SD3212, a new antiarrhythmic drug, raises atrial fibrillation threshold in isolated rabbit hearts

Ryohei Matsuo, Takeshi Shirayama, Keiji Inoue, Yayoi Matoba, Hiroto Imai, Hirokazu Shiraishi, Tetsuya Tatsumi, and Masao Nakagawa

Second Department of Medicine, Kyoto Prefectural University of Medicine, Kawaramachi, Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

Summary. SD3212 is a new antiarrhythmic drug which has class I, III, and IV effects. The purpose of this study was to elucidate the electrophysiological effects of this compound on a rabbit atrial fibrillation model, and to test a hypothesis that atrial fibrillation threshold is a quantitative indicator of atrial vulnerability. Whole hearts were excised from rabbits, and the aortas cannulated to perfuse the coronary arteries. Atrial fibrillation was induced with a burst stimulation of 50 Hz for 1 s while 3 μM acetylcholine (ACh) was perfused. When the right atrial appendage was paced at 200-ms intervals, SD3212 prolonged interatrial conduction time: control 30 ± 1.2 ms, ACh 33 ± 1.4 ms, ACh + SD 1 μM 37 ± 2.4 ms, ACh + SD 3 μM 52 ± 8.1 ms. The drug also prolonged the effective refractory period: control 80 ± 3.0 ms, ACh 48 ± 3.8 ms, ACh + SD 1 μM 65 ± 4.7 ms, ACh + SD 3 μM 98 ± 15 ms. The rate of induction of atrial fibrillation by rapid pacing was 26% in Tyrode’s solution, 85% in the presence of ACh, and 38% in the presence of ACh + SD 1 μM. The atrial fibrillation threshold decreased from 8.6 ± 0.8 mA (control) to 2.5 ± 0.7 mA in the presence of ACh. It increased again to 7.8 ± 1.0 mA in the presence of SD3212 (1 μM). SD3212 prolonged both the conduction time and refractory period. A reversed use-dependency was not prominent. These features caused antifibrillatory effects. Thus, the atrial fibrillation threshold seems to be a good quantitative indicator of atrial vulnerability.

Key words: Antiarrhythmic drug – SD3212 – Atrial fibrillation – Electrophysiology

Introduction

Atrial fibrillation is one of the arrhythmias frequently encountered in a clinical setting. The efficacy of antiarrhythmic drugs, however, has remained as low as 50% when used to maintain sinus rhythm during long-term follow-up [1–3]. Although amiodarone and Vaughan-Williams class Ic drugs have been reported to have relatively higher efficacy against atrial fibrillation [4–6], a high rate of recurrence was reported [3]. In addition, adverse effects of amiodarone could be a limit to its broad use [7]. Also, class Ic drugs are not recommended for treatment of the patients suffering from ischemic heart diseases [8]. Thus, it is necessary to develop new drugs to overcome these drawbacks.

A recently synthesized compound, SD3212 or (−)-(S)-2-[5-methoxy-2-[3-[methyl [2-[3,4(methylene dioxy)phenoxy]-ethyl] amino] propoxyl] phenyl]-4-methyl-2H-1,4-benzothiazin-3(4H)-one hydrogen fumarate, has been reported to have class I, III, and IV effects. It reduced maximum upstroke velocity of rabbit ventricular action potential in a use-dependent manner [9]. Action potential duration was prolonged at any stimulation rate [10], i.e., this drug has no reverse use-dependency as other class III drugs commonly do [11]. A patch-clamp study revealed that SD3212 potently blocked acetylcholine-receptor-operated potassium channels (IK_ACh), but not muscarinic receptors [12]. This drug also exerted calcium antagonistic actions in the heart [12, 13] and in the vascular smooth muscle cells [14]. Antiarrhythmic effects of this unique drug have been tested in animal models, focusing on ventricular arrhythmias [15–17]. While the effects against atrial arrhythmias have not been studied, the characteristics of this drug as a multiple channel blocker prompted us to investigate the efficacy against atrial fibrillation.

Our laboratory and others have reported that a rapid stimulation of the atrium at a rate of 50 Hz for 1 s can induce atrial fibrillation in small animals [18–21] and in...
human patients [22]. The duration of atrial fibrillation is
dependent on current amplitude of the stimulating
pulses, and the atrial fibrillation threshold can be a
useful predictor of efficacy of antiarrhythmic drugs
against atrial fibrillation [20, 22]. Although a definition
of atrial fibrillation is somewhat arbitrary, atrial vul-
nerability can be measured quantitatively as atrial
fibrillation threshold [20, 22]. The purpose of this study
is to elucidate the efficacy of SD3212 against atrial
fibrillation in rabbits, and to verify the applicability
of atrial fibrillation threshold to evaluate drug
efficacy.

\section*{Materials and methods}

\subsection*{Preparations}

Japanese white rabbits weighing from 2.0 to 2.8 kg were
used for the experiments. Each rabbit received an intra-
peritoneal injection of heparin (5000 U). After 10 to
15 min, sodium pentobarbital (50 mg/kg body weight)
was injected intravenously to anesthetize the rabbits
deeply. The heart was quickly removed, and the aorta
was cannulated to a Langendorff apparatus to perfuse
coronary arteries with Tyrode’s solution at a rate of
15 ml/min. Ten minutes were allowed to pass and ex-
periments were started while the heart was beating
normally. Temperature was controlled at 37°C. The
composition of the perfusing Tyrode’s solution was as
follows (in mM): NaCl 137, KCl 2.7, CaCl\textsubscript{2} 1.8, MgCl\textsubscript{2}
1.0, Na\textsubscript{2}HPO\textsubscript{4} 0.5, NaHCO\textsubscript{3} 12, glucose 5.6. pH was
adjusted to 7.4 ± 0.05 by gassing with a mixture of 95%
O\textsubscript{2} and 5% CO\textsubscript{2}.

The experimental protocol was approved by the
Committee for Animal Research of the Kyoto Prefec-
tural University of Medicine, and followed all Rules and
Regulations of the Committee.

\subsection*{Electrophysiological measurements}

The electrophysiological parameters were obtained as
in previous reports from our laboratory [20, 22]. Briefly,
epicardial electrograms at the right and left atrial
appendages were recorded with bipolar electrodes
(diameter 0.2 mm, 1 mm apart), manufactured from
enamel-coated silver wire. A stimulating bipolar elec-
trode made from silver wire with a diameter of 0.3 mm,
and two wires were placed 1 mm apart to give rectangu-
lar electric pulses of 2 ms width. The stimulating
electrodes were placed at the tip of the right atrial
appendage. When necessary, the same electrodes were
also placed at the left atrial appendage, or at the ante-
rior part of the orifice of the superior vena cava (high on
the right atrium). The electrocardiogram was amplified
by a Biophysiograph 180 amplifier (NEC, Tokyo,
Japan) with a high cut-off filter of 3 kHz and a time
constant of 0.003 s. The electrocardiogram was moni-
tored with a VC-10 oscilloscope (Nihon Koden, Tokyo,
Japan). A thermal array recorder (RTA 1000; Nihon
Koden) was used to obtain paper records. Electrical
stimulation was generated by a SEN 7230 stimulator
(Nihon Koden), and was applied to the heart through an
isolator (SS-102J; Nihon Koden).

Effective refractory period (ERP) was measured as
the longest coupling interval of an extra stimulation
which did not cause any atrial excitation after eight
consecutive pacing pulses were given. Interatrial con-
duction time was defined as the time between the stimu-
lation artifact and the beginning of atrial electrogram
at the other appendage of atrium, which was recorded
during continuous pacing at cycle lengths of 200, 300,
and 400 ms. Data were obtained for an average conduc-
tion time of 10 consecutive beats.

Atrial fibrillation was induced with rapid stimulation
at a rate of 50 Hz for 1 s after eight conditioning stimul-
i (cycle length 300 ms) were given. The stimulation site
was fixed at the right atrial appendage. The current
intensity of rapid stimulations to induce atrial
fibrillation was increased from 0.1 mA to 1.0 mA by
0.1-mA increments, then it was stepped up to 1.5 mA,
followed by further increase from 2 mA to 10 mA by
1-mA increments. The duration of fibrillatory activity at
each current intensity was measured in all experimen-
tal cases to examine the profile of the current–duration
relationship. One minute was allowed before the next
application of rapid pacing to avoid atrial “electrical
remodeling” [23]. This experiment was performed on
27 animals after acetylcholine and/or SD3212 was per-
fused. Ten minutes were allowed until electrophysi-
ological measurements were stabilized in the presence
of acetylcholine and 40 min after the introduction of
SD3212. The atrial fibrillation threshold was defined
as the least additional current above the stimulation
threshold to induce repetitive atrial firings lasting more
than 1 s. The interval between repetitive atrial firings
should be less than 200 ms without regularity. Except
for this rapid pacing to measure atrial fibrillation thresh-
old, the intensity of pacing stimuli was set at twice the
diastolic threshold.

Preliminary experiments (n = 6) showed that the
measurements of atrial fibrillation threshold were re-
producible within one step difference of current intensi-
ties during a period of 2 h for each animal. In the
continuous presence of 3 μM acetylcholine in the bath,
atrial fibrillation threshold was increased from 0.8 ±
0.3 mA to 0.9 ± 0.2 mA. The effective refractory period
also increased in parallel from 53 ± 13 ms to 60 ± 3 ms.
These increases could be due to desensitization of mus-
carinic acetylcholine receptors. At the particular cur-