as adjusting factor. *P*-values were obtained by comparisons among adjusted means. A *P*-value less than 0.05 was considered as statistically significant.

For heart rate, systolic and diastolic blood pressure, two separate analyses were carried out: one for night-time period (from 10:00 p.m. to 7:00 a.m. of the day after) and another for the entire 24-h period post-dose (from 10:00 p.m. to 10:00 p.m. of the day after).

All the statistical analyses were performed using SAS system software, version 6.12. Data were generally expressed as mean ± standard error of the mean (S.E.M.).

3. Results

Fig. 1 shows the time course of mean heart rate, diastolic and systolic blood pressure measured during the night after placebo administration. Hemodynamic variables were registered for 1 h before (9:00–10:00 p.m.) and for 9 h after (10:00 p.m.–7:00 a.m.) placebo administration (10:00 p.m.). During the night-time period, a progressive decrease in heart rate was observed (from 96 ± 3 to 82 ± 5 beats/min, $P = 0.019$). Mean diastolic blood pressure decreased sharply during the second hour after placebo administration, reaching a minimum of 75 ± 3 mm Hg. After that, a progressive increase of blood pressure was observed up to the baseline values (83 ± 2 mm Hg). During the night-time period, mean systolic blood pressure remained almost unchanged.

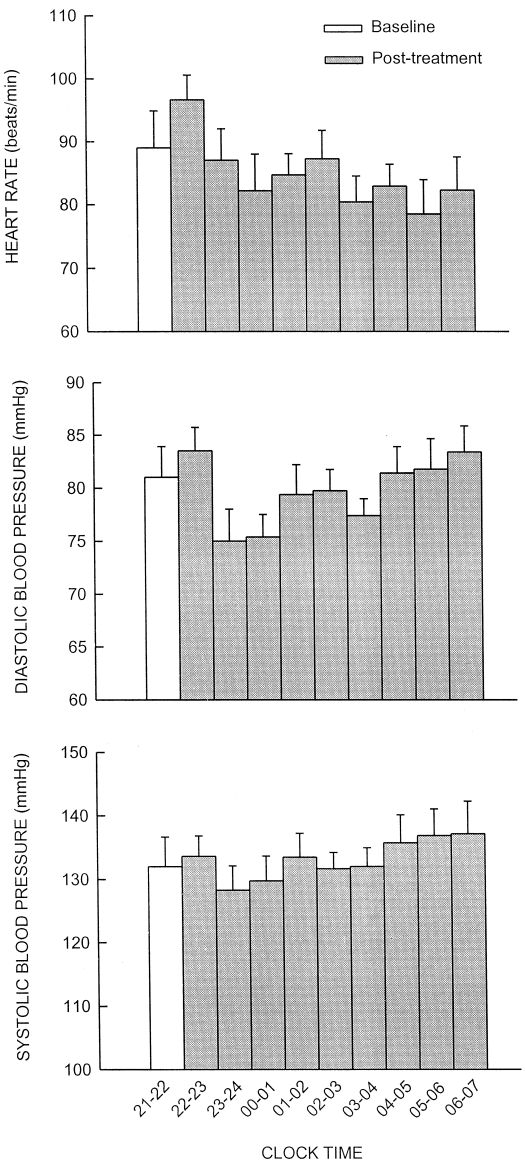

Fig. 1. Time course of mean (±S.E.M.) heart rate, diastolic and systolic blood pressure during the night-time period (between 10:00 p.m. and 7:00 a.m.) in eight dogs receiving placebo.

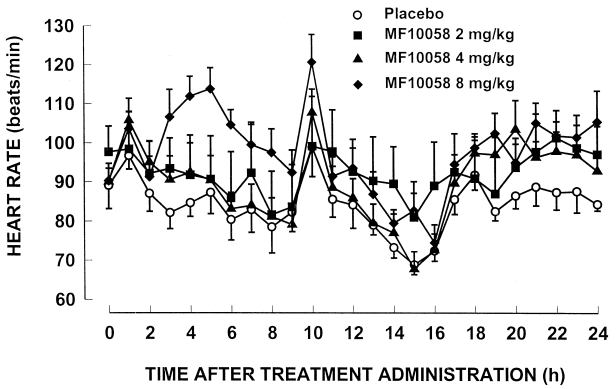

Fig. 2. Time course of mean (±S.E.M.) heart rate observed during 24 h after oral administration (10:00 p.m.) of placebo (○) and MF 10058 at the doses of 2 mg/kg (■), 4 mg/kg (▲) and 8 mg/kg (◆).

Fig. 2 shows the effect of MF 10058 on heart rate for the entire 24-h post-treatment period. During the night-time period (10:00 p.m.–7:00 a.m.), mean adjusted heart rate was 87 ± 4 beats/min after placebo vs. 104 ± 4 beats/min after 8 mg/kg MF 10058 ($P = 0.013$). The maximal tachycardic effect of the 8 mg/kg dose was observed 5 h after drug administration (114 ± 5 beats/min, +27% vs. baseline), the effect remaining significant, compared to placebo, for the entire 24-h observation period (86 ± 4 beats/min after placebo vs. 99 ± 4 beats/min after 8 mg/kg, $P = 0.030$). The time-course of mean heart rate after the lower doses was systematically above that of placebo, but the effect did not reach statistical significance.

A peak of tachycardia, that was observed at 10 h after treatment with both placebo and MF 10058, occurred in correspondence with the change from the dark period to the light period.

MF 10058 administration did not significantly affect diastolic and systolic blood pressure measured during the night-time period.

Regarding the cardiac conduction times, MF 10058 administration significantly shortened QT interval during the 12-h registration period, the adjusted means being 196 ± 3 ms after placebo, 197 ± 3 ms after 2 mg/kg ($P = 0.713$), 190 ± 3 ms after 4 mg/kg ($P = 0.103$) and 188 ± 3 ms after 8 mg/kg ($P = 0.049$). The effect of MF 10058 on PR interval was borderline with adjusted mean values of 118 ± 2 ms after placebo, 120 ± 2 ms after 2 mg/kg ($P = 0.475$), 113 ± 2 ms after 4 mg/kg ($P = 0.069$) and 114 ± 2 ms after 8 mg/kg ($P = 0.101$). The

QRS interval was not significantly modified by MF 10058, compared to placebo, at any dose tested.

4. Discussion

This study demonstrated that oral administration of MF 10058 reverses nocturnal bradycardia and increases daytime heart rate in conscious freely moving dogs. The hemodynamic effects are dose-dependent with the effect of the 8 mg/kg dose lasting for 24 h. The same paradigm of physiological bradycardia has been employed for assessing the cardiovascular effects of other selective muscarinic M_2 antagonists, administered by oral route (Watanabe et al., 1998). Other groups used pharmacologically induced bradycardia models. Intravenous methoctramine prevented the bradycardic action of the opioid agonist fentanyl in a dose-dependent manner (Hendrix et al., 1995). Intravenous AF-DX 116 significantly reversed the clonidine-induced bradycardia in conscious dogs (Giachetti et al., 1986; Micheletti et al., 1986).

We evaluated the cardiac activity of MF 10058 in a model of physiological bradycardia, evaluating the ability of the compound in reversing the heart rate decrease that naturally occurs during the night in conscious dogs. For this species, a consistent nocturnal pattern, characterised by an overnight fall in cardiac frequency and a compensatory increase in stroke volume, has been documented (Anderson et al., 1990). The role of sympathovagal modulation of cardiovascular activity during the nocturnal phase has been elucidated for other species. Studies using power spectral analysis have demonstrated that heart rate variability during the dark period is mainly influenced by the parasympathetic nervous activity in miniature swine (Kuwahara et al., 1999) and by the sympathetic nervous system in rats (Hashimoto et al. 1999). The complex interaction between sympathetic and parasympathetic activities on heart rate variability during sleep was also investigated in humans (Otzenberger et al., 1998). Studies using spectral analysis of R–R intervals have reported that power spectrum contains both low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.50 Hz) peaks (Akselrod et al., 1981). LF and HF peaks are considered markers of sympathetic and parasympathetic activity, respectively (Pomeranz et al., 1985; Bootsma et al., 1994). The LF/HF ratio is commonly considered an index of sympathovagal balance (Malliani et al., 1994; Stein et al., 1995). It has been demonstrated that HF and filtered QRS interval (f-QRS) increase during the night and decrease during the day, whereas heart rate and the LF/HF ratio are greater during the day than at night (Nakagawa et al., 1998). During rapid eye movement (REM) sleep, the sympathetic activity increases, as revealed by high LF/HF values, whereas during non-REM sleep, the parasympathetic activity is predominant, as revealed by a decrease of LF/HF values (Berlad et al., 1993; Toscani et al., 1996; Vanoli et al., 1995).

Parasympathetic activation has four main effects on the cardiovascular system: vasodilatation, decrease in heart rate, decrease in the rate of conduction in the specialised tissues of the sinoatrial and atrioventricular nodes and decrease in the force of cardiac contraction (Levy and Warner, 1994). Cardiac conduction system and cardiac muscle express muscarinic M_2 receptors in humans and most other animal species (Deighton et al., 1990; Levey, 1993), with the exception of birds (Jeck et al., 1988). Parasympathetic innervation and receptor density are higher in atria than in ventricles (DeJonge et al., 1986). The activity of these muscarinic receptors is mediated by the interaction of acetylcholine with a sub-family of G proteins (G_i) and the consequent inhibition of adenylyl cyclase and activation of K^+ conductance. Thus, the inhibition of cardiac muscarinic receptors elicits, in general, a positive chronotropic and inotropic effect.

The effects of MF 10058 on heart rate was accompanied by a reduction of cardiac conduction times with the effect on the QT interval being significant and that on PR interval borderline. The QT interval measures the duration of the ventricular action potential. Severe bradycardia prolongs the QT interval by reducing the repolarising Na^+/K^+ pump activity and causing more complete decay of the repolarising delayed rectifier K^+ current (Tan et al, 1995). The suppression of muscarinic activity shortens ventricular refractoriness in human right ventricular apex and abolishes the negative effect of acetylcholine on canine Purkinje fibers automaticity (Tse et al, 1976; Gadsby et al, 1978). Thus, MF 10058 could affect the QT interval by speeding up the heart rate and/or by acting directly on ventricular refractoriness.

The PR interval reflects the duration of conduction from atrium through the atrioventricular junction and bundle branches. The prolongation of the atrioventricular conduction time, induced by vagus stimulation, is entirely mediated through muscarinic M_2 receptors in isolated canine preparations (Narita et al, 1991). The muscarinic M_2 antagonism of MF 10058 well explains the observed positive dromotropic effects and suggests its potential use for treatment of nodal conduction disturbances.

The antibradycardic effect shown in the dog confirms previous results obtained in rats. In this species, bradycardia and hypotension were pharmacologically induced by acetylcholine infusion. MF 10058, intravenously and intraduodenally administered, was able to antagonise bradycardia and hypotension in a dose-dependent manner. After intravenous administration, the ED_{50} values for bradycardia and hypotension were 55 ± 4 and 80 ± 1 μg/kg, respectively. In the intraduodenal studies, MF 10058 counteracted the effects of acetylcholine administration with a maximum activity at the dose of 15 mg/kg (Mandelli et al., 2000).

In the present study, MF 10058 did not produce significant effects on nocturnal diastolic or systolic blood pressure. The parasympathetic control of blood pressure and

vascular tone involves heterogeneous populations of muscarinic receptors, the role of which differ in different species and vascular sites. In several types of large conduit arteries of different animal species, the muscarinic M_3 receptor subtype has been demonstrated in endothelial cells to mediate cholinergic vasodilatation by releasing an endothelium-derived relaxing factor (Eglen and Whiting, 1990). Cholinergic vasodilatation can be elicited by presynaptic inhibition of norepinephrine release from sympathetic nerve endings. In canine femoral vein, a decrease in sympathetic outflow seems to involve a muscarinic M_2 receptor subtype (Eglen and Whiting, 1990). Acetylcholine can produce vasoconstriction directly, by stimulating receptors on vascular smooth muscle (Kalsner, 1989) and indirectly through muscarinic receptors located on the vascular endothelium (Lüscher et al., 1992). Muscarinic M_1 receptors subtype seems to mediate a vasoconstriction in canine venous tissue (Eglen and Whiting, 1990). Thus, the response to muscarinic agonists or antagonists depends to the relative abundance of the receptor subtypes, to the functional response that it evokes, and finally, to the selectivity of the compound for the different muscarinic subtypes. Taking into account the complexity of these regulatory mechanisms, we can merely speculate that MF 10058, being highly selective for muscarinic M_2 receptors, could differentiate between cardiac and vascular receptors. Thus, even at the dose maximally active in reversing nocturnal bradycardia, it could be virtually ineffective in modifying nocturnal blood pressure. Similarly, intravenous AF-DX 116 did not reduce the betanechol-induced vasodilatation at a dose two-fold higher than that maximally effective on bradycardia in anaesthetised dogs (Micheletti et al., 1986).

The antibradycardic effect of MF 10058, described in the present study, occurs at a dose devoid of side effects. A good functional selectivity was also found in rats in which doses as high as 30 times the antibradycardic effective dose did not modify cholinergic functions mediated by muscarinic M_1 or M_3 receptors (Mandelli et al., 2000). Compared to AF-DX 116, a standard muscarinic M_2 selective blocking agent, MF 10058 showed a better selectivity profile either in binding studies and in vivo experiments. Moreover, MF 10058 produced very weak central analgesic activity in mouse, suggesting a poor penetration of the blood brain barrier (Mandelli et al., 2000).

Collectively considered, these results indicate that MF 10058 can be classified as a peripherally acting cardioselective muscarinic antagonist, with antibradycardic properties both after parenteral and oral administration at the doses devoid of side effects. This compound appears to be a promising agent for the treatment of bradycardic disorders, like sinus bradycardia, related to enhanced vagal tone. MF 10058 could be used for long-term control of mild to moderate dysfunction of cardiac conduction system and as a temporary therapy until permanent pacemaker implantation.

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