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Nuclear Medicine and Biology 33 (2006) 21–27
NUCLEAR MEDICINE — AND — BIOLOGY
www.elsevier.com/locate/nucmedbio

Synthesis and evaluation of radioiodinated cyclooxygenase-2 inhibitors as potential SPECT tracers for cyclooxygenase-2 expression

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Received 26 August 2005; received in revised form 30 September 2005; accepted 5 October 2005

Abstract

Although several COX-2 inhibitors have recently been radiolabeled, their potential for imaging COX-2 expression remains unclear. In particular, the sulfonamide moiety of COX-2 inhibitors may cause slow blood clearance of the radiotracer, due to its affinity for carbonic anhydrase (CA) in erythrocytes. Thus, we designed a methyl sulfone-type analogue, 5-(4-iodophenyl)-1-[4-(methylsulfonyl)phenyl]-3-trifluoromethyl-1*H*-pyrazole (IMTP). In this study, the potential of radioiodinated IMTP was assessed in comparison with a ^{125}I-labeled celecoxib analogue with a sulfonamide moiety (^{125}I-IATP).

Methods: The COX inhibitory potency was assessed by measuring COX-catalyzed oxidation by hydrogen peroxide. The biodistribution of ^{125}I-IMTP and ^{125}I-IATP was determined by the ex vivo tissue counting method in rats. Distribution of the labeled compounds to rat blood cells was measured.

Results: The COX-2 inhibitory potency of IMTP (IC_{50}=5.16 μM) and IATP (IC_{50}=8.20 μM) was higher than that of meloxicam (IC_{50}=29.0 μM) and comparable to that of SC-58125 (IC_{50}=1.36 μM). The IC_{50} ratios (COX-1/COX-2) indicated the high isoform selectivity of IMTP and IATP for COX-2. Significant levels of ^{125}I-IMTP and ^{125}I-IATP were observed in the kidneys and the brain (organs known to express COX-2). The blood clearance of ^{125}I-IMTP was much faster than that of ^{125}I-IATP. Distribution of ^{125}I-IATP to blood cells (88.0%) was markedly higher than that of ^{125}I-IMTP (18.1%), which was decreased by CA inhibitors.

Conclusions: Our results showed a high inhibitory potency and selectivity of IMTP for COX-2. The substitution of a sulfonamide moiety to a methyl sulfone moiety effectively improved the blood clearance of the compound, indicating the loss of the cross reactivity with CA in ^{125}I-IMTP. ^{123}I-IMTP may be a potential SPECT radiopharmaceutical for COX-2 expression.

Keywords: Cyclooxygenase-2 (COX-2); Inhibitor; Radioiodination; SPECT; Radiopharmaceutical

1. Introduction

Cyclooxygenases (COXs) catalyse the key rate-limiting step in the conversion of arachidonic acid into prostaglandins and thromboxanes. To date, at least 2 distinct isoforms of the COXs—a constitutive form (COX-1) and an inducible isoform (COX-2)—and several of their variants have been discovered [1]. COX-1 is constitutively expressed in most tissues and is responsible for maintaining homeostasis, whereas COX-2 is induced in response to inflammatory stimuli. Besides being associated with inflammation, COX-2 has been implicated in a number of pathological processes, including many human cancers, atherosclerosis, and cerebral and cardiac ischemia [2–5]. We also reported

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doi:10.1016/j.nucmedbio.2005.10.004

the neuronal expression of COX-2 in rodent and primate models of cerebral ischemia [6–10].

Accordingly, the noninvasive imaging of COX-2 expression should help in understanding the pathophysiology of the diseases and contribute to the clinical use of COX-2 inhibitors [11]. In this regard, several COX-2 inhibitors were recently radiolabeled with F-18 and their potentials for positron emission tomography (PET) tracers were preliminarily evaluated [12–14]. The results for the potentials of these labeled compounds, however, are not necessarily consistent from one laboratory to another. In addition, the short half-life of ^{18}F may hamper the determination of the specific binding of the tracer to COX-2, because it is known that the COX-2 inhibitors show time-dependent inhibition of COX-2 [11]. The longer half-lives of single photon emission tomography (SPECT) nuclides, such as Tc-99m or I-123, may be more suitable for radiotracers to image COX-2. From these points of view, we intended to develop radioiodinated COX-2 inhibitors as SPECT tracers for imaging COX-2 expression.

As for SPECT tracers, Yang et al. [15] proposed a ^{99m}Tc-labeled celecoxib (celebrex) analogue as a potential tracer for COX-2 expression. Kabalka et al. [16] recently reported the radiosynthesis of a ^{123}I-labeled celecoxib analogue. However, the detailed characteristics of these tracers, including affinity and selectivity to COX-2, have not

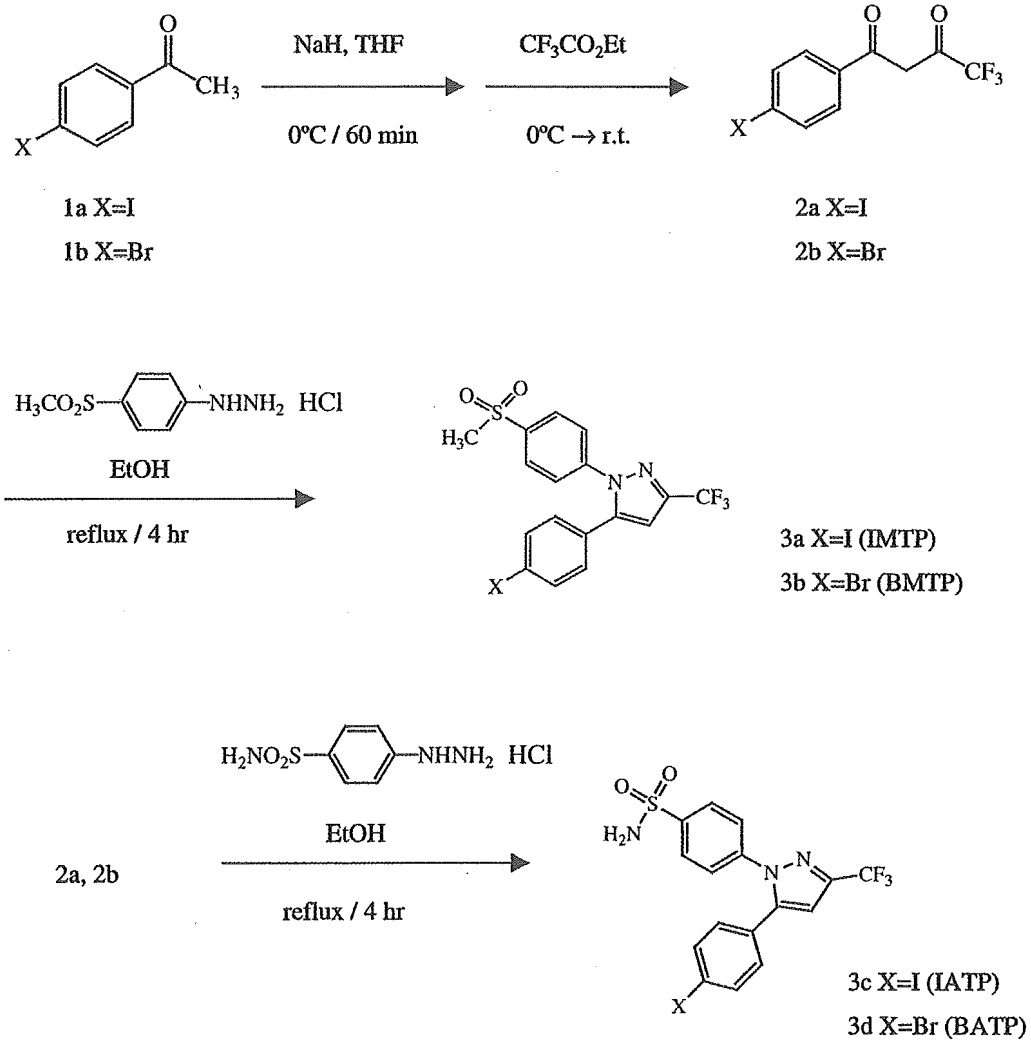

Fig. 1. Synthesis of IMTP (compound **3a**), BMTP (compound **3b**), IATP (compound **3c**) and BATP (compound **3d**).
Compound 1a, iodoacetophenone
Compound 1b, bromoacetophenone
Compound 2a, 4,4,4-trifluoro-1-(4-iodophenyl)-butane-1,3-dione
Compound 2b, 4,4,4-trifluoro-1-(4-bromophenyl)-butane-1,3-dione
Compound 3a, 5-(4-iodophenyl)-1-[4-(methylsulfonyl)phenyl]-3-trifluoromethyl-1H-pyrazole
Compound 3b, 5-(4-bromophenyl)-1-[4-(methylsulfonyl)phenyl]-3-trifluoromethyl-1H-pyrazole
Compound 3c, 5-(4-iodophenyl)-1-[4-(aminosulfonyl)phenyl]-3-trifluoromethyl-1H-pyrazole
Compound 3d, 5-(4-bromophenyl)-1-[4-(aminosulfonyl)phenyl]-3-trifluoromethyl-1H-pyrazole

been determined and their usefulness remains unclear. In particular, the sulfonamide moiety of celecoxib may cause slow blood clearance of the radiotracer, due to its affinity for carbonic anhydrase in erythrocytes [17,18].

Thus, we designed a methyl sulfone-type analogue, 5-(4-iodo-phenyl)-1-[4-(methylsulfonyl)phenyl]-3-trifluoromethyl-1*H*-pyrazole (IMTP), iodinated at position 4 of the 5-phenyl ring as a SPECT tracer for imaging COX-2 expression (Fig. 1). In this study, radioiodinated IMTP was synthesized, and its potential was assessed in comparison with a ^{125}I-labeled cerecoxib analogue with a sulfonamide moiety (^{125}I-IATP).

2. Materials and methods

2.1. General

Sodium ^{125}I-iodide (642.8 GBq/mg) was purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA). All chemicals used were of reagent grade.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a JNM-EX 400 spectrometer (JEOL, Tokyo, Japan), and the chemical shifts were reported in parts per million (ppm) downfield from an internal tetramethylsilane standard. Fast atom bombardment (FAB) mass spectra were recorded with a JMS-HX/HX110A model spectrometer (JEOL).

2.2. Synthesis

2.2.1. 5-(4-Iodophenyl)-1-[4-(methylsulfonyl)phenyl]-3-trifluoromethyl-1H-pyrazole (IMTP)

IMTP was synthesized according to the procedure outlined in Fig. 1. To dry tetrahydrofuran (THF, 5 mL) were added NaH (19.5 mg, 0.49 mmol) and iodoacetophenone 1a (100 mg, 0.4 mmol). The mixture was stirred at 0°C for 60 min, and then ethyl trifluoroacetate (145 μL, 1.22 mmol) was added dropwise. After stirring at 0°C for 12 h and at room temperature for 12 h, the reaction mixture was acidified with 1N HCl and then neutralized with 1N NaOH. The reaction mixture was extracted with chloroform. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give brownish oil. The crude product was purified by silica gel column chromatography (AcOEt/hexane/triethylamine=1:6:0.01) to give 2a as brownish oil in a yield of 35%. ^{1}H NMR ($CDCl_3$) δ, 7.71 (d, J=7.3 Hz, 2H), 7.51 (d, J=7.6 Hz, 2H), 6.30 (s, 1H).

Compound 2a (45.7 mg, 0.134 mmol) and 4-methylsulfonyl-phenylhydrazine hydrochloride (29.8 mg, 0.134 mmol) were dissolved in ethanol (3 ml) and heated under reflux for 4 h. The mixture was allowed to cool before concentration. The crude product was purified by silica gel flash column chromatography (AcOEt/hexane=1:2) to give IMTP 3a as a colorless solid in a yield of 51%. ^{1}H NMR ($CDCl_3$) δ, 7.97 (d, J=8.8 Hz, 2H), 7.74 (d, J=8.5 Hz, 2H), 7.53 (d, J=8.8 Hz, 2H), 6.97 (d, J=8.3 Hz, 2H), 6.79 (s, 1H), 3.08 (s, 3H). FAB-MS calcd for $C_{17}H_{12}IF_3N_2O_2S$ [MH^+]: m/z 493, found 493.

2.2.2. 5-(4-Bromophenyl)-1-[4-(methylsulfonyl)phenyl]-3-trifluoromethyl-1H-pyrazole (BMTP)

BMTP was synthesized in the same manner as IMTP, using bromoacetophenone 1b (100 mg, 0.5 mmol) as a starting material instead of iodoacetophenone 1a (Fig. 1). Compound 2b was obtained in a yield of 31%. ^{1}H NMR ($CDCl_3$) δ, 7.75 (d, J=8.1 Hz, 2H), 7.59 (d, J=7.8 Hz, 2H), 6.43 (s, 1H). Product 2b was then reacted with 4-methylsulfonylphenylhydrazine hydrochloride to give BMTP 3b as a colorless solid in a yield of 78%. ^{1}H NMR ($CDCl_3$) δ, 7.97 (d, J=8.8 Hz, 2H), 7.54 (d, J=8.5 Hz, 2H), 7.53 (d, J=8.8 Hz, 2H), 7.11 (d, J=8.8 Hz, 2H), 6.79 (s, 1H), 3.07 (s, 3H). FAB-MS calcd for $C_{17}H_{12}BrF_3N_2O2S$ [MH^+]: m/z 445, found 445.

2.2.3. 5-(4-Iodophenyl)-1-[4-(aminosulfonyl)phenyl]-3-trifluoromethyl-1H-pyrazole (IATP)

This compound was synthesized by the same method as for IMTP, except that 4-aminosulfonylphenylhydrazine hydrochloride was used instead of 4-methylsulfonylphenylhydrazine hydrochloride (Fig. 1). The product 2a was reacted with 4-aminosulfonylphenylhydrazine hydrochloride to give IATP 3c as a colorless solid in a yield of 85%. ^{1}H NMR ($CDCl_3$) δ, 7.93 (d, J=8.5 Hz, 2H), 7.73 (d, J=8.5 Hz, 2H), 7.47 (d, J=8.5 Hz, 2H), 6.97 (d, J=8.5 Hz, 2H), 6.78 (s, 1H), 4.99 (s, 2H). FAB-MS calcd for $C_{16}H_{11}IF_3N_3O_2S$ [MH^+]: m/z 494, found 494.

2.2.4. 5-(4-Bromophenyl)-1-[4-(aminosulfonyl)phenyl]-3-trifluoromethyl-1H-pyrazole (BATP)

This compound was synthesized using the same method as for BMTP, except that 4-aminosulfonylphenylhydrazine hydrochloride was used instead of 4-methylsulfonylphenylhydrazine hydrochloride. The product 2b was reacted with 4-aminosulfonylphenylhydrazine hydrochloride to give BATP 3d as a colorless solid in a yield of 48%. ^{1}H NMR ($CDCl_3$) δ, 7.94 (d, J=8.5 Hz, 2H), 7.53 (d, J=8.3 Hz, 2H), 7.47 (d, J=8.5 Hz, 2H), 7.11 (d, J=8.3 Hz, 2H), 6.78 (s, 1H), 4.89 (s, 2H). FAB-MS calcd for $C_{16}H_{11}IF_3N_3O_2S$ [MH^+]: m/z 446, found 446.

2.3. Radiolabeling

The radioiodinated IMTP and IATP were obtained by a halogen exchange reaction with sodium ^{125}I-iodine according to the methods of Kiyono et al. [19]. Briefly, BMTP or BATP was added to a mixture of sodium ^{125}I-iodine, ammonium sulfate and copper (II) sulfate pentahydrate in water in a vial. The reaction mixture was heated for 2 h at 140°C. After cooling, the reaction mixture was filtered with a 0.22-μm filter (Ultrafree-MC 0.22-μm filter unit, Millipore, Benford, TX). The filtered solution was applied to a reverse-phase high-performance liquid chromatography (HPLC) column (Cosmosil 5C_{18}-AR-300 Packed Column, 250×10 mm id,

Nacalai Tesque, Kyoto, Japan) and eluted at a flow rate of 2.0 ml/min with 10 mM KH_2PO_4/acetonitrile=1:1 for the purification of ^{125}I-IMTP (R_t=54 min for BMTP, 64 min for IMTP) and 10 mM KH_2PO_4/acetonitrile=53:47 for that of IATP (R_t=58 min for BATP, 70 min for IATP).

The radiochemical purity of the labeled compound was determined by TLC and analytical HPLC. The TLC was performed on a silica gel plate, developed with AcOEt/hexane=1:2 (R_f=0.6 for IMTP and 0.4 for IATP). Analytical HPLC was performed on a 150×4.6-mm id Cosmosil AR-300 column (Nacalai Tesque, Kyoto, Japan) eluted at a flow rate of 1.0 ml/min with 10 mM KH_2PO_4/acetonitrile=1:1 for ^{125}I-IMTP (R_t=18.0 min) and 10 mM KH_2PO_4/acetonitrile=53:47 for ^{125}I-IATP (R_t=17.9 min).

2.4. COX inhibitory potency

Peroxidase inhibitory activities of IMTP and IATP were assessed by measuring the COX-catalyzed oxidation of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) by hydrogen peroxide using a commercially available kit (Colorimetric COX Inhibitor Screening Assay Kit, Cayman Chemical). Briefly, 10 μl of ovine COX-1 or COX-2 solution was added to a 96-well plate with 150 μl of 0.1 mol/L Tris buffer at pH 8.0, 10 μl of heme solution in DMSO and 10 μl of the test compound (final concentration: 10^{-4}–10^{-9} mol/L). After 5 min of incubation at 25°C, 20 μL of TMPD and 20 μL of 1.1 mM arachidonic acid were added to the mixture. The oxidation of TMPD was monitored by measuring the absorbance of the mixture with a plate reader at 600 nm. SC-58125, meloxicam and indomethacin were used as reference compounds.

2.5. Animal experiments

Animal studies were conducted in accordance with institutional guidelines, and the experimental procedures were approved by the Kyoto University Animal Care Committee.

Biodistribution studies were performed on male Sprague-Dawley rats. ^{125}I-IMTP (74 kBq/rat) or ^{125}I-IATP (74 kBq/rat) was administered to rats under chloral hydrate anesthesia by tail vein injection. At appropriate time points after the administration, the rats were sacrificed by exsanguinations under chloral hydrate anesthesia. Blood and organs were excised and weighed, and the radioactivity was measured with an auto well gamma counter (ARC2000, Aloka, Tokyo, Japan).

Table 1
COX inhibitory potency and selectivity of IMTP, IATP and reference compounds

Compounds	IC_{50} (μM)		IC_{50} ratio (COX-1/COX-2)
	COX-1	COX-2	
IMTP	>100	5.16±2.83	>19
IATP	>100	8.20±1.43	>12
SC58125	>100	1.36±0.44	>73
Meloxicam[a]	>100	29.0	>3.5
Indomethacin[a]	0.08	11.9	0.007

Mean±S.D. of three independent experiments.
[a] Mean of two independent experiments.

Table 2
Biodistribution of ^{125}I-IMTP in rats (%dose/g tissue)

	Time after injection (min)			
	10	30	60	180
Blood	0.08±0.02	0.08±0.01	0.06±0.01	0.04±0.01
Plasma	0.12±0.03	0.12±0.01	0.09±0.01	0.06±0.01
Heart	0.49±0.11	0.50±0.05	0.38±0.06	0.23±0.04
Lung	0.48±0.14	0.42±0.08	0.34±0.04	0.28±0.05
Liver	1.59±0.29	1.53±0.27	1.02±0.15	0.59±0.11
Kidney	0.65±0.14	0.60±0.07	0.42±0.06	0.34±0.05
Pancreas	0.59±0.13	1.26±0.56	0.67±0.11	0.88±0.35
Spleen	0.28±0.06	0.27±0.05	0.23±0.07	0.15±0.04
Stomach	0.27±0.10	0.19±0.04	0.23±0.06	0.17±0.05
Intestine	0.27±0.06	0.38±0.20	0.27±0.04	0.25±0.05
Muscle	0.07±0.02	0.22±0.04	0.17±0.03	0.16±0.01
Thyroid	0.37±0.25	0.54±0.13	0.69±0.30	0.58±0.26
Brain	0.25±0.06	0.23±0.04	0.17±0.03	0.10±0.02
Brain/blood[a]	3.19±0.17	2.87±0.31	2.74±0.24	2.67±0.09

Mean±S.D. for four to five animals.
[a] Brain-to-blood ratio.

2.6. Distribution to blood cells

Distribution of ^{125}I-IATP and ^{125}I-IMTP to blood cells and the effects of several compounds on the distribution were measured by using rat whole blood. Acetazolamide and chlorthalidone were used as reference compounds for binding to carbonic anhydrase (CA), chlorpromazine for binding to the cellular membrane of red blood cells and phenothiazine for binding to hemoglobin. Heparinized whole blood from male Sprague-Dawley rats was preincubated at 37°C with gentle shaking for 5 min and then ^{125}I-IMTP (0.74 kBq) or ^{125}I-IATP (0.74 kBq) was added. After incubation at 37°C for 10 min, chlorpromazine, phenothiazine, acetazolamide or chlorthalidone was added in final concentrations of 10 to 300 μg/ml and then incubated at 37°C for 10 min. A small portion of the blood samples was counted in an auto well gamma counter (Cobra II Auto-

Table 3
Biodistribution of ^{125}I-IATP in rats (%dose/g tissue)

	Time after injection (min)			
	10	30	60	180
Blood	0.63±0.08	0.53±0.03	0.44±0.03	0.45±0.05
Plasma	0.14±0.02	0.12±0.01	0.11±0.01	0.10±0.01
Heart	0.86±0.12	0.62±0.03	0.57±0.03	0.56±0.03
Lung	0.77±0.06	0.58±0.03	0.53±0.05	0.55±0.04
Liver	1.89±0.28	1.31±0.14	1.13±0.12	1.15±0.15
Kidney	0.92±0.11	0.64±0.03	0.55±0.05	0.58±0.06
Pancreas	0.77±0.07	0.79±0.06	0.71±0.06	0.78±0.18
Spleen	0.58±0.07	0.44±0.03	0.39±0.03	0.36±0.02
Stomach	0.24±0.04	0.19±0.06	0.24±0.04	0.22±0.06
Intestine	0.26±0.04	0.29±0.04	0.32±0.07	0.36±0.04
Muscle	0.23±0.06	0.28±0.01	0.27±0.03	0.29±0.03
Thyroid	0.58±0.18	0.47±0.14	0.60±0.07	0.51±0.17
Brain	0.23±0.02	0.22±0.02	0.21±0.01	0.20±0.01
Brain/blood[a]	0.36±0.05	0.42±0.03	0.48±0.03	0.45±0.05

Mean±S.D. for five animals.
[a] Brain-to-blood ratio.

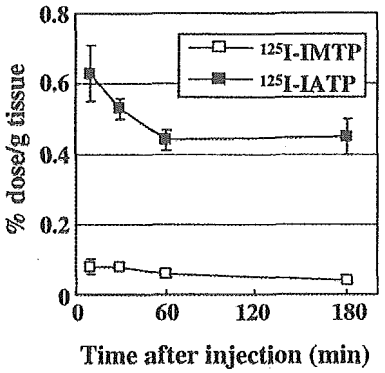

Fig. 2. Levels of ^{125}I-IMTP and ^{125}I-IATP in the blood. Mean±S.D. for four to five animals.

Gamma, Packard, Tokyo, Japan), and the rest were centrifuged for 1 min and the plasma separated. A small portion of the plasma samples was also counted. Hematocrit was measured using an i-STAT portable clinical analyzer (i-STAT, East Windsor, NJ). Distribution of the labeled compounds to blood cells was calculated as follows:

$$T = [1 - C_P/C_B \times (100 - H_t)/100] \times 100$$

where T is the distribution% to blood cells, C_P and C_B are the radioactivity in blood and plasma, respectively, and H_t is the hematocrit value.

2.7. Statistical analysis

Data are presented as mean values with the standard deviation, unless otherwise noted. Statistical analysis was performed by one-way ANOVA followed by Bonferroni–Dunn test for post hoc comparisons. Statistical significance was defined as a two-tailed P value <.05/6 (i.e., .0083).

3. Results

3.1. Synthesis and radiolabeling

IMTP, BMTP, IATP and BMTP were obtained with overall yields of 18%, 25%, 26% and 17%, respectively, from the starting material 1a or 1b. The radiosynthesis of ^{125}I-IMTP and ^{125}I-IATP was achieved with an iodine–bromide exchange reaction. ^{125}I-IMTP and ^{125}I-IATP were obtained with no carrier being added by the following separation from the precursors (BMTP and BATP) using reverse-phase HPLC. The radiochemical yields were 42% for ^{125}I-IMTP and 35% for ^{125}I-IATP, and the radiochemical purities were >95% for both of the labeled compounds.

3.2. COX inhibitory potency

IMTP and IATP inhibited COX-2 in a concentration-dependent manner, while they showed no inhibitory potency for COX-1 even at the highest concentration examined. Table 1 summarizes the IC_{50} values of the test compounds. The IC_{50} values of IMTP and IATP were 5.16 and 8.20 μM for COX-2 and >100 μM for COX-1. The COX-2 inhibitory potency of IMTP and IATP was higher than that of meloxicam (IC_{50}=29.0 μM) and comparable to that of SC-58125 (IC_{50}=1.36 μM). The IC_{50} ratio (COX-1/COX-2) for IMTP, IATP, SC-58125 and meloxicam was >19, 12, 73 and 3.5, indicating a high isoform selectivity of IMTP and IATP for COX-2.

3.3. Biodistribution

The biodistribution of ^{125}I-IMTP and ^{125}I-IATP is shown in Tables 2 and 3, respectively. The level of radioactivity for ^{125}I-IMTP in the blood decreased more rapidly than that for ^{125}I-IATP (Fig. 2). The radioactivity in the blood was 0.04 %dose/g tissue for ^{125}I-IMTP and 0.45 %dose/g tissue for ^{125}I-IMTP at 180 min after the tracer administration. At 10 min after the injection, high levels of the radioactivity were found in the liver and kidneys for both compounds. ^{125}I-IATP showed relatively higher levels of radioactivity in the heart and lung. Both compounds showed no marked accumulation in the stomach and thyroid. Significant levels of radioactivity were found in the brains of rats, with brain-to-blood ratios of 2.67–3.19 for ^{125}I-IMTP and 0.36–0.48 for ^{125}I-IATP.

3.4. Distribution to blood cells

Distribution of ^{125}I-IATP to blood cells (88.0%) was markedly higher than that of ^{125}I-IMTP (18.1%) as shown

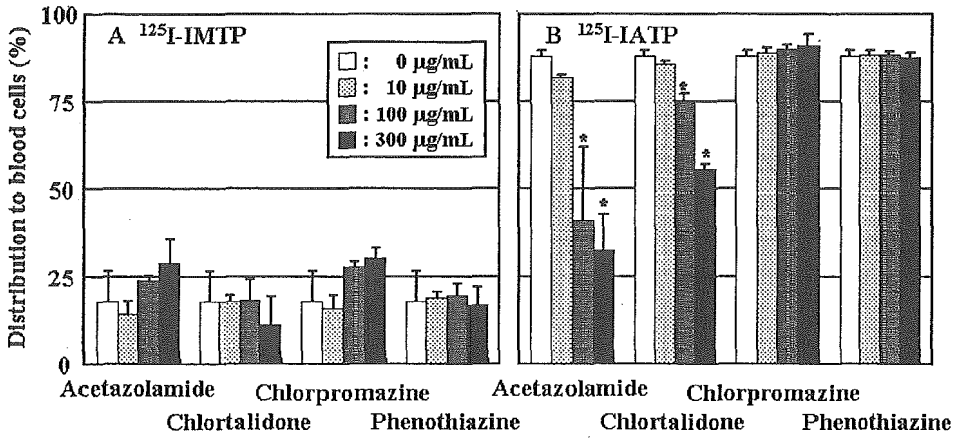

Fig. 3. Distribution of ^{125}I-IMTP (A) and ^{125}I-IATP (B) to blood cells. Mean±S.D. of three measurements. *P<.05/6 (i.e., .0083).

in Fig. 3. The distribution of ^{125}I-IATP to blood cells was significantly decreased by CA inhibitors (acetazolamide and chlorthalidone), but not by chlorpromazine or phenothiazine. The distribution of ^{125}I-IMTP to blood cells was not affected by any of the compounds used in the present study.

4. Discussion

In the present study, we synthesized a methyl sulfone-type COX-2 inhibitor, 5-(4-iodophenyl)-1-[4-(methylsulfonyl)phenyl]-3-trifluoromethyl-1*H*-pyrazole (IMTP). The potential of radioiodinated IMTP for imaging COX-2 expression was evaluated in comparison with a ^{125}I-labeled celecoxib analogue with a sulfonamide moiety (^{125}I-IATP). The major findings in the present study can be summarized as follows: (1) IMTP had a high inhibitory potency and selectivity for COX-2. (2) ^{125}I-IMTP showed a biodistribution compatible with the known distribution of COX-2. (3) The blood clearance of ^{125}I-IMTP was much faster than that of ^{125}I-IATP. (4) ^{125}I-IATP showed markedly higher distribution to blood cells than ^{125}I-IMTP, which was decreased by CA inhibitors. These results demonstrate that the substitution of the sulfonamide moiety to a methyl sulfone moiety effectively improved the blood clearance of the compound, indicating the loss of the cross reactivity with CA in ^{125}I-IMTP. Methyl sulfone-type COX-2 inhibitors may be a preferential candidate as radiopharmaceuticals for COX-2 expression.

The methyl sulfone moiety and sulfonamide moiety at position 4 of the 1-phenyl ring are considered to be optimal for COX-2 selectivity [11]. In this regard, several COX-2 inhibitors with a methyl sulfone or sulfonamide moiety were recently radiolabeled and preliminarily evaluated as imaging agents [12–14,16]. However, the effects of these moieties on the pharmacokinetics of the labeled tracers have not been determined. Our results clearly showed that the substitution of the sulfonamide moiety to the methyl sulfone moiety effectively improved the blood clearance of the compound (Fig. 2). In addition, the high distribution of ^{125}I-IATP to blood cells was significantly inhibited by CA inhibitors (Fig. 3). Recently, it was reported that sulfonamide-type celecoxib analogues show high affinity to carbonic anhydrase (CA) [18]. Agents containing sulfonamides (e.g., acetazolamide) have been widely used in clinical medicine to inhibit carbonic anhydrase (CA) [17,18]. The slow blood clearance of ^{125}I-IATP can be ascribed to the affinity of its sulfonamide moiety to CA in erythrocytes [17,18]. These results indicate the feasibility of methyl sulfone-type COX-2 inhibitors as radiopharmaceuticals for COX-2 expression.

Although COX-2 is an inducible isoform, it is predominantly found in the normal brain and kidneys [20]. The preferential uptakes of ^{125}I-IMTP and ^{125}I-IATP in these organs were compatible with the expression of COX-2 in these organs. The high brain-to-blood ratio of ^{125}I-IMTP indicates the feasibility of this compound for COX-2 imaging in the brain. On the other hand, no marked ^{125}I-IMTP accumulation was observed in the stomach or thyroid, indicating its stability to in vivo deiodination. The present results using ^{125}I-IMTP are consistent with those using ^{18}F-SC-58125, which showed preferential uptakes in the brain and kidneys with rapid blood clearance [12]. SC-58125 is a methyl sulfone-type COX-2 inhibitor that has the same structure as IMTP except that the fluorine in SC-58125 is replaced with iodine in IMTP. These results further confirm the potentials of methyl sulfone-type COX-2 inhibitors as radiopharmaceuticals for COX-2 expression.

In the present study, we determined the biodistribution of the labeled compounds at several time points within 3 h, considering that small animals generally show rapid pharmacokinetics compared with that in humans. Consequently, we demonstrated that ^{125}I-IMTP showed preferential uptakes in the brain and kidneys with much faster blood clearance than ^{125}I-IATP. Time points <3 h appear to be appropriate to extrapolate the pharmacokinetics in humans from those in rats. We generally perform experiments to block the uptake of a candidate compound in tissues by coinjecting the nonradioactive compound, in order to confirm its specific distribution. In the present study, however, we did not perform such blocking experiments, because the physiological expression levels of COX-2 are relatively low compared with those in the pathological state. Such blocking experiments do not appear to be suitable to demonstrate the specific distribution of radiolabeled COX-2 inhibitors. McCarthy et al. [12] failed to obtain in vivo blocking data to show the specific binding of a radiotracer (^{18}F-SC58125) to COX-2 in rats. Contrarily, de Vries et al. [13] indicated the specific binding of ^{18}F-desbromo-DuP-697 by blocking experiments in rats. Experiments in animal models with higher COX-2 expression may be necessary to assess the specific binding of tracers to COX-2. We must await further studies to achieve this goal. Experiments to demonstrate the advantage of longer half-lives of SPECT nuclides are also required.

The COX-2 inhibitory potency of IMTP and IATP was higher than that of meloxicam and was comparable to that of SC-58125, suggesting that the introduction of iodine at position 4 of the 5-phenyl ring did not largely affect the COX-2 inhibitory potency. In addition, IC_{50} ratios (COX-1/COX-2) for IMTP and IATP showed high isoform selectivity of these compounds for COX-2 (Table 1), indicating that the selectivity of IMTP and IATP for COX-2 is comparable to celecoxib [21,22]. These results were consistent with the consideration on the structure–activity relationship reported by Herschman et al. [11] and suggest that the introduction of iodine at position 4 of the 5-phenyl ring is acceptable.

5. Conclusion

A radioiodinated COX-2 inhibitor, ^{125}I-IMTP, was synthesized. Our results showed a high inhibitory potency

and selectivity of IMTP for COX-2. In addition, radioiodinated-IMTP was stable for in vivo deiodination and showed rapid blood clearance. These results indicate that radioiodinated IMTP, a methyl sulfone-type COX-2 inhibitor, meets the basic requirements for an effective radiopharmaceutical and deserves further elucidation as a SPECT radiopharmaceutical for imaging COX-2 expression.

Acknowledgments

This study was supported in part by Grants-in-Aid for General Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors wish to thank Dr. H Hashimoto of Chemical Research Laboratories, Japan Tobacco, Inc., for useful discussion.

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Original Paper

Cerebrovascular Diseases

Cerebrovasc Dis 2005;19:225–228
DOI: 10.1159/000083887

Received: April 1, 2004
Accepted: October 5, 2004
Published online: February 8, 2005

A Case-Control Analysis of Intra-Arterial Urokinase Thrombolysis in Acute Cardioembolic Stroke

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Key Words
Intra-arterial urokinase thrombolysis · Cardioembolic stroke · Acute ischemic stroke · National Institute of Health Stroke Scale score · Modified Rankin scale score

Abstract
Background: Intra-arterial urokinase (IA-UK) thrombolysis is frequently given in Japan to selected patients with acute cerebral artery occlusion. However, it is not clear whether or not IA-UK thrombolysis has an efficacy for acute stroke patients. The purpose of this study was to assess the effects of IA-UK thrombolysis in acute cardioembolic stroke patients, by performing a case-control analysis using data from Japan's Multicenter Stroke Investigator's Collaboration (J-MUSIC). ***Methods:*** 16,922 acute ischemic stroke patients were enrolled into J-MUSIC. From these patients, we selected 91 patients (UK group) who met the following criteria: treatment with IA-UK; 20–75 years of age; cardioembolic stroke; presenting with a carotid stroke; admission within 4.5 h of symptom onset, and a National Institutes of Health Stroke Scale (NIHSS) score of 5–22 points on admission. A control group of 182 patients without IA-UK treatment and matched to the NIHSS score, gender, and age was chosen. We compared the modified Rankin scale (mRS) score at discharge and the mortality between the 2 groups. ***Results:*** In both groups, the mean age was 65 ± 8 years, and the median NIHSS score was 14. The mean interval between symptom onset and UK administration was 3.4 ± 1.3 h, and the IA-UK dose was 392,000 ± 200,000 units. The mRS score at discharge was lower in the UK group than in the control group (mean, SD, median; 2.8, 2.9, 2 in UK group vs. 3.3, 1.8, 4, in the control, respectively p = 0.031). A favorable outcome (mRS of 0–2) was more frequently observed in the UK group (50.5%) than in the control group (34.1%, p = 0.0124). No difference in the mortality rate was seen between the UK group (11.0%) and the control group (13.3%). As well, there was no difference in the length of hospital stay between the UK group (46 ± 41 days, mean ± SD) and the control group (42 ± 42 days, mean ± SD). ***Conclusions:*** IA-UK thrombolytic therapy may improve the outcome in hyperacute cardioembolic stroke patients.

Intravenous (IV) thrombolytic therapy using recombinant tissue plasminogen activator (rt-PA) has been shown to be an effective treatment for ischemic stroke if used within 3 h of stroke onset [1, 2]. Recently, prolyse in acute cerebral thromboembolism (PROACT) I and II reported that local and intra-arterial (IA) thrombolytic therapy with pro-urokinase (proUK) could improve the outcome

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for ischemic stroke patients if used within 6 h of symptom onset [3, 4]. In Japan, the use of rt-PA for acute ischemic stroke has not been approved by the government. Therefore, IA thrombolytic therapy with urokinase (IA-UK) is mainly performed as a replacement of IV-rt-PA thrombolysis for acute cerebral artery occlusion, and in particular, for embolic occlusion of the middle cerebral artery. Several investigators have reported that IA-UK therapy was safe and effective for acute ischemic stroke [5–15]. However, their sample sizes were small, and not all their studies were randomized controlled trials.. Therefore, it remains unclear whether IA-UK therapy is effective for acute stroke patients. The aim of this study was to assess the efficacy of IA-UK thrombolysis for acute stroke patients by a case-control analysis using data from J-MUSIC [16, 17].

Subjects and Methods

We conducted a multicenter, prospective, hospital-based registration study (J-MUSIC) from May 1999 to April 2000 in which 156 hospitals from all over Japan participated [16, 17]. A total of 16,922 consecutive patients with acute ischemic stroke and transient ischemic attack within 7 days of onset were registered in this study.

The following data were assessed in all the patients, using common data-sheets prepared by the protocol committee: (1) age and gender; (2) time from onset to hospital arrival; (3) a history of stroke; (4) National Institutes of Health Stroke Scale (NIHSS) score on admission; (5) site of acute lesions on CT or MRI; (6) stroke subtype (clinical category); (7) thrombolytic therapy (IV and IA rt-PA, IA UK) within 12 h of onset; and (8) outcome at discharge.

Clinical categories were defined by using clinical and radiographic diagnosis rubrics according to the classification of cerebrovascular diseases III developed by National Institute of Neurological Disorders and Stroke [18]. The main subtypes included: lacunar, atherothrombotic, cardioembolic, and other stroke. The modified Rankin Scale (mRS) [19] score and mortality were used to assess clinical outcome at hospital discharge.

We selected patients treated with IA-UK (UK group) and patients who had been treated without thrombolytic therapy (control group) from 16,922 patients. The UK group was identified as the patients treated with IA-UK who met the following criteria: aged 20–75 years; presence of a cardioembolic stroke or a carotid stroke; admission within 4.5 h of symptom onset, and an NIHSS score of 5–22 points on admission. We randomly selected control patients who had no thrombolytic therapy, such as IA-UK, IA-rt-PA, and IV-rt-PA and were matched to the UK group patients with respect to age, gender, and NIHSS score. The number of control group patients was set to be twice the number of the UK group patients.

Statistical Analysis

Analyses were made with a commercially available software package (Stat-View, version 4.5; ASA Institute, Cary, N.C.). We compared the mRS score, mortality, and length of hospital stay between the two groups. The statistical significance for differences between the two groups was assessed by the Wilcoxon signed-rank test for the mRS score, the χ^2 test for favorable outcome (mRS score 0–2) and mortality, and the paired t-test for length of hospital stay. A p value <0.05 was considered statistically significant.

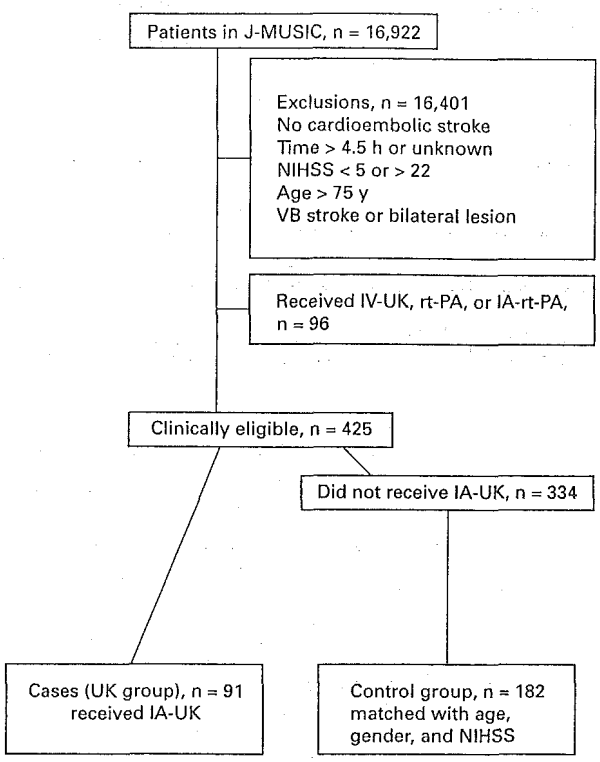

Fig. 1. Flow chart showing process of patient selection.

Results

Ninety-one patients met the criteria for inclusion into the UK group and 182 patients were selected for the control group (fig. 1). Table 1 shows the baseline characteristics of the two groups. In each group, the average age was 65 ± 8 years. The median NIHSS score for the two groups was 14. In the UK group, the mean interval between the onset of symptoms and UK administration was 3.4 ± 1.3 h, and the IA-UK dose was 392,000 ± 200,000 units. The mRS score at discharge was lower in the UK group than in the control group (mean, SD, median; 2.8, 2.9, 2 vs. 3.3, 1.8, 4, respectively; p = 0.031). Patients with

Table 1. Characteristics of the two groups

	UK group	Control group	p
Patients	91	182	
Gender, F/M	24/67	48/134	
Age, years (mean ± SD)	65 ± 8	65 ± 8	
NIHSS score on admission	14	14	
Interval time from stroke onset to hospital, h (mean ± SD)	1.1 ± 0.2	1.1 ± 0.3	
Interval time from stroke onset to treatment, h (mean ± SD)	3.4 ± 1.3	–	
Range	1–6		
Mega units of UK administered (mean ± SD)	0.39 ± 0.20	–	
Mean, SD, median of mRS score at discharge	2.8, 2.9, 2	3.3, 1.8, 4	0.031
mRS ≤2, %	50.5	34.1	0.012
Mortality, %	11.0	13.2	0.745
Length of hospital stay, days (mean ± SD)	46 ± 41	42 ± 42	0.347

favorable outcome were more frequently found in the UK group than in the control group (50.5 vs. 34.1%, p = 0.0124). However, no difference between the two groups was observed in the mortality rate (11.0 vs. 13.2%) or the length of hospital stay (46 ± 41 vs. 42 ± 42 days, mean ± SD).

We analyzed the relationship between time interval from stroke onset to IA-UK thrombolytic therapy and patients' outcome. The percentage of favorable outcome was higher in patients treated within 2 h of stroke onset than in those between 2–4 h and over 4 h [63% (17/27), 45% (21/47), and 47% (8/17)]. However, no significant differences among them were observed (p = 0.30).

Discussion

This case-control study based on the data from J-MUSIC demonstrates the effectiveness of IA-UK thrombolysis in acute stroke patients. Patients with IA-UK thrombolysis had an increased frequency of good outcomes, approximately 1.5 times greater than patients without IA-UK thrombolysis. However, no difference in mortality rate was observed between patients with and without IA-UK thrombolysis.

The PROACT II study [4] demonstrated a significant benefit from treatment with IA proUK in patients with a middle cerebral artery occlusion treated within 6 h of stroke onset. Their proUK group had a higher recanalization rate (66 vs. 18%) with a greater number of patients with good outcomes (mRS score 0–2) after 3 months of stroke onset (40 vs. 25%). However, the incidence of symptomatic intracranial hemorrhage was 10% in the proUK group, but only 2% in the placebo group.

In 1988, del Zoppo et al. [5] studied 20 patients and showed that local IA fibrinolytic therapy using UK or streptokinase might lead to cerebral arterial recanalization in patients with an acute carotid territory thrombotic stroke. Mori et al. [6] also assessed 22 patients and reported on the safety and efficacy of UK thrombolytic therapy for acute thromboembolic occlusion of the middle cerebral artery. Recently, Gonner et al. [8] performed IA-UK thrombolytic therapy in 43 ischemic stroke patients within 6 h of symptom onset, and reported that therapy was effective except in patients with a carotid artery occlusion. Arnold et al. [20] analyzed the clinical and radiological findings, and assessed the functional outcome 3 months after IA-UK thrombolysis for 100 consecutive patients. They concluded that IA-UK thrombolytic therapy was safe and could be efficacious. The results of the present study also lead us to conclude that local IA thrombolytic therapy using UK could be effective for acute ischemic stroke.

The therapeutic time window of IV thrombolytic therapy with rt-PA is within 3 h [1, 2]. However, in the PROACT II study proUK could be administered within 6 h of stroke onset [4]. Therefore, IA thrombolytic therapy may allow the extension of the therapeutic time window for treating acute stroke from 3 to 6 h. In the future, thrombolysis using proUK as well as UK may provide an alternative to IV thrombolysis with rt-PA in selected patients with acute ischemic stroke.

Our study has some limitations. Firstly, the aim of the J-MUSIC [15] study was to determine the present state of stroke managements in Japan, and not to investigate the effectiveness of thrombolytic therapy. Secondly, we did not require to describe the presence and frequency of symptomatic cerebral hemorrhage after thrombolytic therapy in J-MUSIC. There was a higher rate of symptomatic intracranial hemorrhage with IA proUK in PROACT II (10.2%) [4] compared to IV-rt-PA in NINDS (6.4%) [2]. However, there is no evidence that the rate of symptomatic brain hemorrhage is lower with IV thrombolysis than with IA thrombolysis. Thirdly, this was not a randomized study. Therefore, there may be some selection bias against choosing stroke patients with complications, such as heart diseases and infection. Patients with

such complications were not likely to be treated with thrombolytic therapy, and outcomes of such patients were not as good as those in patients without such complications. Furthermore, control patients did not always undergo angiography. The catheter placement itself might be benefit for destruction of the clot. Moreover, physicians who assessed patients' outcome were not blinded to treatment. Therefore, it is possible that efficacy of IA-UK thrombolysis is overestimated.

In conclusion, IA thrombolysis using UK could potentially be effective for acute ischemic stroke patients, and would allow the possible extension of the 3-h therapeutic window. This would lead to an increased number of patients being eligible for thrombolytic therapy.

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Reprinted from JOURNAL OF NEUROLOGY, NEUROSURGERY AND PSYCHIATRY, May 2005, Vol 76, p 679-683

PAPER

Atrial fibrillation as a predictive factor for severe stroke and early death in 15 831 patients with acute ischaemic stroke

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J Neurol Neurosurg Psychiatry 2005;**76**:679–683. doi: 10.1136/jnnp.2004.048827

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Received 29 June 2004
Revised version received 8 September 2004
Accepted 8 September 2004

Background: Atrial fibrillation (AF) is a common arrhythmia and a major risk factor for stroke.
Aims: To assess whether AF in patients with acute ischaemic stroke is associated with severe stroke and early death.
Materials/methods: Patients with acute ischaemic stroke (15 831) who were registered in the Japan Multicenter Stroke Investigators' Collaboration registry were analysed. The AF group comprised 3335 (21.1%) patients (median age, 75 years) and the non-AF group comprised 12 496 (78.9%) patients (median age, 70 years). The association between AF and severe stroke and early death was investigated by means of multivariate logistic regression analysis.
Results: The admission National Institutes of Health Stroke Scale (NIHSS) score of the AF group was higher than that of the non-AF group (median, 12 *v* 5; $p < 0.0001$). Multivariate logistic regression analyses found that female sex, advanced age, AF, and a history of stroke were independent factors associated with severe stroke (NIHSS score, ≥ 11). The mortality rate within 28 days after admission was 11.3% in the AF group and 3.4% in the non-AF group ($p < 0.0001$). Multivariate logistic regression analyses identified older age, AF, and NIHSS score at admission as independent factors associated with early death.
Conclusion: AF was a predictive factor for severe stroke and early death in acute ischaemic stroke. Careful cardiac evaluation and appropriate treatment are needed to improve outcome in patients with acute stroke and AF.

Atrial fibrillation (AF) is a common arrhythmia that is associated with a high risk of stroke, particularly cardioembolic stroke.[1] AF carries an annual risk of thromboembolic complications of 3–6%, which is five to seven times greater than that of controls with sinus rhythm.[2–4] AF is present in 15–21% of patients affected by stroke.[5–11] In a clinical study in Hokkaido, a northern part of Japan, the incidence of ischaemic events in 20 000 patients with AF, who were followed up in cardiovascular clinics, was 4.6% during a 1.7 year follow up period.[12]

Patients with AF have an increased risk of major, disabling stroke, often caused by large infarctions in the middle cerebral artery territory.[13–15] Some studies demonstrated that AF was associated with an increased risk of death in the first four weeks after stroke.[5 16] This association was explained by several factors, including the advanced age in stroke patients with AF, large infarction, severe neurological deficits, and poor functional outcomes. However, it is not clear whether AF itself is associated with early death in patients with acute ischaemic stroke.

We conducted a nationwide prospective survey from May 1999 to April 2000 in Japan (Japan Multicenter Stroke Investigators' Collaboration; J-MUSIC) to clarify the current status of stroke management and outcome. In total, 16 922 patients were registered to J-MUSIC.[17 18] We performed retrospective and secondary analyses to assess whether AF was associated with severe stroke and early death in patients with acute ischaemic stroke, using data from the large sample of hospitalised patients registered in J-MUSIC.

MATERIALS AND METHODS

We conducted a multicentre prospective hospital based registration study (J-MUSIC) from May 1999 to April 2000 in which 156 hospitals participated.[17 18] In total, 16 922 consecutive patients with acute ischaemic stroke and transient ischaemic attack (TIA) within seven days of onset were registered in our study.[17] We excluded 1091 TIA patients, and thus enrolled 15 831 patients with acute stroke into our present study. First, we divided patients into two groups based on the presence of AF: the AF group and the non-AF group. We compared the baseline and clinical characteristics and outcome between these two groups. Second, we used multivariate logistic regression analyses to investigate the association between AF and severe stroke, and early death. Early death was defined as death within 28 days after admission. Informed consent was obtained from all patients participating in our study.

For all patients, the following data from the common stroke protocol were assessed: (1) age and sex; (2) past history of stroke; (3) National Institutes of Health Stroke Scale (NIHSS) score on admission; (4) time from stroke onset to arrival at hospital; (5) cardiovascular risk factors (hypertension, diabetes mellitus, hyperlipidaemia, AF, and current smoking); (6) treatment, including thrombolytic treatment, heparin, and aspirin; (7) death within 28 days after admission; (8) hospital discharge status; and (9) residence after hospital discharge.

AF included both paroxysmal AF and persistent AF identified by electrocardiography (ECG) and/or 24 hour ECG monitoring during admission. Patients with a history of paroxysmal AF confirmed by ECG were also classified as patients with AF. The hospital discharge status was evaluated by using the modified Rankin scale score (mRS)[19] and death. Good outcome was defined as mRS scores of 0, 1, and 2. Residence after hospital discharge was classified into two groups: the patient's own home and an institution (including nursing home and another hospital for rehabilitation and medical management).

Abbreviations: AF, atrial fibrillation; CI, confidence interval; ECG, electrocardiography; J-MUSIC, Japan Multicenter Stroke Investigators' Collaboration; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; TIA, transient ischaemic attack

Table 1 Baseline characteristics of patients with and without AF

Characteristics	Total N=15831	With AF N=3335	Without AF N=12496	p Value
Mean (SD; median) age (years)	70.7 (11.5; 71)	74.5 (9.8; 75)	69.6 (11.7; 70)	<0.0001
<45 (%)	1.9	0.4	2.3	
45–54 (%)	7.6	2.8	8.9	
55–64 (%)	19.5	14.4	20.8	
65–74 (%)	33.2	31.8	33.5	
75–84 (%)	28.2	36.0	26.1	
>84 (%)	9.8	14.6	8.5	
Female (%)	38.7	46.5	38.0	0.0003
History of stroke (%)	34.2	35.6	30.1	<0.0001
Risk factors				
Hypertension (%)	61.2	48.7	64.5	<0.0001
Diabetes mellitus (%)	24.7	16.9	26.8	<0.0001
Hyperlipidaemia (%)	16.6	9.5	18.5	<0.0001
Smoking (%)	17.3	10.2	19.2	<0.0001
NIHSS score (mean, SD, median)	8.3, 7.9, 5	13.7, 9.7, 12	6.9, 6.7, 5	<0.0001
0–6 (%)	57.4	31.3	64.4	
7–10 (%)	15.4	12.7	16.2	
11–15 (%)	9.9	15.3	8.5	
16–22 (%)	9.6	21.0	6.5	
≥23 (%)	7.7	19.7	4.5	

AF, atrial fibrillation; NIHSS, National Institutes of Health Stroke Scale.

Statistical analysis

Statistical analyses were performed with a commercially available software package (Stat-view, version 4.5; SAS Institute, Cary, North Carolina, USA). The Mann-Whitney U test was used to detect differences in age and NIHSS scores among the groups. All other differences were assessed using the χ^2 test. We divided the patients into five groups based on stroke severity (NIHSS score: ⩽ 6, 7–10, 11–15, 16–22, and ⩾ 23) according to those in the TOAST study.[20] Multivariate logistic regression models were used to identify factors associated with mild stroke (NIHSS scores, ⩽ 6) and severe stroke (NIHSS scores, ⩾ 11) at admission. Furthermore, multivariate logistic regression models were applied to identify factors associated with early death after admission. Next, we examined the early death rate in patients with and without AF by five groups based on stroke severity. According to the results, we divided patients into subgroups and applied multivariate logistic regression models to identify factors associated with early death for each group. Variables ($p < 0.20$) associated with stroke severity and early death in the univariate analysis were selected to be evaluated in the multivariate logistic regression analyses. Differences were considered significant at the level of $p < 0.05$.

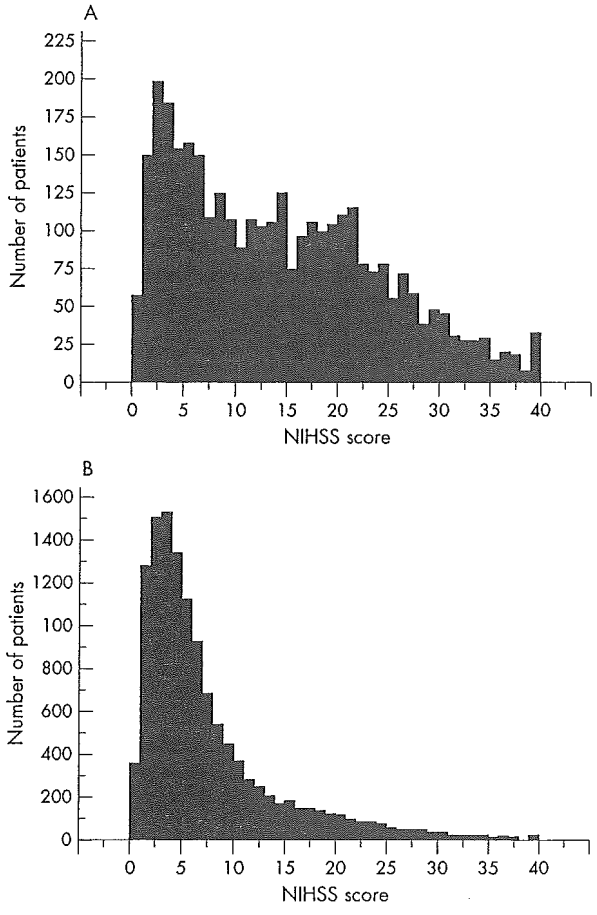

Figure 1 Distribution of National Institutes of Health Stroke Scale (NIHSS) scores for (A) patients with atrial fibrillation (AF) and (B) patients without AF.

RESULTS

The mean age of all patients (6130 women and 9701 men) was 70.7 (SD, 11.5) years (median, 71; range, 18–107). The AF group comprised 3335 patients (21.1%) and the non-AF group comprised 12 496 patients (78.9%). Table 1 shows the baseline features of the patients with and without AF.

Severity of stroke (NIHSS on admission)

The mean (SD) and median NIHSS scores for all patients were 8.3 (7.9) and 5. The scores were significantly higher in the AF group (mean, 13.7; SD, 9.7; median, 12) than in the non-AF group (mean, 6.9; SD, 6.7; median, 5) ($p < 0.0001$; fig 1). AF was seen in 11.5% of patients with NIHSS scores of 0–6, 17.4% of patients with scores of 7–10, 32.5% of patients with scores of 11–15, 46.3% of patients with scores of 16–22, and 54.0% of patients with scores ⩾ 23 ($p < 0.0001$). Thus, the frequency of AF rose steeply as NIHSS scores increased. The mean (SD) and median NIHSS scores were 7.4 (8.2) and 4 in patients aged less than 45 years, 5.8 (6.2) and 4 in patients aged 45–54 years, 6.6 (6.9) and 4 in patients aged 55–64 years, 7.8 (7.6) and 5 in patients aged 65–74 years, 9.6 (8.3) and 7 in patients aged 75–84 years, and 12.3 (9.0) and 10 in patients aged 85 years or older ($p < 0.0001$). Multivariate logistic regression analysis identified female sex, increased age, AF, and history of stroke as independent factors associated with severe stroke (table 2).

Table 2 Multivariate logistic regression analysis models for probability of mild and severe neurological deficits

Variable	NIHSS score ≤6			NIHSS score ≥11		
	OR	95% CI	p Value	OR	95% CI	p Value
Female sex	0.85	0.789 to 0.914	<0.0001	1.25	1.150 to 1.356	<0.0001
Age difference of 1 year	0.97	0.970 to 0.976	<0.0001	1.03	1.025 to 1.033	<0.0001
Hypertension	1.15	1.068 to 1.230	0.0002	0.79	0.728 to 0.853	<0.0001
Diabetes mellitus	0.92	0.846 to 0.992	0.031	0.92	0.836 to 1.008	0.0728
Hypercholesterolaemia	1.22	1.109 to 1.338	<0.0001	0.77	0.687 to 0.862	<0.0001
Atrial fibrillation	0.29	0.268 to 0.318	<0.0001	4.43	4.067 to 4.828	<0.0001
Current smoking	1.09	0.985 to 1.195	0.097	0.83	0.735 to 0.929	0.001
History of stroke	0.72	0.667 to 0.772	<0.0001	1.32	1.216 to 1.433	<0.0001

CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio.

Time between stroke onset and hospital arrival

Thirty five per cent of patients were admitted within three hours of stroke onset. The cumulative frequency was 48.1% within six hours, 58.4% within 12 hours, 71.7% within 24 hours, 83.6% within 48 hours, and 91.0% within 72 hours. Both the frequency of AF and the NIHSS score at admission were higher in patients admitted within 24 hours of onset than in those admitted after 24 hours (frequency of AF: 85.6% *v* 68.1%; $p < 0.0001$; NIHSS score: mean, 9.5; SD, 8.5; median 6 *v* mean, 5.5; SD, 5.4; median, 4; $p < 0.0001$).

Treatment within 12 hours of stroke onset

When we define thrombolytic treatment as intravenous tissue plasminogen activator or intra-arterial urokinase or tissue plasminogen activator, 7.3% of the AF group and 1.3% of the non-AF group received thrombolytic treatment during the superacute phase of their stroke.

Treatment within seven days of stroke onset

Heparin was administered to 38.1% of the AF group and to 10.6% of the non-AF group ($p < 0.0001$), whereas aspirin was given to 7.0% of the AF group and to 10.5% of the non-AF group ($p < 0.0001$).

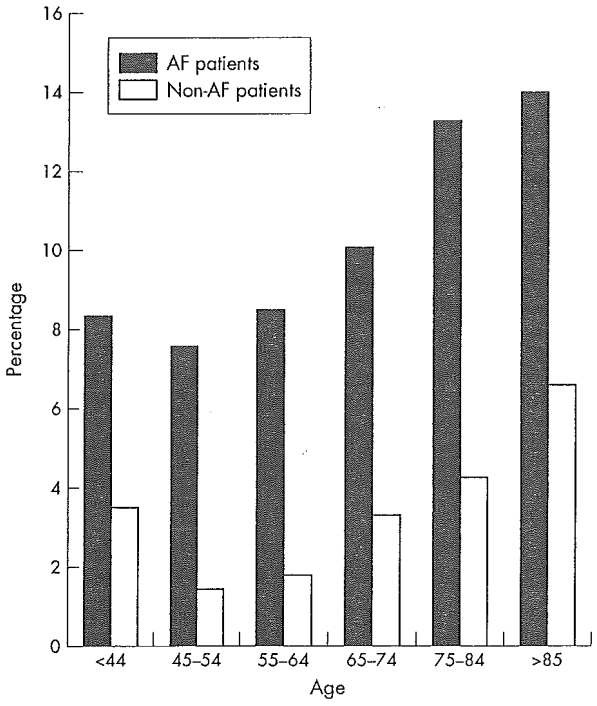

Figure 2 Death within 28 days of admission and age for patients with atrial fibrillation (AF) and non-AF patients.

Length of hospital stay

The mean (SD) length of hospital stay for all patients was 35.3 (34.1) days (median, 25; range, 1–429). The mean (SD) length of hospital stay for the AF group was 40.5 (37.8) days (median, 29; range, 0–374) and for the non-AF group 34.0 (32.9) days (median, 24; range, 0–429) ($p < 0.0001$).

Death within 28 days of admission

A total of 804 patients (5.1%) died within 28 days of admission. Early death was more frequent in patients admitted within 24 hours of onset than in those admitted after 24 hours (6.5% *v* 1.5%; $p < 0.0001$). Their mean (SD) survival time was 8.3 (7.5) days (median, 5; range, 0–28). The mortality rate was 11.3% in the AF group and 3.4% in the non-AF group ($p < 0.0001$). The mortality rate was higher in the patients with AF than in those without AF for each age group ($p < 0.0001$ for each group; fig 2). Univariate analysis showed that older age, higher NIHSS score, presence of AF, history of stroke, thrombolytic treatment, and the use of heparin were higher in the early death group (table 3). Multivariate logistic regression analysis identified age, AF, and NIHSS score at admission as independent factors associated with early death (table 3). Figure 3 shows the death rate in patients with and without AF stratified by NIHSS score group. In patients with NIHSS scores of 0–6 and 7–10, the death rate of patients with AF was higher than that of those without ($p = 0.0001$ and $p = 0.0123$, respectively). However, no significant differences were seen in those with NIHSS scores of 11–15, 16–22, and > 23. Therefore, we

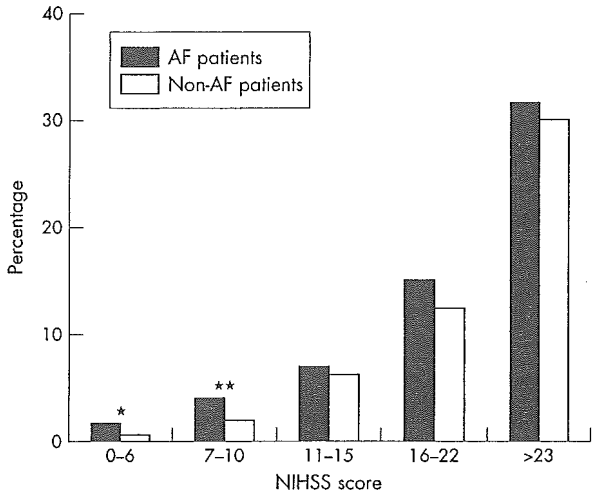

Figure 3 Death in acute stroke in patients with and without atrial fibrillation (AF), according to the severity of stroke at admission. The death rate of patients with AF is higher than that of the non-AF patients in the subgroup of patients with mild stroke.

Table 3 Multivariate logistic regression analysis models for probability of early death

	Univariate analysis			Multivariate logistic regression analysis		
	Early death					
Variable	Yes (n=804)	No (n=15004)	p Value	OR	95% CI	p Value
Female	45.9%	38.4%	<0.0001	1.00	0.841 to 1.178	0.957
Age in years (mean, SD, median)	74.8, 10.8, 75	70.4, 11.5, 71	<0.0001	1.01*	1.005 to 1.020	0.002
Hypertension	52.6%	61.7%	<0.0001	0.95	0.809 to 1.119	0.957
Diabetes mellitus	20.9%	24.9%	0.011	1.11	0.912 to 1.354	0.297
Hypercholesterolaemia	8.6%	17.7%	<0.0001	0.75	0.570 to 0.990	0.042
Atrial fibrillation	47.8%	19.6%	<0.0001	1.27	1.061 to 1.510	0.009
Current smoking	10.2%	17.7%	<0.0001	0.88	0.680 to 1.148	0.353
History of stroke	35.0%	31.1%	0.021	–	–	–
Thrombolytic treatment	17.0%	4.8%	<0.0001	1.34	1.007 to 1.795	0.045
Aspirin	1.9%	10.1%	<0.0001	0.30	0.175 to 0.503	<0.0001
Heparin	23.5%	16.0%	<0.0001	0.91	0.750 to 1.108	0.352
NIHSS score on admission (mean, SD, median)	21.5, 9.7, 22	7.6, 7.2, 5	<0.0001	1.15†	1.137 to 1.155	<0.0001

*Analysis by difference of 1 year; †analysis by difference of 1 point.
CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio.

performed multivariate logistic regression analysis for probability of early death in patients with NIHSS scores < 11 and ≥ 11. AF was an independent factor associated with early death in patients with NIHSS scores < 11, but not in those with NIHSS scores ≥ 11 (table 4).

Hospital discharge status

The proportions of patients with each mRS score at discharge in the AF and non-AF groups were as follows: 9.4% and 15.7% for score 0, 18.4% and 33.2% for score 1, 11.6% and 14.8% for score 2, 7.7% and 9.1% for score 3, 16.9% and 14.7% for score 4, 19.6% and 7.6% for score 5, and 16.4% and 5.0% for death, respectively. Good outcome was observed in 39.4% of patients with AF and in 63.7% of patients without AF, respectively ($p < 0.0001$). Multivariate logistic regression analysis showed that older age (odds ratio (OR), 1.03; 95% confidence interval (CI), 1.02 to 1.04), the presence of AF (OR, 1.3; 95% CI, 1.14 to 1.55), diabetes mellitus (OR, 1.2; 95% CI, 1.02 to 1.44), and NIHSS score at admission (OR, 1.1; 95% CI, 1.14 to 1.15) were independent factors associated with death.

Residence after hospital discharge

Sixty two per cent of all patients were discharged home and 38% were sent to an institution. Of the patients without AF, 66.4% returned to their own homes, whereas only 45.1% of patients with AF returned home ($p < 0.0001$).

DISCUSSION

Our study showed that AF was clearly associated with an increased risk of severe neurological deficits. The fact that patients with AF have more severe stroke than those without AF supports the hypothesis that the pathogenetic mechanism of stroke may be different. First, strokes in patients with AF may chiefly be cardioembolic, which causes a sudden occlusion of large cerebral arteries without sufficient collateral blood flow, resulting in more severe strokes.[1 12–14] Several studies have reported that stroke patients with AF more often have large cortical infarcts on computed tomography, and less frequently have lacunar infarction compared with patients without AF.[5 8 10 15] Second, a previous study found a significant reduction in hemispheric cerebral blood flow in patients with AF compared with those with sinus rhythm.[21] The effect of the decreased hemispheric cerebral blood flow may contribute to the development of large infarcts and neurological severity in patients with AF.

Our present study showed that older age and higher NIHSS score at admission were independent factors associated with early death, results that agreed with previous reports.[20 22 23] Furthermore, we identified AF as an independent factor for early death, in particular, for patients with NIHSS scores 0–10. Previous studies have reported that AF was associated with an increased risk of early death.[5 7 9 16] This was explained by its association with severe neurological deficits and the older age of patients with AF. However, not all investigators agreed that AF itself increased the risk of death.[8 24 25] In our present study, after adjustment for age and NIHSS score using multivariate logistic analysis, AF was identified as an independent risk factor for early death, which was compatible with the results of the Oxfordshire community stroke project.[10]

Heart diseases are more frequent in patients with AF than in those without.[26 27] Some studies have suggested that

Table 4 Multivariate logistic regression analysis models for probability of early death in patients with NIHSS <11 and ≥11

	NIHSS score <11			NIHSS score ≥11		
Variable	OR	95% CI	p Value	OR	95% CI	p Value
Age (years)	1.02*	1.004 to 1.041	0.014	1.01*	0.998 to 1.014	0.141
Hypertension	–	–	–	0.96	0.805 to 1.145	0.650
Hypercholesterolaemia	0.59	0.323 to 1.082	0.088	0.85	0.624 to 1.153	0.293
Atrial fibrillation	1.88	1.210 to 2.924	0.005	1.11	0.928 to 1.338	0.248
Current smoking	–	–	–	0.88	0.657 to 1.181	0.397
Thrombolytic treatment	3.58	1.474 to 8.677	0.005	1.18	0.885 to 1.584	0.255
Aspirin	0.52	0.242 to 1.127	0.0976	0.22	0.105 to 0.440	<0.0001
Heparin	1.26	0.776 to 2.034	0.3523	0.80	0.653 to 0.986	0.037
NIHSS score on admission	1.23†	1.153 to 1.319	<0.0001	1.11†	1.098 to 1.124	<0.0001

*Analysis by difference of 1 year; †analysis by difference of 1 point.
CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio.

cardiac causes of death in the acute phase of stroke predominate in patients with AF compared with those without AF.[28 29] Tomita *et al* reported that 77% of 2677 patients with AF had heart diseases such as hypertensive heart disease, ischaemic heart disease, valvular heart disease, sick sinus syndrome, and cardiomyopathy.[12] In our study, the mortality rate in patients with NIHSS scores of < 11 was higher in patients with AF than in non-AF patients. Death in such patients may be caused by stroke complications, such as heart diseases and pneumonia, rather than stroke itself. Careful cardiac evaluation and treatment are needed in acute stroke patients with AF even if patients' neurological deficits are mild. Furthermore, Lin *et al* reported that the one year survival rate was lower in patients with AF compared with those without AF.[30] Secondary prevention of embolic events is one of the most important issues for patients with AF.

Our present study has some limitations. First, the definition of AF in our study included both chronic AF and paroxysmal AF. The frequency of paroxysmal AF is reported to be about half that of chronic AF in Japan.[12] Thus, the presence of paroxysmal AF may not have been fully recognised in our present study. Second, we did not assess stroke recurrence and the cause of death, such as severe stroke, stroke recurrence, heart failure, renal failure, pneumonia, and pulmonary embolism. Some studies reported no difference in the frequency of stroke recurrence between acute stroke patients with and without AF,[9 22 29] whereas other studies disagreed with these findings.[31 32] Further studies are needed to investigate stroke recurrence and the cause of death related to AF. Third, Saxena *et al* reported that AF was a high risk carried early death, which could be explained by older age and large infarcts.[22] Unfortunately, we did not examine the neuroimaging findings, such as computed tomography and magnetic resonance imaging. Fourth, the NIHSS score at admission may be affected by some previous neurological deficits, particularly in patients with a history of stroke. The severity of stroke at admission in patients with AF might be estimated to be severe compared with those without AF because patients with AF more often had a history of stroke than those without AF. Finally, early aspirin use is of benefit in acute ischaemic stroke.[33] However, few patients in our study were treated with aspirin during the acute phase of stroke. The death rate in our present study might have been lower if aspirin had been used more frequently in patients in the acute phase of stroke.

In conclusion, our study showed that AF was a predictive factor for both severe stroke and early death in acute ischaemic stroke. Therefore, careful cardiac evaluation and appropriate treatment are needed to improve the outcome in patients with acute stroke and AF.

ACKNOWLEDGEMENTS

This study was supported by Health Sciences Research Grants (1998–2000) from the Ministry of Health, Labour and Welfare, Japan.

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Competing interests: none declared

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Original Paper

Cerebrovascular Diseases

Cerebrovasc Dis 2005;19:171–178
DOI: 10.1159/000083252

Received: February 12, 2004
Accepted: September 7, 2004
Published online: January 11, 2005

Mortality and Cause of Death after Hospital Discharge in 10,981 Patients with Ischemic Stroke and Transient Ischemic Attack

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Key Words
Brain infarction · Transient ischemic attack · Stroke, acute · Stroke management

Abstract
Background: The aim of this study was to examine the 1-year cumulative mortality rate and cause of death, and to identify the predictive factors for death after hospital discharge following ischemic stroke and transient ischemic attack (TIA) using data from the Japan Multicenter Stroke Investigators' Collaboration study. ***Methods:*** We prospectively registered 16,922 consecutive patients with acute ischemic stroke or TIA from May 1999 to April 2000 in 156 Japanese hospitals. We mailed a questionnaire to the 15,322 patients who were alive at hospital discharge. ***Results:*** 10,981 patients (6,945 men, 4,036 women, age 70 ± 11 years, median 71, range 19–100 years) were enrolled in the follow-up study. The mean follow-up period was 271 ± 110 days (median 272 days; range 1–487 days). The 1-year cumulative mortality was 6.8% (7.0% for 10,234 stroke patients and 3.5% for 747 TIA patients). The causes of death were: cerebrovascular disease, 24.1%; pneumonia, 22.6%; heart disease, 18.1%; cancer, 11.0%, and miscellaneous causes, 24.1%. Multivariate analysis suggested that male gender, age, diabetes mellitus, atrial fibrillation, history of stroke, nonlacunar stroke, functional disability and transfer to another hospital or nursing home on discharge were significant independent predictors of death during the follow-up period. ***Conclusions:*** The major causes of death after hospital discharge were found to be cerebrovascular diseases, pneumonia and heart diseases. Thus, in order to improve survival after hospital discharge, in addition to appropriate management of vascular risk factors following stroke, it appears to be important to take measures to prevent pneumonia and to discharge patients to their own home, if possible.

Stroke is the third leading cause of death and a major cause of long-term disability in Japan. Stroke mortality has gradually but markedly decreased during the past 3 decades [1]. In western countries, the 1-year survival rate after ischemic stroke has been reported to be 50–70% [2–9]. Many investigators have shown that older age, a greater degree of functional disability, a nonlacunar stroke, the presence of heart diseases, diabetes mellitus (DM), atrial fibrillation (AF) and a history of stroke or transient isch-

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emic attack (TIA) may increase the risk of death after a stroke [4, 5, 7, 10, 11]. Unfortunately, in Japan, there is a paucity of reliable follow-up information about mortality and the cause of death following an acute ischemic stroke.

With the cooperation of 156 hospitals we conducted a large prospective hospital-based registration study to develop an acute ischemic stroke/TIA database [12, 13]. Several studies have demonstrated that the risk of mortality after stroke is highest during the first year, and that, thereafter, the risk remains almost constant [2, 3, 5, 7–9, 11]. Therefore, we examined the 1-year mortality rate and cause of death, and identified the factors that could be used as predictors of death occurring after an ischemic stroke or TIA.

Subjects and Methods

Of 16,922 patients with an acute ischemic stroke or TIA admitted within 7 days of onset, who were consecutively registered in 156 hospitals during the period from May 1999 to April 2000, 1,177 patients died during admission [12, 13]. Twelve of the 156 initially collaborating hospitals declined to participate in the follow-up study, and thus the 423 patients from these hospitals were excluded from the follow-up study. Therefore, 15,322 patients were enrolled in the study. To identify the factors that could predict death within 1 year after stroke, we used data recorded during hospitalization [12, 13]. The data included: (1) age and gender; (2) history of stroke; (3) NIH stroke scale (NIHSS) score on admission; (4) stroke subtype (clinical categories of ischemic stroke); (5) vascular risk factors, including hypertension, DM, hypercholesterolemia, current cigarette smoking and AF; (6) functional outcome at hospital discharge, and (7) residence after hospital discharge.

Clinical categories (stroke subtypes) were defined using clinical and radiographic diagnostic rubrics according to the 'Classification of Cerebrovascular Diseases III' developed by the National Institute of Neurological Disorders and Stroke [14]. Stroke subtypes included lacunar, atherothrombotic, cardioembolic and other strokes. The functional outcome at the time of hospital discharge was evaluated using the modified Rankin scale (mRS) [15]. Residence after hospital discharge was categorized into 2 groups, either own home or an institution (including nursing home and another hospital for chronic rehabilitation and medical management).

The following risk factors were studied: hypertension (defined as the use of antihypertensive agents, a systolic blood pressure reading ≥160 mm Hg or a diastolic blood pressure reading ≥95 mm Hg before stroke onset or 2 weeks after stroke onset); DM (defined as the use of oral hypoglycemic agents or insulin, or a glycosylated hemoglobin level ≥6.4%); hypercholesterolemia (defined as the use of antihyperlipidemic agents or a serum cholesterol level ≥220 mg/dl); current cigarette smoking, and potential cardiac sources of emboli (including nonvalvular AF, acute myocardial infarction, old myocardial infarction with intraventricular thrombus, mitral valve disease, prosthetic cardiac valve, pacemaker and dilated cardiomyopathy).

During the study, the central office reported the names and registration numbers of patients enrolled in the follow-up study to the doctor in charge of each participating hospital. To obtain patients' follow-up information as of September 1, 2000, the doctors in charge mailed a questionnaire to all participating patients. Responses from the patients or their families were collected by the doctor in charge of each hospital. They deleted the patient's name and address from the data sheet to protect patient privacy and sent completed forms to the central office by September 30, 2000. The follow-up information that was collected included: (1) whether the patient was alive or dead and, if dead, the date and cause of death; (2) the patient's level of activities of daily living. The cause of death was classified into five groups: cerebrovascular diseases; cancer; heart disease; pneumonia, and miscellaneous causes. The level of disability was evaluated by the patients and their families using the mRS.

Statistical analyses were performed with a commercially available software package (StatView, version 4.5; SAS Institute, Cary, N.C., USA). The Kaplan-Meier method was applied to estimate the survival rate and to determine the 1-year cumulative mortality rate for all patients and subgroups. We examined a variety of factors associated with death by using a univariate analysis. A multivariate analysis using the Cox proportional hazard model was also performed to identify the independent risk factors for death and to calculate the hazard ratios. The risk factors included in the multivariate analysis were gender, age, hypertension, DM, hypercholesterolemia, current cigarette smoking, history of stroke, mRS score at hospital discharge, stroke subtype and residence after hospital discharge. These factors were used as covariates in the analyses. The Mann-Whitney U test or the Kruskal-Wallis test was applied to detect differences in age among the subgroups. The frequency of the stroke subtypes and an association between causes of death and the mRS score or residence after hospital discharge was assessed by the χ^2 test. A p value of <0.05 was considered statistically significant.

Results

Of the 15,322 patients contacted for the follow-up study, we received replies from 11,266 patients (73.5%). We excluded 285 incomplete patients' replies. Thus, the data from 10,981 patients (71.7%) – 6,945 men (63.2%), 4,036 women (36.8%) – were used for the analysis. Table 1 shows the baseline clinical characteristics of the enrolled and nonenrolled patients. The enrolled patients' age (mean ± standard deviation) was 70.4 ± 11.1 years (median 71 years; range 19–100 years). Women (73.2 ± 11.4 years; median 75 years; range 20–100 years) were older than men (68.8 ± 10.6 years; median 70 years; range 19–100 years; $p < 0.0001$). The follow-up period for all patients was 271 ± 110 days (median 272 days; range 1–487 days).

Cardiovascular risk factors in the enrolled subjects included: hypertension in 60.0%; DM in 24.0%; hypercholesterolemia in 18.1%; AF in 18.3%, and current smoking in 18.0%. As well, 29.7% of the patients had a

Table 1. Baseline characteristics of enrolled and nonenrolled patients

Characteristics	Nonenrolled patients (n = 4,764)	Enrolled patients (n = 10,981)	p value
Mean age, years	69.9 (12.4)	70.3 (11.1)	0.321
Male gender, %	59.6	63.2	<0.0001
History of stroke, %	30.9	29.7	0.149
Risk factors, %			
Hypertension	60.4	62.0	0.487
DM	25.7	24.0	0.022
Hyperlipidemia	15.8	18.1	0.001
AF	20.2	18.3	0.007
Smoking	18.4	17.9	0.490
NIHSS score at admission			<0.0001
Median	7	5	
Mean	7.8 (7.5)	6.8 (6.6)	
Stroke subtype, %			0.001
Lacunar stroke	36.6	39.5	
Atherothrombotic	31.0	31.3	
Cardioembolic	19.5	17.1	
Other	5.9	5.3	
TIA	7.1	6.8	
mRS score at discharge, %			<0.0001
mRS 0	18.7	20.4	
mRS 1	28.2	32.2	
mRS 2	13.2	14.8	
mRS 3	9.5	8.7	
mRS 4	17.4	14.6	
mRS 5	12.9	9.1	
Institution after hospital discharge	45.7	31.0	<0.0001

1,177 patients who died during admission were excluded. Figures in parentheses indicate SD.

history of previous stroke. Lacunar stroke (39.5%) was the most frequent stroke subtype, followed by atherothrombotic (31.3%), cardioembolic (17.1%) and other strokes (5.3%). TIA was diagnosed in 6.8% of the patients.

The distribution of the mRS scores at hospital discharge was as follows: 20.4% of patients scored 0; 32.2% scored 1; 14.8% scored 2; 8.7% scored 3; 14.6% scored 4, and 9.1% scored 5. When functional disability was classified into 2 categories, namely mRS of 0–2 indicating independence and 3–5 indicating dependence, 7,410 patients (67.6%) were independent, while 3,553 (32.4%) were classified as dependent. Figure 1 shows the relationship between the NIHSS score on admission and the mRS score at hospital discharge.

Sixty-nine percent of patients were discharged home, and 31% were sent to an institution. Of the patients with mRS scores 0–2, 90.6% returned to their own home, while only 24.5% of patients with mRS scores 3–5 returned home. Among patients with mRS scores 0–2, the patients who returned to their own home were slightly younger (68.3 ± 10.7 years; median 69 years; range 19–100 years) than those who were discharged to an institution (69.5 ± 10.7 years; median 70 years; range 30–100 years; p = 0.0105). However, among patients with mRS scores 3–5, no difference in age was observed between those discharged to their own home (74.9 ± 10.4 years; median 76 years; range 21–98 years) and those sent to an institution (74.6 ± 10.7 years; median 76 years; range 21–100 years; p = 0.6429).

A total of 604 patients died during the follow-up period. Overall, the 1-year cumulative mortality rate after discharge was 6.8 ± 0.3% (mean ± SEM), i.e. 7.0 ± 0.3% for the 10,234 stroke patients and 3.5 ± 0.8% for the 747 TIA patients. Table 2 shows the 1-year cumulative mortality rate by gender and age groups. The mortality rate increased progressively with age, reaching a maximum at

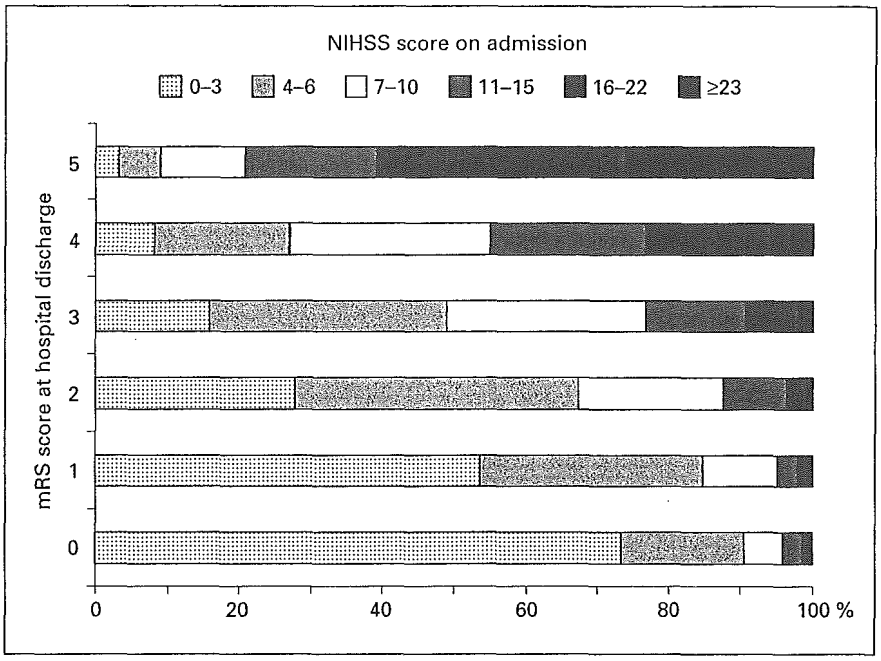

Fig. 1. NIHSS score on admission and mRS at hospital discharge.

Table 2. One-year cumulative mortality rates risk (±SD) by gender and age

Age	Male	Female	p value
≤59 years	1.7±0.4	1.0±0.5	0.3143
60–69 years	3.8±0.5	2.4±0.7	0.0562
70–79 years	6.9±0.6	5.7±0.7	0.4964
≥80 years	18.0±1.5	14.4±1.3	0.0331

80 years and older in both men and women; it was higher in men than in women (p = 0.0331) in patients aged 80 years and older.

The most frequent causes of death were cerebrovascular diseases (24.1%) followed by pneumonia (22.6%), heart diseases (18.1%), cancer (11.0%) and miscellaneous causes (24.1%).

We compared the mortality rate among stroke subtypes; the 1-year cumulative mortality was highest in cardioembolic stroke patients (12.5%) as compared to those with the remaining subtypes (4.0% in lacunar stroke, 7.8% in atherothrombotic stroke, 8.1% in other and 3.0% in TIA, $p < 0.001$).

Patients with an mRS score of 5 at discharge showed a markedly higher mortality (25.3%) compared to those with other scores (1.6% in those who scored 0; 1.9% in those who scored 1; 3.4% in those who scored 2; 4.5% in those who scored 3; 9.2% in those who scored 4; $p < 0.0001$). The cumulative survival rate was higher in patients with mRS scores of 0–2 than in those with mRS scores of 3–5.

At the time of follow-up, 15.5% of patients had an mRS score of 0; 28.9% of patients had a score of 1; 14.1% had a score of 2; 12.4% had a score of 3; 12.6% had a score of 4, and 11.0% had a score of 5. Comparing the mRS scores at follow-up with those at discharge, the number of patients who were independent (mRS 0–2) decreased from 67.6 to 58.5%, and those who were dependent (mRS 3–5) increased from 32.4 to 36.0%. Follow-up mRS scores improved in 10.8% of patients, remained unchanged in 63.6%, and deteriorated in 25.6% of the patients as compared to the mRS scores at discharge (death was assigned an mRS score of 6).

There was a significant difference in the cause of death between patients with mRS scores of 0–2 and those with 3–5 at hospital discharge ($p < 0.0001$; fig. 2). For patients with mRS scores of 0–2, the most frequent cause of death was cancer (23.1%), while for those with mRS scores of 3–5, it was pneumonia (25.5%).