Comparison of Cardiovascular Effects of a Novel Class Ic Antiarrhythmic Agent, NIK-244, with Those of Flecainide in Isolated Canine Heart Preparations Cross-Circulated with a Donor Dog

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ABSTRACT — To assess the cardiovascular effects of a new class I antiarrhythmic agent, NIK-244, and to compare them with those of flecainide, canine isolated, sinoatrial node, papillary muscle and atrioventricular node preparations cross-circulated with a donor dog were used. NIK-244 injected intraarterially into the isolated preparations showed dose-related negative chronotropic, negative inotropic, and coronary vasodilator effects, which are comparable to those of flecainide, and it also showed a dose-related negative dromotropic effect on both atrio-His (AH) and His-ventricular (HV) conduction. The prolongation of AH interval by NIK-244 was significantly more potent than that by flecainide, while that of the HV interval by NIK-244 was slightly more potent, but not significantly, compared with that by flecainide. NIK-244 administered intravenously into the donor dog showed bradycardic and depressor effects in both the donor dog and the cross-circulated sinus node and papillary muscle preparations, which are comparable to the effects of flecainide. Although the negative dromotropic effects of NIK-244 on both the donor dog heart and the cross-circulated atrioventricular node preparation started more slowly, they were more potent and longer-lasting than those of flecainide. Our results suggest that NIK-244 may be a more powerful and longer-lasting antiarrhythmic agent than flecainide, since the antiarrhythmic action of class I drugs is considered to result from inhibition of the fast inward current, which is the most important depolarizing current responsible for the intraatrial and His-Purkinje-ventricular conduction.

NIK-244 (ethacizin), 2-(ethoxycarbonylamino)-10-(3-(diethylamino)propionyl) phenothiazine hydrochloride, has been developed in the U.S.S.R. and is a new antiarrhythmic drug of the phenothiazine group chemically very close to ethmozin (1, 2). Our study (3) suggests that NIK-244 is a class Ic antiarrhythmic drug that

Abbreviations used are: AH, atrio-His; HV, His-ventricular; SA, sinoatrial; PM, papillary muscle; AV, atrioventricular; RCA, right coronary artery; SAR, sinoatrial rate; ASA, anterior septal artery; DT, developed tension; PSA, posterior septal artery; HR, heart rate; mAP, mean arterial pressure; CBF, coronary blood flow.
produces no change in APD and is a slow kinetic drug. NIK-244 was reported to be effective on a variety of experimental (4–6) and clinical arrhythmias (1, 7). In addition, to assess their antiarrhythmic effectiveness of a drug, it is also important to know its effects on cardiohemodynamics because arrhythmias often occur in impaired hearts and *vice versa*.

The purpose of the present study was to assess the cardiovascular effects of NIK-244 in comparison with those of flecainide. We used isolated canine SA node, PM and AV node preparations cross-circulated with a donor dog, which permit precise measurement of drug effects under both in vivo and in vitro conditions (8–11). Particularly, we could examine both the cardiohemodynamic effects in the donor dog and the direct cardiac effects in the isolated heart preparations simultaneously when the drugs were administered into the donor dog.

**MATERIALS AND METHODS**

Experiments were carried out on isolated canine SA node, PM and AV node preparations cross-circulated with heparinized arterial blood of a donor dog.

*The isolated in vitro preparations*

The hearts were obtained from mongrel dogs of either sex, weighing approximately 10 kg, which were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and given calcium heparin (500 U/kg, i.v.). They were excised after exsanguination and plunged into cold Tyrode’s solution kept at about 4°C.

The SA node preparation consisted of the entire right atrium. The sinus node artery was cannulated through the RCA. The spontaneous beating rate, i.e., SAR, was measured with a cardiotachograph (San-еi Instruments, 1321) triggered by bipolar atrial electrograms sutured on the atrial epicardium close to the sinus node.

The PM preparation consisted of the anterior papillary muscle of the right ventricle attached to the interventricular septum. The ASA was directly cannulated. The PM preparation was electrically driven at a fixed rate of 120 beats/min by a stimulator (Dia Medical, DHM-226-3) and an isolation unit (Dia Medical, DPS-110) with rectangular pulses of 1–3 V (about 20% above the threshold voltage) at 5-msec duration through bipolar stimulating electrodes sutured onto the base of the PM. DT of the PM preloaded with a 2-g weight was measured isometrically using a force displacement transducer (Dia Medical, DRM-100S).

The AV node preparation consisted of both the right atrium and interventricular septum. The RCA and the ASA were directly cannulated, while the PSA was cannulated through the left circumflex artery. The AV node preparation was electrically driven at a fixed rate of 150 beats/min by a stimulator (Dia Medical, DHM-226-3) and an isolation unit (Dia Medical, DPS-110) with rectangular pulses of 1–3 V (about 20% above the threshold voltage) at 5-msec duration through bipolar stimulating electrodes sutured onto the crista terminals of the right atrium. Bipolar electrograms were recorded from the right atrium (A), His bundle (H) and the base of the anterior PM (V). These electrograms were fed to an automatic interval meter (Dia Medical, DHM-226-1), which measures AH and HV intervals individually with an analysis pitch of 1 msec. Since the PSA mainly supplies the AV node area and the ASA mainly supplies the His-Purkinje-ventricle system, drugs selectively injected into the PSA affect the AH interval, while drugs injected into the ASA predominantly affect the HV interval. However, drugs injected into the ASA occasionally altered the AH interval in addition to the HV interval, probably due to anastomosis which exists between the ASA and PSA.

*Donor dogs*

Adult mongrel dogs of either sex, weighing 14–23 kg, were used as donor dogs. Dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and supplemented with 4–5 mg/kg every hour. At the start of cross-cir-
Calculation, calcium heparin (500 U/kg, i.v.) was given and 200 U/kg was supplemented every hour. Respiration was controlled using a dog respirator (Harvard, 607). The HR, mAP at the femoral artery, and lead II ECG were continuously monitored with a polygraph (San-ei Instruments, 361-6). The PQ interval and QRS width of the donor dog heart were directly measured from the lead II ECG recorded at a paper speed of 100 mm/sec every 5 min and when necessary.

Cross-circulation
Preparations were placed in a double-wall glass jacket maintained at 38°C by circulating warm water, and they were cross-circulated through each cannulated artery with heparinized blood pumped from the carotid artery of the donor dog. The SA node and PM preparations were obtained from the same animal, while the AV node preparation was obtained from a different animal. Perfusion pressure was kept at 100 mmHg with a peristaltic pump (Cole-Parmer, 7553-00) and a Starling pneumatic resistance placed parallel to the perfusion circuit. Venous blood from the preparations and excess blood passing through the pneumatic resistance were collected in a blood reservoir and returned to the donor dog through the jugular vein. The rate of CBF through each nutrient artery was continuously measured with an electromagnetic flowmeter (Nihon-Kohden, MVF-1100).

Direct cardiac effects on in vitro preparations
NIK-244 (1 to 300 µg) or flecainide (1 to 300 µg) was injected into each nutrient artery of the preparations over 4 sec with a microsyringe (Terumo). Maximal changes in each parameter were measured and expressed as a percent of their basal values before injection, and the dose-response curves for the chronotropic, inotropic, dromotropic and vasodilator effects were drawn.

Cardiohemodynamic effects in the donor dogs
NIK-244 (1 mg/kg) or flecainide (1 mg/kg), in an estimated canine antiarrhythmic dose (5, 12), was administered to the donor dog through the jugular vein for a period of 30 sec. Each drug caused cardiohemodynamic effects on the donor dog and also produced direct cardiac effects in the in vitro preparations after a time lag of 1 to 2 min. The time course of their effects on HR, mAP, PQ and QRS of the donor dog and on SAR of the SA node preparation, DT and CBF of the PM preparation, AH and HV intervals and CBF through the ASA of the AV node preparation were simultaneously recorded.

Drugs and statistics
Drugs used were NIK-244 (Nikken Chemical K.K., Tokyo, Japan) and flecainide acetate (Eisai K.K., Tokyo, Japan). The doses of drugs causing a 15% decrease in SAR, ED₁₅(SAR); those causing a 50% decrease in DT, ED₅₀(DT); those causing a 50% increase in CBF, ED₅₀(CBF); those causing a 50% increase in AH interval, ED₅₀(AH); and those causing a 50% increase in HV interval, ED₅₀(HV), were calculated for each experiment using the least squares method. The data were presented as the mean ± S.E.M., and the statistical comparisons of mean values were evaluated by a paired t-test or unpaired t-test, and P values less than 0.05 were considered significant.

RESULTS
Effects of NIK-244 or flecainide injected directly into each nutrient artery of the in vitro preparations
The SA node preparation showed spontaneous regular automaticity. When NIK-244 or flecainide was injected into the rubber tube connecting the RCA, SAR dose-relatedly decreased. A typical experiment of NIK-244 is shown in Fig. 1A. Dose-response curves for the negative chronotropic effects of NIK-244 and flecainide are shown in Fig. 1B. At 300 µg, sinus arrest was induced in 4 out of 7 preparations by NIK-244 and 3 out of 11 by flecainide. There was no significant difference between the ED₁₅(SAR) values of NIK-244
Fig. 1. Negative chronotropic effect of NIK-244 and flecainide in the sinoatrial node preparation. The basal sinoatrial rate (SAR) before NIK-244 was 96 ± 6 beats/min (n = 7) and that before flecainide was 98 ± 4 (n = 11). (A) Original tracing of NIK-244. B) Dose-response curves of NIK-244 and flecainide. *P < 0.05, **P < 0.01, significantly different from each basal value. —○— NIK-244; —△— flecainide.

Table 1. Comparison of negative chronotropic, negative inotropic, coronary vasodilator, and negative dromotropic effects of NIK-244 and flecainide in isolated canine blood-perfused sinoatrial (SA) node, papillary muscle (PM) and atrioventricular (AV) node preparations

<table>
<thead>
<tr>
<th>Effect</th>
<th>NIK-244 (ug)</th>
<th>Flecainide (ug)</th>
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<tbody>
<tr>
<td>ED_{15}(SAR)</td>
<td>290 ± 57</td>
<td>221 ± 29</td>
</tr>
<tr>
<td>ED_{50}(DT)</td>
<td>280 ± 41</td>
<td>361 ± 25</td>
</tr>
<tr>
<td>ED_{50}(CBF)</td>
<td>201 ± 38</td>
<td>260 ± 43</td>
</tr>
<tr>
<td>ED_{50}(AH)</td>
<td>49 ± 9*</td>
<td>229 ± 76</td>
</tr>
<tr>
<td>ED_{50}(HV)</td>
<td>201 ± 43</td>
<td>304 ± 70</td>
</tr>
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Table 1. (footnote)
ED_{15}(SAR): The dose (µg) that caused a 15% decrease in the sinoatrial rate (SAR) of the SA node preparation. ED_{50}(DT): The dose (µg) that caused a 50% decrease in the developed tension (DT) of the PM preparation. ED_{50}(CBF): The dose (µg) that caused a 50% increase in the blood flow through the anterior septal artery of the PM preparation. ED_{50}(AH): The dose (µg) that caused a 50% increase in the AH interval of the AV node preparation. ED_{50}(HV): The dose (µg) that caused a 50% increase in the HV interval of the AV node preparation. Asterisks next to the values for NIK-244 represent significant differences from the respective values of flecainide. *P < 0.05.
and flecainide (Table 1).

When NIK-244 or flecainide was injected into the ASA of the PM preparation, DT decreased and CBF increased, dose-relatedly. The changes of DT were longer-lasting than those of CBF. A typical experiment of NIK-244 is shown in Fig. 2A. Dose-response curves for the negative inotropic effect and for the coronary vasodilator effect of NIK-244 and flecainide are shown in Fig. 2, B and C, respectively. There was no significant difference between the ED$_{50}$(DT) values of NIK-244 and flecainide (Table 1). There was also no significant difference between the ED$_{50}$(CBF) values of both drugs (Table 1).

When NIK-244 or flecainide was injected

![Fig. 2. Negative inotropic and coronary vasodilator effects of NIK-244 and flecainide in the papillary muscle (PM) preparation. The basal developed tension (DT) of the PM before NIK-244 was 5.9 ± 0.5 g (n = 10) and that before flecainide was 7.1 ± 0.7 g (n = 9). The basal coronary blood flow (CBF) through the anterior septal artery before NIK-244 was 5.4 ± 0.5 ml/min (n = 8) and that before flecainide was 5.7 ± 0.5 ml/min (n = 9). (A) Original tracings of the inotropic (upper) and coronary vasodilator effects (lower) of NIK-244. (B) Dose-response curves for the negative inotropic effects. (C) Dose-response curves for the coronary vasodilator effects. *P < 0.05, **P < 0.01, significantly different from each basal value. -○- NIK-244; △ flecainide.](image-url)
into the PSA of the AV node preparation, the AH interval increased dose-relatedly, while the HV interval was little affected. A typical experiment of NIK-244 is shown in Fig. 3A. Dose-response curves for the effects of NIK-244 and flecainide on the AH interval are shown in Fig. 3B. At 30 μg, AH block was induced in 2 out of 7 preparations by NIK-244 and in no preparation by flecainide; at 100 μg, in 5 out of 6 by NIK-244 and in none by flecainide; and at 300 μg, in 2 out of 7 by flecainide. The ED$_{50}$(AH) of NIK-244 was significantly less than that of flecainide (Table 1).

On the other hand, when NIK-244 or flecainide was injected into the ASA of the AV node preparation, the HV interval dose-relatedly increased, while the AH interval increased only with larger doses of NIK-244 or flecainide. A typical experiment of NIK-244 is shown in Fig. 4A, in which the HV interval predominantly increased. Dose-response curves for the effects of NIK-244 and fleca
cainide on HV intervals are shown in Fig. 4B. There was no significant difference between the ED$_{50}$(HV) values of NIK-244 and flecainide (Table 1).

**Effects of intravenous administration of NIK-244 and flecainide into the donor dogs**

**Effects of NIK-244 on the donor dog:** All the parameters of the donor dog changed within 30 sec after i.v. administration of NIK-244. HR gradually decreased during the observation period, and the value at 20 min was 92% of the basal level (Fig. 5A). Mean arterial pressure decreased, reaching a peak response (90% of the basal value) at 1 min, and then gradually returned to the basal level (Fig. 5B). PQ and QRS intervals increased, and reaching their peaks (127 and 132% of the basal values) at 10 min and 5 min, respectively; and these increases were maintained during the observation period (Fig. 5, C and D).

**Effects of flecainide on the donor dog:** Similar to NIK-244, all the parameters of the donor dog changed within 30 sec after i.v. administration of flecainide. HR rapidly de-

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**Fig. 4.** Negative dromotropic effect on HV interval of NIK-244 and flecainide in the AV node preparation. The basal HV interval before NIK-244 was 53 ± 5 msec (n = 5) and that before flecainide was 44 ± 5 msec (n = 7). (A) Typical experiment of NIK-244 injected into the anterior septal artery (ASA). (B) Change in HV interval (%) injected into the ASA. *P < 0.05, **P < 0.01, significantly different from each basal value.
creased, reaching a peak (94% of the basal value) at 3 min, and then gradually returned to the basal level (Fig. 5A). Mean arterial pressure initially decreased for 0 to 30 sec, followed by an increase toward the basal level for 30 sec to 1 min, then decreased again, reaching a peak (97% of the basal value) at 5 min and then gradually returned to the basal level (Fig. 5B). PQ and QRS rapidly increased, reaching their peaks (117 and 118% of the basal values) at 2 min and 1 min, respectively, then gradually returned to the basal level (Fig. 5, C and D).

The percent change in HR at 1 min after i.v. NIK-244 was significantly less than that after flecainide (Fig. 5A). The percent changes in mAP at 1 to 2 min after i.v. NIK-244 were significantly larger than those after flecainide (Fig. 5B). The percent changes in PQ interval at 0.5 to 2 min after i.v. NIK-244 were significantly less than those after flecainide, while the percent changes in PQ interval at 5 to 20 min were significantly larger than those after flecainide (Fig. 5C). The percent change in QRS width at 0.5 min after i.v. NIK-244 was significantly less than that after flecainide, while the percent changes in QRS width at 10 to 20 min after i.v. NIK-244 were larger than those after flecainide (Fig. 5D).

Effects of NIK-244 given into the donor dog on the in vitro preparations: After i.v. administration of NIK-244, SAR started to decrease
within 1 to 2 min after injection, reaching a minimum (94% of the basal value) at 10 min, and then gradually returned to the basal level (Fig. 6A). DT also decreased, reaching a minimum (83% of the basal value) at 7 min and then gradually returned to the basal level (Fig. 6B). CBF transiently increased, reaching a peak (126% of the basal value) at 3 min, then gradually returned to the basal level (Fig. 6C). AH and HV intervals increased, reaching their maximum values (147% and 158% of the basal values) at 10 and 7 min, respectively; and these increases were maintained during the observation period (Fig. 6, D and E).

Second degree AH conduction block occurred in 1 out of 4 preparations at 15 min and persisted during the observation period.

The maximal percent changes of parameters in the in vitro preparation after i.v. administration of 1 mg/kg of NIK-244 into the donor dog were the same as those produced by about 20 μg to 300 μg of NIK-244 directly injected into the nutrient arteries of the preparations.

Effects of flecainide given into the donor dog on the in vitro preparations: SAR decreased, reaching a minimum (93% of the basal value) at 3 min, and then gradually returned to the
basal level (Fig. 6A). DT decreased, reaching the peak decrease (88% of the basal value) at 5 min and then gradually returned to the basal level (Fig. 6B). CBF transiently increased, reaching a peak (107% of the basal value) at 2 min, then gradually returned to the basal level (Fig. 6C). AH and HV rapidly increased, reaching their maximum (127% and 125% of the basal values) at 5 min, and then gradually returned to their respective basal levels (Fig. 6, D and E).

The maximal percent changes of the parameters in the in vitro preparation after i.v. administration of 1 mg/kg of flecainide into the donor dog were the same as those produced by about 10 μg to 200 μg of flecainide directly injected into the nutrient arteries of the preparations.

There was no significant difference in the percent changes of SAR between NIK-244 and flecainide at the corresponding time after i.v. administration (Fig. 6A). The percent change in DT at 10 min after i.v. NIK-244 was significantly larger than that after flecainide (Fig. 6B). The percent changes in CBF at 3 to 7 min after i.v. NIK-244 were significantly larger than those after flecainide (Fig. 6C). The percent changes in AH interval at 0.5 to 3 min after i.v. NIK-244 were significantly less than those after i.v. flecainide, while the percent changes in AH interval at 7 to 20 min after i.v. NIK-244 were larger than those after i.v. flecainide. The percent changes in HV interval at 0.5 to 3 min after i.v. NIK-244 were significantly less than those after i.v. flecainide, while the percent changes in HV interval at 7 to 20 min after i.v. NIK-244 were significantly larger than those after i.v. flecainide (Fig. 6E).

DISCUSSION

The present study using the blood-perfused isolated heart preparations revealed the direct and indirect effects of NIK-244 on cardiac properties in comparison with flecainide. NIK-244 injected directly into the SA node and PM preparations showed negative chronotropic, negative inotropic and coronary vasodilator effects, which were comparable to those of flecainide. Similar results on the negative inotropic action of NIK-244 were reported by others (4, 13, 14). NIK-244 injected directly into the AV node preparations showed a negative dromotropic effect on AH and HV conductions. The effect on AH interval of NIK-244 was significantly more potent than that of flecainide, while the effect on HV interval of NIK-244 was slightly more potent, but not significantly, compared with flecainide. NIK-244 administered i.v. into the donor dog showed bradycardic and depressor effects on the donor dog similar to flecainide, while although the negative dromotropic effects on the PQ interval and QRS width of NIK-244 started more slowly, they were more potent and longer-lasting than those of flecainide. In the in vitro preparations cross-circulated by arterial blood of the donor dog given NIK-244 or flecainide, i.v., cardiodepressant and coronary vasodilator effects also appeared.

NIK-244 that was i.a.-injected into the RCA decreased SAR of the SA node preparation in a dose-dependent manner. NIK-244 that was i.v.-administered into the donor dog also caused bradycardia in the donor dog and decreased SAR of the cross-circulated SA node preparation. These results are consistent with a previous report that NIK-244 has a direct negative chronotropic effect in the intact canine heart in situ (2). Urthaler et al. (2) partially attributed this effect of NIK-244 to its depressing actions on sinus node automaticity which were mainly mediated through a decreased activity of the slow current-dependent cells of the sinus node.

Flecainide significantly decreased the mAP only at 2 min, while NIK-244 significantly decreased the mAP at 0.5 to 5 min. Since the direct negative chronotropic and inotropic effects of NIK-244 were almost the same as those of flecainide, respectively, and NIK-244 has been reported not to interfere with reflex-mediated cardiovascular adaptive changes (15), NIK-244 must possess a more potent direct vasodilator effect on peripheral vasculature compared to flecainide. Whether this
effect of NIK-244 on the vasculature is favorable clinically or not must be determined by further studies.

It has been reported that almost all the class I antiarrhythmic agents transiently increased CBF, when they were directly injected into isolated canine blood-perfused heart preparations (8, 11). However, some of them have not shown significant coronary vasodilator effects even in the isolated preparations, when administered intravenously (11). In our study, i.a. and i.v. NIK-244 as well as flecainide transiently increased CBF of the preparation.

In the AV node preparation, NIK-244 prolonged the AH as well as HV intervals, which is consistent with the previous report by Urthaler et al. (2). Antiarrhythmic action of class I drugs is considered to result from their membrane effects; namely, an inhibition of the fast inward current, because we showed in earlier studies that the antiarrhythmic effect of class I drugs on canine coronary ligation and digitalis induced arrhythmias occurred at concentrations decreasing the Na current (5, 12). Since Na current is the most important depolarizing current responsible for the intraatrial and His-Purkinje-ventricle conduction, our results that the effect of i.a. NIK-244 on AH was significantly more potent than that of i.a. flecainide, while the effect of i.a. NIK-244 on HV was slightly more potent compared with i.a. flecainide, suggest that NIK-244 at least possesses an electrophysiologic profile similar to that of the well-established class I agent flecainide.

NIK-244 i.v. administered to the donor dog showed significantly slower onset and longer-lasting negative dromotropic effects on PQ and QRS of the donor heart and on AH and HV conductions of the cross-circulated AV node preparation than flecainide, although the pharmacokinetics of the two drugs are similar (5, 12). These results are in good accordance with our earlier report on dog Purkinje cells (3), in which the slowly developing block of INa by NIK-244 and a much slower recovery from use-dependent block than flecainide were demonstrated. Therefore, these findings indicate that NIK-244 should be classified as a slower kinetic drug than flecainide, and this may be related to the significantly more potent negative dromotropism by i.a. NIK-244 in AH conduction than that by i.a. flecainide.

In summary, NIK-244 has negative chronotropic, inotropic and vasodilator effects which are comparable to flecainide, while its negative dromotropic effect is more potent and slower in on-set and longer-lasting than flecainide. As these properties of the negative dromotropic effect might explain the previous report that NIK-244 is a more potent antiarrhythmic drug on canine coronary ligation-induced, and digitalis-induced arrhythmias in terms of the low dose or concentration to suppress arrhythmias than flecainide (5, 12). As a result, NIK-244 may prove to be a powerful antiarrhythmic agent as has already been shown in studies using a limited number of patients (1, 7).

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