ORIGINAL RESEARCH ARTICLE

Drug Safety 2009; 32 (8): 675-690
0114-5916/09/0008-0675/$49.95/0

Thiazolidinediones and Cardiovascular Events in Patients with Type 2 Diabetes Mellitus

A Retrospective Cohort Study of over 473 000 Patients Using the National Health Insurance Database in Taiwan

Fei-Yuan Hsiao,[1,2] *Weng-Foung Huang*,[1] *Yu-Wen Wen*,[3] *Pei-Fen Chen*,[3] *Ken N. Kuo*[3] and *Yi-Wen Tsai*[3,4]

1 Institute of Health and Welfare Policy/Center for Health and Welfare Policy Research, National Yang-Ming University, Taipei, Taiwan
2 Pharmaceutical Health Services Research Department, University of Maryland School of Pharmacy, Baltimore, Maryland, USA
3 Center for Health Policy Research and Development, National Health Research Institutes, Mioali, Taiwan
4 Institute of Health and Welfare Policy, National Yang-Ming University, Taipei, Taiwan

Abstract

Background and objective: Concern has been expressed over the cardiovascular risks associated with rosiglitazone and pioglitazone. This study investigates the association between oral antihyperglycaemics (rosiglitazone, pioglitazone, sulfonylureas and metformin) with myocardial infarction, congestive heart failure, angina pectoris, stroke and transient ischaemic attack.

Methods: We used Taiwan's 2000–5 National Health Insurance database to conduct a population-based, retrospective cohort study of 473 483 newly diagnosed patients with type 2 diabetes mellitus. We classified study patients into five basic groups based on the agents they were prescribed during the study period: (i) rosiglitazone monotherapy; (ii) pioglitazone monotherapy; (iii) sulfonylurea-based therapy; (iv) metformin-based therapy; and (v) sulfonylurea and metformin-based therapy. Cox proportional hazards models were used to evaluate the association between the use of rosiglitazone or pioglitazone and the occurrence of cardiovascular events.

Results: Patients receiving rosiglitazone monotherapy were at higher risk for any cardiovascular event (hazard ratio [HR] 1.89; 95% CI 1.57, 2.28), myocardial infarction (HR 2.09; 95% CI 1.36, 3.24), angina pectoris (HR 1.79; 95% CI 1.39, 2.30) and transient ischaemic attack (HR 2.57; 95% CI 1.33, 4.96) than those receiving metformin monotherapy. Overall, add-on rosiglitazone and pioglitazone were associated with comparable cardiovascular risk. Based on our point estimates, pioglitazone as an add-on therapy was found to have a favourable, but nonsignificant, effect on outcome.

Conclusions: Our findings extend the evidence from current literature to a real-world setting and support data from clinical trials that the disadvantages or harm caused by thiazolidinediones, especially rosiglitazone, may outweigh their benefits in patients with type 2 diabetes.

Background

The thiazolidinediones (TZDs), oral antihyperglycaemic agents that act as insulin sensitizers, improve glycaemic control by reducing the body's resistance to insulin.[1] However, results from a recent meta-analysis,[2] together with results from an unplanned interim analysis of data from an ongoing clinical trial,[3] and new US FDA warnings about a possible increased risk of myocardial infarction from the use of rosiglitazone[4] and pioglitazone,[5] have caused much concern regarding possible adverse cardiac effects of TZDs. The concern is well justified as cardiovascular disease is one of the most important causes of morbidity and mortality among patients with type 2 diabetes mellitus.[6]

There were early debates about the safety of TZDs, particularly for patients with congestive heart failure (CHF). In 2001, the US FDA issued warnings that TZDs (rosiglitazone and pioglitazone) may cause fluid retention in patients with advanced heart failure (New York Heart Association [NYHA] class III or IV).[7-9] Later in 2003, a statement made jointly by the American Heart Association and the American Diabetes Association also recommended that TZDs should not be prescribed to diabetic patients with severe heart failure (NYHA class III or IV).[10] A meta-analysis of pooled data from randomized trials[11] and previous observational studies[12-15] also indicated that patients receiving TZDs were at increased risk for developing CHF. In 2007, a black-box warning was issued by the US FDA to warn patients with pre-existing CHF against the use of TZDs.[4,5]

In addition to CHF, recent studies, especially meta-analyses, have raised the concerns about potential increased risk of cardiovascular events in diabetic patients treated with TZDs. Meta-analyses by Nissen and Wolski,[2] Singh et al.[16] and Psaty and Furberg[17] have all suggested that the use of rosiglitazone puts diabetic patients at increased risk of myocardial infarction (MI), whereas a meta-analysis by Lincoff et al.[18] indicated that pioglitazone had a favourable effect on ischaemic cardiovascular events. Debate was first centered on whether the conclusions of these meta-analyses provided adequate and reliable answers to the risk of harmful cardiovascular events since they had several limitations, including misclassification of cardiovascular endpoints, relatively short follow-up and lack of patient-level data.[19-20] On the other hand, to date most studies examining the adverse cardiovascular outcomes associated with TZDs have been clinical trials. How their results apply to day-to-day clinical practice is not clear.

Using secondary-data analysis to assess the effects of TZD on a 'real-world' population, Lipscombe et al.[21] and Winkelmayer et al.[22] conducted studies to evaluate the association between TZDs and risk of adverse cardiovascular events. Their studies provided valuable evidence regarding the safety of using TZDs in the treatment of type 2 diabetes in an elderly population. However, questions on the effects of long-term use of TZDs in younger patients (<65 years of age) or in patients with newly diagnosed diabetes were left unanswered. Like most studies involving TZDs, the effects of dose and duration of TZD medication on cardiovascular events are left unanswered. In addition, a conclusive direct head-to-head comparison between rosiglitazone and pioglitazone has not been reported. Together, these limitations indicate a great need for more information on the safety of TZDs and a need for a comparison of the relative cardiovascular risk posed by the two TZDs.

Using Taiwan's National Health Insurance (NHI) claims database, we conducted a 6-year (2000–5), population-based, retrospective cohort

study of patients newly diagnosed with type 2 diabetes to investigate the association between four oral antihyperglycaemic therapies (rosiglitazone, pioglitazone, sulfonylurea and metformin) on MI, congestive heart failure (CHF), angina pectoris, stroke and transient ischaemic attack (TIA). We specifically designed and conducted this study to evaluate differences in cardiovascular events in users of rosiglitazone and pioglitazone.

Methods

Study Design

We classified type 2 diabetic patients into five groups based on the agents they were mainly prescribed during the study period: (i) rosiglitazone monotherapy; (ii) pioglitazone monotherapy; (iii) sulfonylurea-based therapy; (iv) metformin-based therapy; and (v) sulfonylurea+metformin-based therapy (figure 1). We compared the adverse cardiovascular events across four monotherapy groups: rosiglitazone, pioglitazone, sulfonylurea alone (without add-on rosiglitazone or pioglitazone), and metformin alone (without add-on rosiglitazone or pioglitazone). We further estimated the dose-response relationship of exposure to the antihyperglycaemic agents and cardiovascular events across these four monotherapy groups (figures 2–4). Defined daily dose (DDD) was used to quantify exposure to oral antihyperglycaemic agents to provide more reliable evidence on the association between TZD use and risk of cardiovascular events. We also compared the risk of cardiovascular events for use of rosiglitazone or pioglitazone as add-ons within sulfonylurea-based, metformin-based and sulfonylurea+metformin-based groups.

Study Population and Cohort Definition

Patients with type 2 diabetes were identified from Taiwan's NHI claims database, which covers nearly 99% of Taiwan's population. The inclusion criteria were newly diagnosed type 2 diabetic patients who had their first ambulatory visits with a diagnosis of diabetes (International Classification of Diseases [9th edition], Clinical Modification [ICD-9-CM] codes: 250.xx) and were prescribed oral antihyperglycaemic agents (sulfonylurea, metformin and/or a TZD) at least three times between 1 March 2001 and 31 December 2005 (n = 473 483). The cohort entry date (index date) for each patient was defined as the date that an oral antihyperglycaemic agent was first prescribed. None of these patients had records showing a diagnosis of diabetes during the year before the index date. Patients were excluded if they had type 1 diabetes (ICD-9-CM codes: 250.x1) or if they had been prescribed insulin only during the study period.

Study Outcomes

Study outcomes of interest were MI, CHF, stroke, angina pectoris, TIA and the composite outcome of any of these five events. All events were defined according to the diagnostic code of hospitalization: MI (ICD-9-CM codes 410.xx and 411.xx), CHF (428.xx, 402.01, 402.11, 402.91, 404.01, 404.11 and 404.xx), stroke (433.xx and 434.xx), angina pectoris (413.xx and 414.xx) and TIA (435.xx and 437.1). For each patient, the primary endpoint was time to the first hospitalization for the cardiovascular event. All patients were followed until they experienced a cardiovascular event or until the end of the study (31 December 2005), whichever came first.

Exposure to Oral Antihyperglycaemic Agents

We identified four hypoglycaemic drugs covered by Taiwan's NHI: rosiglitazone, pioglitazone, sulfonylurea and metformin. Together, they accounted for 93.21% of the total prescriptions of antihyperglycaemic agents during the study period. Although we were primarily interested in comparing the effects of exposure to rosiglitazone or pioglitazone on cardiovascular events, we included prescription patterns of non-TZD antihyperglycaemic agents because the prescriptions for patients with type 2 diabetes were complicated. A very high proportion of our study population were found to be using rosiglitazone or pioglitazone as add-ons with other oral

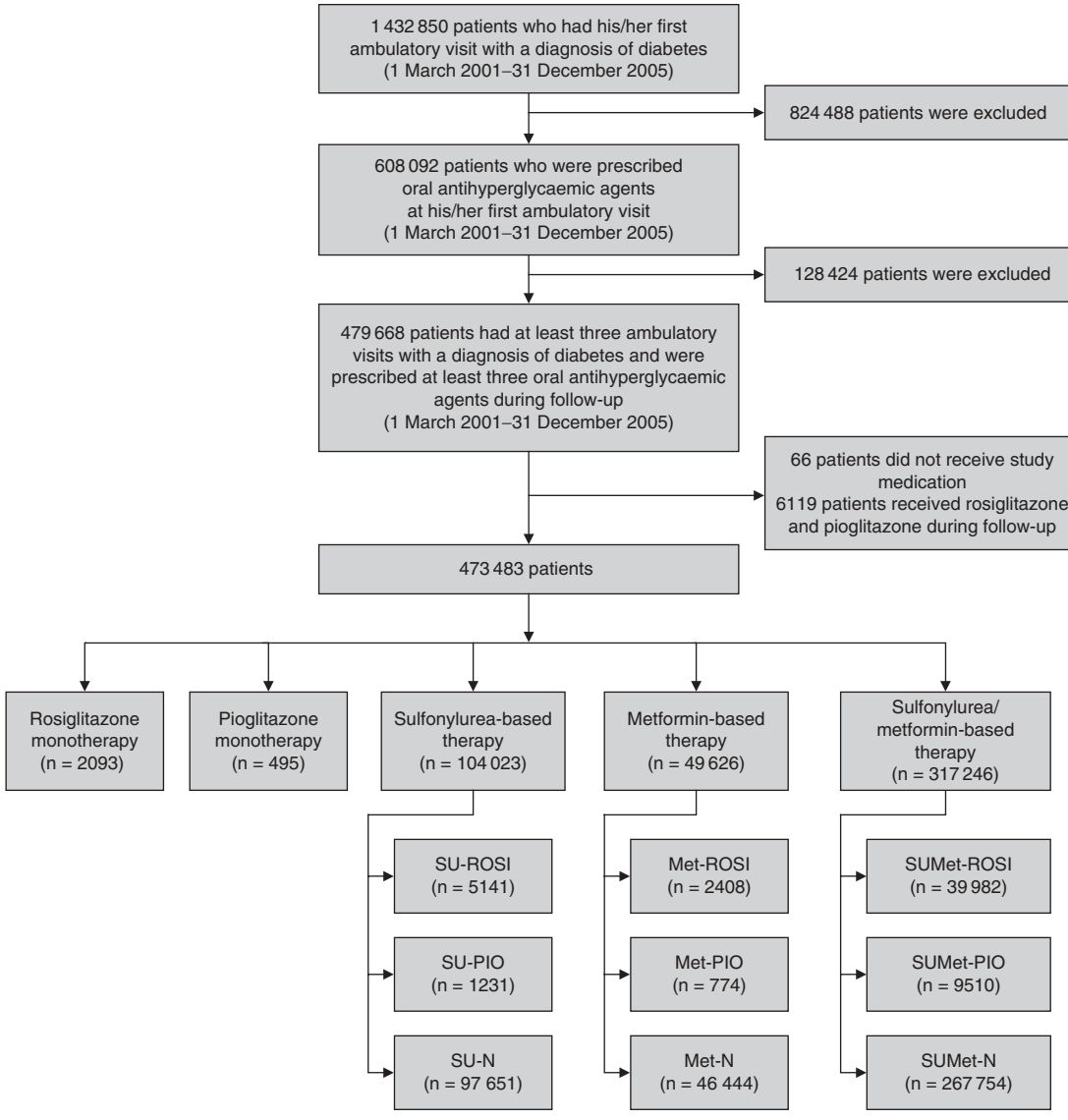

Fig. 1. Patient enrolment and classification. 473 483 newly diagnosed type 2 diabetic patients were classified into five basic groups based on the agents they were prescribed during the study period: (i) rosiglitazone monotherapy; (ii) pioglitazone monotherapy; (iii) sulfonylurea-based therapy; (iv) metformin-based therapy; and (v) sulfonylurea+metformin based therapy. Patients using rosiglitazone or pioglitazone as add-ons within SU-, Met- and SUMet - based therapy were also identified. **Met-N** = metformin without rosiglitazone or pioglitazone; **Met-PIO** = metformin and pioglitazone; **Met-ROSI** = metformin and rosiglitazone; **SU-N** = sulfonylurea without rosiglitazone and pioglitazone; **SU-PIO** = sulfonylurea and pioglitazone; **SU-ROSI** = sulfonylurea and rosiglitazone; **SUMet-N** = sulfonylurea and metformin without rosiglitazone and pioglitazone; **SUMet-PIO** = sulfonylurea, metformin and pioglitazone; **SUMet-ROSI** = sulfonylurea, metformin and rosiglitazone.

antihyperglycaemic agents (mostly a sulfonylurea and/or metformin) [figure 1].

The exposure groups were classified by pattern of antihyperglycaemic agent prescription from the index date to the end of follow-up (study period): (i) rosiglitazone monotherapy, meaning that the patients had been prescribed rosiglitazone alone during the study period; (ii) pioglitazone

monotherapy, meaning that the patients had been prescribed pioglitazone during the study period; (iii) sulfonylurea-based therapy, meaning that the patients had been prescribed sulfonylurea during the study period, including those who had ever been prescribed rosiglitazone or pioglitazone and those who had never been prescribed a TZD; (iv) metformin-based therapy, meaning that the patients had been prescribed metformin during the study period, including those who had been prescribed a TZD (metformin+rosiglitazone or metformin+pioglitazone) and those who had never been prescribed a TZD; and (iv) sulfonylurea+metformin-based therapy, meaning that the patients had been prescribed both metformin and a sulfonylurea, but not necessarily simultaneously. Subjects in this group could also include those who had ever been prescribed a TZD during the study period (sulfonylurea+metformin+rosiglitazone, sulfonylurea+metformin+pioglitazone) and those who had never been prescribed a TZD (sulfonylurea+metformin without a TZD). Those who have switched between rosiglitazone and pioglitazone or had a combined use of rosiglitazone and pioglitazone, regardless of whether they were monotherapy or add-on users, were excluded in our study to avoid a carry-over effect.

We established a prescription profile that listed each antihyperglycaemic agent prescribed to the patient during the study period. The profile included accumulated days and accumulated dosage of each antihyperglycaemic agent. The dosage of oral antihyperglycaemic agents was quantified by calculation of a defined daily dose (DDD) as defined by the WHO[23] DDD is the average maintenance daily dose for a drug used for its main indication and may not necessarily reflect the recommended or prescribed daily dose. Accumulated DDDs of each antihyperglycaemic agent during the follow-up for each study subject was used as a continuous variable in the statistical models to measure the exposure to the antihyperglycaemic agents more precisely.

Co-variates

We adjusted for potential confounders by including in our analysis the following variables:

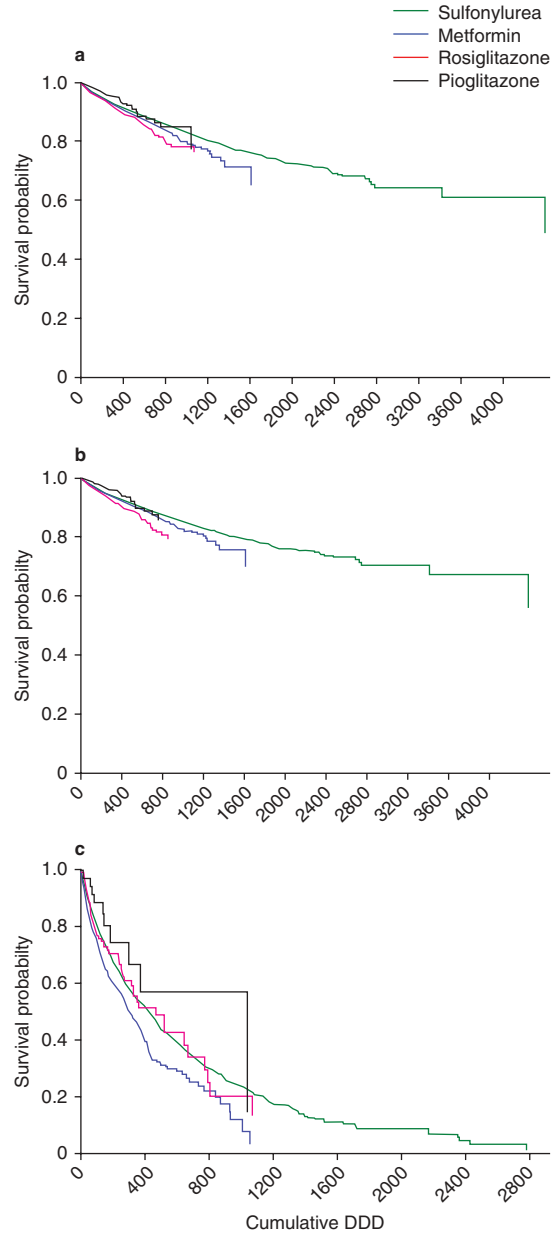

Fig. 2. Survival analysis of any cardiovascular event vs accumulated exposure (defined daily doses [DDDs]) to antidiabetic monotherapy shows the (**a**) survival curves for all monotherapy users; (**b**) curves for those with no prior history of the same cardiovascular event; (**c**) curves for those with a prior history of the same cardiovascular event. The survival probability curves for patients with a history of any cardiovascular event (figure 2c) dropped rapidly for all four monotherapy groups, demonstrating that the risk for any cardiovascular event increases significantly faster among those with a prior history of that event.

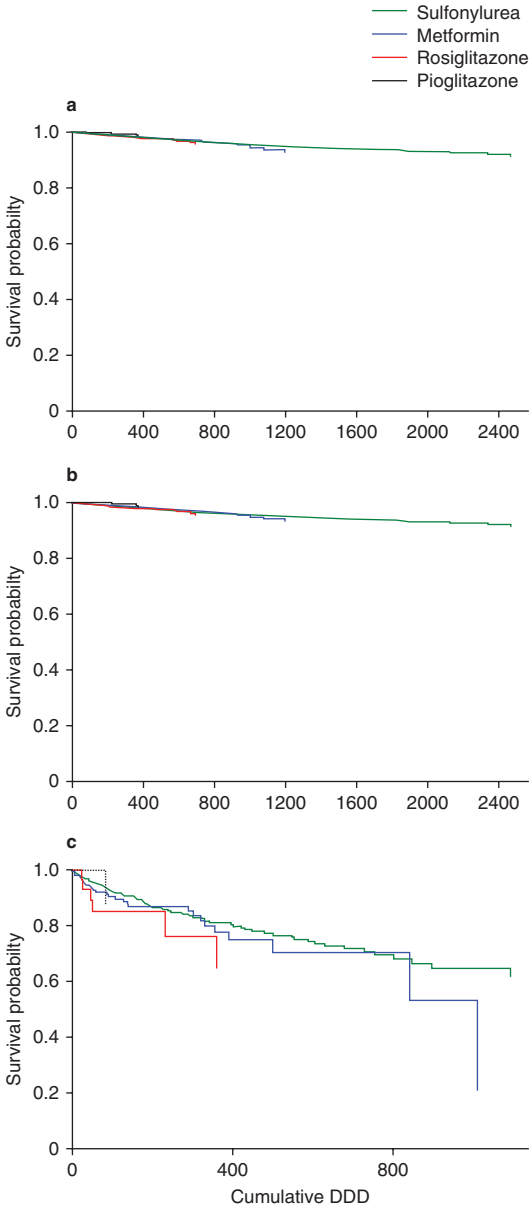

Fig. 3. Survival analysis of myocardial infarction vs accumulated exposure (defined daily doses [DDDs]) to antidiabetic monotherapy shows the (**a**) survival curves for all monotherapy users; (**b**) curves for those with no prior history of myocardial infarction; (**c**) curves for those with a prior history of myocardial infarction. The survival probability curves for patients with a history of myocardial infarction (figure 3c) dropped rapidly for all four monotherapy groups, demonstrating that the risk for myocardial infarction increases significantly faster among those with a prior history of this event.

patient demographics (age and sex), prior cardiovascular events, prior cardiovascular procedures (percutaneous transluminal coronary angioplasty and coronary artery bypass graft surgery), pre-existing medical conditions, and prior history of drug use (e.g. low-dose aspirin [acetylsalicylic acid], anticoagulants, ACE inhibitors/angiotensin II type 1 receptor antagonists, β-adrenergic receptor antagonists, other antihypertensive agents and lipid-lowering agents). We obtained data for pre-existing cardiovascular events from the participant's inpatient claims data submitted in the 12 months leading up to the index date. Pre-existing cardiovascular events included MI, CHF, stroke, angina pectoris and TIA. Other pre-existing medical conditions were obtained from the participant's inpatient and outpatient claims data during the 12 months leading up to the index date. They were hypertension (ICD-9-CM: 401.xx-405.xx), hyperlipidaemia (272.0x, 272.2x, 272.4x) and chronic kidney diseases (585.xx).

Statistical Analysis

Treatment group differences in patient characteristics were assessed with chi-square tests for categorical variables and t-tests for continuous variables. Crude event rates and mean time to event onset also were compared across treatment groups. Cox proportional hazards models were used to evaluate the association between being prescribed TZDs and occurrence of cardiovascular events. In the Cox proportional hazards model, the hazard for one individual is assumed to be proportional to the hazard for any other individual. Based on the study design, we first conducted survival analyses on each outcome to compare the outcome of monotherapy – rosiglitazone, pioglitazone, a sulfonylurea alone or metformin alone. We examined the association between cardiovascular risk and exposure to TZDs (accumulated DDDs) by comparing TZD monotherapies and other antidiabetic monotherapies. We compared cardiovascular risks between add-on rosiglitazone and pioglitazone within the sulfonylurea-based, metformin-based, and sulfonylurea+metformin-based groups.

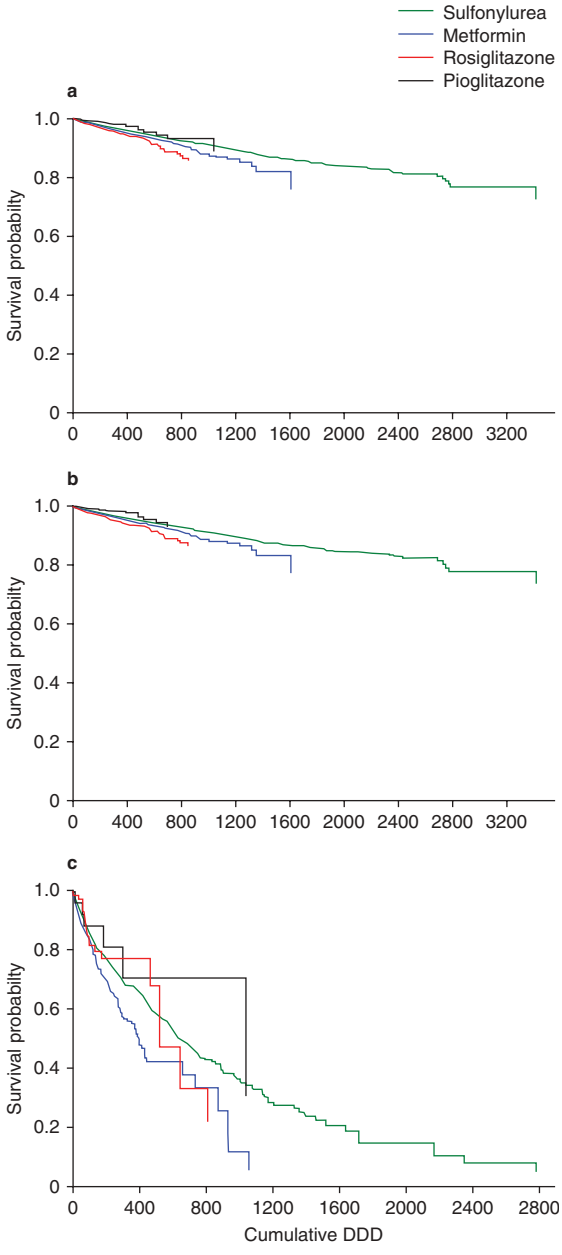

Fig. 4. Survival analysis of angina pectoris vs accumulated exposure (defined daily dose [DDDs]) to antidiabetic monotherapy shows the (**a**) survival curves for all monotherapy-users; (**b**) curves for those with no prior history of angina pectoris; (**c**) curves for those with a prior history of angina pectoris. The survival probability curves for patients with a history of angina pectoris (figure 4c) dropped rapidly for all four monotherapy groups, demonstrating that the risk for angina pectoris increases significantly more quickly among those with a prior history of this event.

All survival analyses were adjusted for patient demographics, prior medical history, prior drug use history, and insulin use during the follow-up. The relative risk comparing the use of TZDs with other antihyperglycaemic agents was estimated as a hazard ratio, with a 95% confidence interval, using the Cox proportional hazard model. A common assumption shared by such models is that the underlying hazard function is the same in each treatment group in time (t). In the analysis of cardiovascular events among TZD users, this assumption was removed by using the stratified Cox proportional hazards model, in which the underlying function is assumed to be different for each treatment group. The results of stratified Cox proportional hazards models for monotherapy users are shown in figures 2–4. All statistical operations were performed using S-plus 7.03 version (Insightful Inc, Seattle, WA, USA) and SAS 8.2 version (SAS Inc., Chicago, IL, USA).

Results

From the selection algorithm, we identified 473 783 newly diagnosed diabetic patients defined as people who had been first diagnosed as having type 2 diabetes during an outpatient visit during the study period and had received any of the four oral antihyperglycaemic treatments investigated herein at least three times (figure 1). Of those, 67.00% (n = 317 246) received sulfonylurea+metformin-based therapy, 21.97% (n = 104 023) received sulfonylurea-based therapy and 10.48% (n = 49 626) received metformin-based therapy (table I). Only 2093 patients (0.44%) received rosiglitazone alone, and 495 (0.10%) received pioglitazone alone. Most rosiglitazone (95.78%, n = 47 531) and pioglitazone (95.88%, n = 11 515) users had also taken either sulfonylurea or metformin during the study period. A higher proportion of those prescribed rosiglitazone alone and pioglitazone alone had a previous history of cardiovascular events compared with the other therapy groups, the most significant difference being the history of MI and angina pectoris. A much higher proportion of those taking TZDs

Table I. Baseline characteristics of 473 483 new type 2 diabetes mellitus patients, stratified by medication taken during the follow-up

	Rosiglitazone monotherapy [n (%)]	Pioglitazone monotherapy [n (%)]	Sulfonylurea-based therapy [n (%); 104 023 (21.97)]			Metformin-based therapy [n (%); 49 626 (10.48)]			Sulfonylurea + metformin-based therapy [n (%); 317 246 (67.00)]		
			w/o TZD	rosiglitazone	pioglitazone	w/o TZD	rosiglitazone	pioglitazone	w/o TZD	rosiglitazone	pioglitazone
Total	2093 (0.44)	495 (0.10)	97 651 (20.62)	5141 (1.09)	1231 (0.26)	46 444 (9.81)	2408 (0.51)	774 (0.61)	267 754 (56.55)	39 982 (8.44)	9510 (2.01)
Characteristic											
Age (y)	61.24 (13.48)	60.75 (12.78)	60.71 (12.96)	59.76 (12.83)	58.05 (12.97)	59.00 (14.46)	57.25 (14.00)	54.94 (13.63)	57.17 (12.78)	54.74 (12.39)	54.07 (12.39)
Female	974 (46.58)	47.98	44 827 (46.00)	2 390 (46.53)	575 (46.79)	24 047 (51.87)	1206 (50.27)	386 (50.00)	121 960 (45.62)	18 959 (47.49)	4 432 (46.65)
1-year prior cardiovascular events											
Any event	138 (6.59)	31 (6.26)	3 157 (3.23)	179 (3.48)	45 (3.66)	1 218 (2.62)	75 (3.11)	18 (2.33)	4 459 (1.67)	604 (1.51)	167 (1.76)
MI	35 (1.67)	8 (1.62)	845 (0.87)	46 (0.89)	14 (1.14)	258 (0.56)	18 (0.75)	4 (0.52)	1 023 (0.38)	137 (0.34)	46 (0.48)
CHF	27 (1.29)	6 (1.21)	782 (0.80)	43 (0.84)	7 (0.57)	241 (0.52)	14 (0.58)	1 (0.13)	951 (0.36)	107 (0.27)	25 (0.26)
Stroke	6 (0.29)	0 (0.00)	129 (0.13)	6 (0.12)	6 (0.49)	52 (0.11)	2 (0.08)	0 (0.00)	150 (0.06)	11 (0.03)	2 (0.02)
Angina pectoris	71 (3.39)	21 (4.24)	1 382 (1.42)	78 (1.52)	21 (1.71)	605 (1.30)	43 (1.79)	14 (1.81)	1 968 (0.74)	310 (0.78)	79 (0.83)
TIA	10 (0.48)	0 (0.00)	332 (0.34)	14 (0.27)	1 (0.08)	172 (0.37)	7 (0.29)	1 (0.13)	673 (0.25)	71 (0.18)	25 (0.26)
1-year prior cardiovascular procedures											
PTCA	16 (0.76)	9 (1.82)	340 (0.35)	19 (0.37)	10 (0.81)	141 (0.30)	12 (0.50)	8 (1.03)	420 (0.16)	60 (0.15)	13 (0.14)
CABG	5 (0.24)	1 (0.20)	147 (0.15)	4 (0.08)	0 (0.00)	40 (0.09)	3 (0.12)	0 (0.00)	191 (0.07)	28 (0.07)	7 (0.07)

Continued next page

Table I. Contd

	Rosiglitazone monotherapy [n (%)]	Pioglitazone monotherapy [n (%)]	Sulfonylurea-based therapy [n (%); 104 023 (21.97)]			Metformin-based therapy [n (%); 49 626 (10.48)]			Sulfonylurea + metformin-based therapy [n (%); 317 246 (67.00)]		
			w/o TZD	rosiglitazone	pioglitazone	w/o TZD	rosiglitazone	pioglitazone	w/o TZD	rosiglitazone	pioglitazone
Total	2093 (0.44)	495 (0.10)	97 651 (20.62)	5141 (1.09)	1231 (0.26)	46 444 (9.81)	2408 (0.51)	774 (0.61)	267 754 (56.55)	39 982 (8.44)	9510 (2.01)
1-year prior medical conditions											
Hypertension	1260 (60.20)	307 (62.02)	45 252 (46.34)	2 365 (46.00)	569 (46.22)	23 613 (50.84)	1140 (47.34)	343 (44.32)	96 740 (36.13)	12 367 (30.93)	2 936 (30.87)
Hyperlipidaemia	606 (28.95)	174 (35.15)	17 256 (17.67)	975 (18.97)	255 (20.71)	12 352 (26.60)	624 (25.91)	221 (28.55)	40 520 (15.13)	5 351 (13.38)	1 435 (15.09)
Chronic kidney diseases	339 (16.20)	68 (13.74)	2 607 (2.67)	411 (7.99)	84 (6.82)	518 (1.12)	56 (2.33)	15 (1.94)	1 938 (0.72)	321 (0.80)	58 (0.61)
1-year prior drug use history											
Low-dose aspirin [acetylsalicylic acid]	669 (31.96)	152 (30.71)	17 532 (17.95)	1 017 (19.78)	227 (18.44)	9 734 (20.96)	515 (21.39)	132 (17.05)	35 294 (13.18)	4 619 (11.55)	1 099 (11.56)
Anticoagulants	31 (1.48)	7 (1.41)	1 051 (1.08)	55 (1.07)	12 (0.97)	465 (1.00)	30 (1.25)	9 (1.16)	1 880 (0.70)	242 (0.61)	64 (0.67)
ACE-Is/ARBs	942 (45.01)	236 (47.68)	23 922 (24.50)	1 438 (27.97)	356 (28.92)	13 191 (28.40)	772 (32.06)	217 (28.04)	50 005 (18.68)	6 693 (16.74)	1 663 (17.49)
β-adrenergic receptor antagonists	808 (38.60)	207 (41.82)	29 020 (29.72)	1 578 (30.69)	403 (32.74)	15 146 (32.61)	736 (30.56)	210 (27.13)	63 215 (23.61)	8 291 (20.74)	1 949 (20.49)
Antihypertensive drugs	275 (13.14)	64 (12.93)	10 859 (11.12)	517 (10.06)	112 (9.10)	4 693 (10.10)	223 (9.26)	70 (9.04)	23 404 (8.74)	2 909 (7.28)	613 (6.45)
Lipid-lowering agents	714 (34.11)	181 (36.57)	12 768 (13.08)	858 (16.69)	236 (19.17)	8 830 (19.01)	509 (21.14)	162 (20.93)	25 357 (9.47)	3 671 (9.18)	964 (10.14)

ACE-Is/**ARBs** = ACE inhibitors/angiotensin II type 1 receptor antagonists; **CABG** = coronary artery bypass graft surgery; **CHF** = congestive heart failure; **MI** = myocardial infarction; **PTCA** = percutaneous transluminal coronary angioplasty; **TIA** = transient ischaemic attack; **TZD** = thiazolidinediones; **w**/**o** = without.

alone also had a history of hypertension, hyperlipidaemia and chronic kidney disease.

Table II shows group differences in the numbers of patients with cardiovascular events and mean time to the occurrence of each event. The three most frequent cardiovascular events were angina pectoris, CHF and MI. Those patients who were prescribed rosiglitazone alone (12.71%) had a higher incidence of cardiovascular events than those who were prescribed pioglitazone alone (8.89%) during the study period. Overall, time to onset of any cardiovascular event was much shorter in those prescribed rosiglitazone monotherapy (mean ± SD, 342.75 ± 330.27 days) or pioglitazone monotherapy (304.68 ± 294.84 days). A greater proportion of those prescribed add-on rosiglitazone had cardiovascular events compared with those prescribed add-on pioglitazone, especially in the metformin-based therapy group.

The results of our survival analyses of the relative risks of each cardiovascular event are summarized in table III. Among those prescribed monotherapy, we compared those prescribed rosiglitazone and pioglitazone with those prescribed metformin or a sulfonylurea in separate models to adjust the potential hazard differences caused by the selection of reference group. Patients prescribed rosiglitazone monotherapy were at higher risk for any cardiovascular event (HR 1.89; 95% CI 1.29, 1.85), MI (HR 2.09; 95% CI 1.36, 3.24), angina pectoris (HR 2.05; 95% CI 1.47, 2.87) and TIA (HR 2.57; 95% CI 1.33, 4.96) than patients prescribed metformin alone. Similar findings were observed when those prescribed sulfonylurea alone were used as a reference group. All increased risks were identified only with rosiglitazone monotherapy. Head-to-head comparison of cardiovascular risks between add-on rosiglitazone and pioglitazone groups was examined within the sulfonylurea-based, metformin-based and sulfonylurea+metformin-based groups. Overall, cardiovascular risks between the add-on rosiglitazone and pioglitazone groups were comparable. The point estimate, however, suggests that add-on pioglitazone had more favourable cardiovascular event outcomes than did add-on rosiglitazone, although the difference did not reach statistical significance. Adding on of pioglitazone to metformin therapy appeared to pose a greater risk of MI (HR = 6.34; 95% CI 1.80, 22.31) than adding on rosiglitazone; however, the wide confidence interval indicated a limited statistical power for this observation.

The survival analysis of cardiovascular risks associated with the prescription of monotherapy with respect to the accumulated DDDs of each of the antihyperglycaemic therapies was stratified according to the prior history of cardiovascular events. We plotted the adjusted survival curves in three graphs (figures 2–4). The first graph for each outcome shows the survival curves for all monotherapies prescribed, the second graph is for those with a prior history of the same cardiovascular event, and the third is for those with no prior history of the same cardiovascular event. The survival curves indicate that rosiglitazone tended to increase the risk of any cardiovascular event (figure 2) and angina pectoris (figure 4). The survival curves for patients without a history of cardiovascular disease (figures 2b, 3b, 4b) shows the same pattern of survival curves as for all monotherapy users (figures 2a, 3a, 4a). Unlike previous graphs, the graphs for patients with a history of cardiovascular events (figures 2c, 3c, 4c) dropped rapidly across the four monotherapy groups. The risk increased significantly faster among those with a history of cardiovascular events. The graph showing any cardiovascular event (figure 2) suggests that the cardiovascular risk was 0.8 after more than 1000 DDD of rosiglitazone had been accumulated.

Discussion

This observational retrospective cohort study extends the research done by Lipscombe et al.[21] by expanding the study to encompass a nationwide population and adding three further cardiovascular events: stroke, angina pectoris and TIA. Using a large nationwide health insurance database, we found a greater association between rosiglitazone monotherapy and increased risk of any cardiovascular event, MI, angina pectoris, and TIA in type 2 diabetic patients compared with pioglitazone monotherapy. Our analysis was consistent with recent studies showing that

Table II. Cardiovascular events among new diabetic patients during the follow-up

	Rosiglitazone monotherapy [n (%)]	Pioglitazone monotherapy [n (%)]	Sulfonylurea-based therapy [n (%); 104 023 (21.97)]			Metformin-based therapy [n (%); 49 626 (10.48)]			Sulfonylurea + metformin-based therapy [n (%); 317 246 (67.00)]		
			w/o TZD	rosiglitazone	pioglitazone	w/o TZD	rosiglitazone	pioglitazone	w/o TZD	rosiglitazone	pioglitazone
Total	2093 (0.44)	495 (0.10)	97 651 (20.62)	5141 (1.09)	1231 (0.26)	46 444 (9.81)	2408 (0.51)	774 (0.61)	267 754 (56.55)	39 982 (8.44)	9510 (2.01)
Follow-up, day (mean ± SD)	730.71 ± 439.76	580.70 ± 352.37	810.61 ± 498.07	946.27 ± 455.92	835.16 ± 443.26	719.11 ± 479.58	885.99 ± 464.99	718.61 ± 439.78	965.33 ± 484.83	1130.76 ± 431.65	1055.72 ± 449.26
Follow-up, patient-days	1 529 385	287 448	79 157 058	4 864 766	1 028 082	33 398 198	2 133 471	556 208	258 471 516	45 210 141	10 039 872
Any cardiovascular events											
Case/subgroup population	266 (12.71)	44 (8.89)	7491 (7.67)	395 (7.68)	67 (5.44)	2507 (5.4)	150 (6.23)	30 (3.88)	11 435 (4.27)	1593 (3.98)	293 (3.08)
Time-to-onset, day (mean ± SD)	342.75 ± 330.37	304.68 ± 294.84	390.26 ± 365.72	498.59 ± 380.17	507.6 ± 399.42	382.73 ± 357.69	580.95 ± 393.79	526.3 ± 364.3	593.64 ± 423.26	752.18 ± 419.5	738.84 ± 426.95
Myocardial infarction											
Case/subgroup population	266 (12.71)	44 (8.89)	1678 (1.76)	100 (1.92)	12 (0.96)	464 (1.02)	25 (1.03)	7 (0.89)	11 435 (4.27)	1593 (3.98)	293 (3.08)
Time-to-onset, day (mean ± SD)	324.62 ± 322.3	377 ± 292.43	455.21 ± 378.9	503.26 ± 366.22	286.25 ± 227.46	443.98 ± 368.54	720.84 ± 470.33	836.57 ± 486.72	645.79 ± 431.61	802.92 ± 407.49	789.02 ± 384.87
Congestive heart failure											
Case/subgroup population	67 (3.33)	13 (2.66)	1872 (1.97)	111 (2.15)	13 (1.04)	578 (1.26)	31 (1.28)	4 (0.51)	3010 (1.12)	455 (1.11)	85 (0.86)
Time-to-onset, day (mean ± SD)	456.34 ± 407.47	251.46 ± 314.82	420.61 ± 377.26	497.86 ± 375.69	741.23 ± 462.4	400.38 ± 363.23	585.58 ± 394.9	820 ± 198.9	622.81 ± 422.17	742.24 ± 407.82	774.59 ± 393.51
Stroke											
Case/subgroup population	16 (0.80)	2 (0.41)	318 (0.34)	26 (0.50)	3 (0.24)	116 (0.25)	12 (0.49)	0 (0)	588 (0.22)	110 (0.27)	15 (0.15)
Time-to-onset, day (mean ± SD)	531.75 ± 395.12	505.5 ± 152.03	447.1 ± 391.7	655.23 ± 324.98	562.33 ± 279.3	420.05 ± 370.53	833.5 ± 418.34	0 ± 0	662.62 ± 431.02	859.79 ± 409.05	873.13 ± 445.16
Angina											
Case/subgroup population	154 (7.52)	22 (4.51)	3721 (3.87)	218 (4.22)	49 (3.93)	1367 (2.97)	103 (4.26)	19 (2.45)	5910 (2.2)	890 (2.19)	186 (1.92)
Time-to-onset, day (mean ± SD)	341.21 ± 330.46	293.82 ± 301.02	376.35 ± 361.85	569.39 ± 418.77	511.88 ± 392.57	379.24 ± 361.03	590.16 ± 374.89	427.26 ± 314.88	592.17 ± 423.46	762.02 ± 4-12.08	767.41 ± 442.97
TIA											
Case/subgroup population	23 (1.14)	5 (1.03)	940 (0.99)	38 (0.73)	6 (0.48)	285 (0.63)	11 (0.45)	7 (0.89)	1637 (0.61)	228 (0.56)	30 (0.30)
Time-to-onset, day (mean ± SD)	415.65 ± 303.35	651.2 ± 321.5	444.44 ± 379.42	530.82 ± 386.13	447.83 ± 320.29	410.7 ± 349.78	503.73 ± 431.28	640.57 ± 453.07	634.83 ± 423.7	784.46 ± 415.09	749.93 ± 411.86

TIA = transient ischaemic attack; **TZD** = thiazolidinediones; **w/o** = without.

Table III. Survival analyses of the cardiovascular events associated with rosiglitazone and pioglitazone[a]

	Any cardiovascular event		Myocardial infarction		Congestive heart failure		Stroke		Angina pectoris		TIA	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Monotherapy												
Reference group: sulfonylurea												
rosiglitazone	1.54 (1.29, 1.85)	0.0000	1.49 (0.99, 2.24)	0.0511	1.22 (0.86, 1.74)	0.2620	1.45 (0.69, 3.05)	0.3200	1.46 (1.15, 1.85)	0.0017	1.90 (1.02, 3.57)	0.0405
pioglitazone	1.03 (0.65, 1.65)	0.8897	0.72 (0.19, 2.77)	0.6313	1.37 (0.58, 3.20)	0.4632	0.59 (0.06, 6.03)	0.6501	0.91 (0.47, 1.74)	0.7678	1.28 (0.34, 4.86)	0.7138
Reference group: metformin												
rosiglitazone	1.89 (1.57, 2.28)	0.0000	2.09 (1.36, 3.24)	0.0007	1.30 (0.89, 1.89)	0.1713	1.61 (0.72, 3.62)	0.2384	1.79 (1.39, 2.30)	0.0000	2.57 (1.33, 4.96)	0.0041
pioglitazone	1.29 (0.81, 2.07)	0.2740	1.00 (0.26, 3.89)	0.9954	1.54 (0.65, 3.64)	0.3156	0.61 (0.06, 6.25)	0.6685	1.15 (0.60, 2.21)	0.6753	1.67 (0.44, 6.41)	0.4454
Sulfonylurea-based therapy												
Reference group: rosiglitazone												
pioglitazone	0.84 (0.59, 1.21)	0.3398	0.69 (0.30, 1.55)	0.3538	0.78 (0.36, 1.69)	0.5179	1.49 (0.25, 8.94)	0.6554	1.11 (0.72, 1.72)	0.6225	0.73 (0.23, 2.30)	0.5811
Metformin-based therapy												
Reference group: rosiglitazone												
pioglitazone	0.89 (0.51, 1.56)	0.6767	6.34 (1.80, 22.31)	0.0033	0.63 (0.14, 2.82)	0.5401	0.01 (0.00, >100)	0.6141	0.79 (0.39, 1.61)	0.5104	2.31 (0.59, 9.03)	0.2211
(Sulfonylurea + metformin)-based therapy												
Reference group: rosiglitazone												
pioglitazone	0.94 (0.80, 1.11)	0.4872	1.04 (0.73, 1.47)	0.8286	1.06 (0.78, 1.44)	0.6933	0.65 (0.32, 1.33)	0.2300	1.07 (0.86, 1.31)	0.5438	0.62 (0.38, 1.02)	0.0570

a All models were adjusted for demographics, prior medical conditions, prior drug use history and insulin use during the follow-up period.

HR = hazard ratio; **TIA** = transient ischaemic attack.

rosiglitazone monotherapy increased the risk of cardiovascular events.[21,24-26] These findings provide additional valuable information to clinicians, patients and the public regarding the safety of TZDs.

Although some studies have investigated the cardiovascular safety of TZDs, they have certain limitations. Previous clinical trials have generally investigated rosiglitazone or pioglitazone alone as an intervention and compared these with a placebo. However, our observational study found that only a very small proportion of the patients in our sample actually used either rosiglitazone or pioglitazone alone. Even in earlier observational studies, it was difficult to recruit patients prescribed either rosiglitazone or pioglitazone alone, especially pioglitazone, and populations of patients prescribed either rosiglitazone or pioglitazone were frequently combined as one group[24-25] For example, Johannes and colleagues[25] compared coronary heart disease risk between the two TZDs as a group and (sulfonylurea+metformin) combination therapy, and found no difference between the two groups (adjusted HR1.02; 95% CI 0.87, 1.30). In summary, direct comparisons of cardiovascular risks between rosiglitazone and pioglitazone are scarce.

Our study is a population-based observational cohort study using insurance claims data from Taiwan's NHI, which covers over 99% of the population. It thus fills a need for a more thorough and comprehensive methodological approach than has been used in most previous published studies of TZD comparisons. By using nationwide data, we could explore the extent of prescribing for the drugs concerned and the complexity of prescription patterns in the real world of clinical practice. This allowed a more in depth and thorough study design. In addition to rosiglitazone and pioglitazone, we also identified three common patterns for the prescription of oral antihyperglycaemic agents (sulfonylurea alone, metformin alone, and a combination of sulfonylurea and metformin). These were included in our research design to enable comparisons to be made with TZDs. This has more real-world relevance than comparisons using placebos. We also compared the groups prescribed rosiglitazone and pioglitazone monotherapy with those prescribed metformin or sulfonylurea monotherapy in separate models to adjust potential hazard differences caused by the selection of the reference group. Our findings suggest that metformin was possibly protective against cardiovascular events since the risk of such events with rosiglitazone monotherapy was apparently greater in the model using metformin monotherapy as the reference group. No such association was identified when comparing cardiovascular risk of rosiglitazone in the model using a sulfonylurea as the reference group. Our design refinements made this study potentially relevant to clinical practice since similar findings have been reported in other types of study.[27] Further studies are required to enable firm conclusions to be drawn concerning major clinical benefits and risks associated with the use of oral antihyperglycaemic agents.

In this study, we were aware of the complexity of prescribing medicines for type 2 diabetic patients, which involves the switching, adding on, and dosage adjustments. In order to more accurately assess the dose-dependent cardiovascular response to oral antihyperglycaemic agents, we used newly diagnosed patients to avoid the strong carry-over effect of previous antidiabetic drug usage in patients with a long history of diabetes. In addition, we stratified current patterns of prescribing oral antihyperglycaemic agents into three basic groups and further stratified three basic groups into those that had add-on rosiglitazone, add-on pioglitazone or no add-on TZD based on the drug exposure during the follow-up period. To be more specific in studying cardiovascular risk, we compared the risks between add-on rosiglitazone or pioglitazone within the sulfonylurea-based, metformin-based and sulfonylurea+metformin-based groups. Since add-on TZDs accounted for a large proportion of our study subjects, had we not considered the add-on prescription pattern our results would have very limited relevance in real-world settings. Overall, cardiovascular risk comparisons between add-on rosiglitazone and pioglitazone within each oral antihyperglycaemic agents-based therapies were similar in our study. The point estimate, however,

suggests a nonsignificant association towards a more favourable cardiovascular effect in those prescribed add-on pioglitazone.

Our findings with rosiglitazone or pioglitazone use as monotherapy or as add-on therapy are consistent with several previous studies, such as the meta-analysis by Lincoff et al.[18] and results from the PROactive trial.[28] These studies found pioglitazone to be associated with a significantly lower risk of cardiovascular events, especially MI. Our large population-based cohort study further expands these studies in several important aspects. Firstly, these studies recruited their study subjects from clinical trials with relatively small sample sizes and short follow-up. Secondly, placebo was used as the reference group in most of the trials which, in addition, did not directly compare rosiglitazone and pioglitazone. However, recent findings from a large cohort of US seniors[22] did not find differences in rates of MI or stroke between patients initiating rosiglitazone or pioglitazone treatment. Thus, to better quantify benefit-risk trade-off associated with the two drugs, more research effort should be placed on distinguishing the differences in the risk of cardiovascular events between users of rosiglitazone and pioglitazone.

Finally, we measured exposure to each oral antihyperglycaemic agent by converting the dosage to DDD, and found that the higher the accumulated exposure to TZDs, the greater the risk of a cardiovascular event, an aspect of TZD use not investigated by previous studies.[22,24,25] The rich dataset we used enabled us to include analysis of the outcome for all type 2 diabetic patients included, with and without a prior history of cardiovascular events. This enabled us to demonstrate a dose-response increase in the risk of cardiovascular event in type 2 diabetic patients with a prior history of cardiovascular diseases and suggests that more caution is needed when prescribing TZDs to these high-risk patient groups.

This study is not without limitations. First, one of the main limitations of using claim data is the measurement error caused by using the amount of medicine prescribed as 'exposure', which presumes patients are completely compliant to the instructions of their physicians. Second, self-selection can occur when patients stop medications due to an early adverse drug event. Similarly, because medications might have been withdrawn by physicians in the early stages for high-risk users who had developed minor risk signals, we were unable to adjust for self-selection as we could not adjust for possible underestimation of the risk of either short-term or long-term use of oral antihyperglycaemic agents on adverse outcomes. Third, although we adjusted for a wide range of potential cardiovascular risk factors, we were not able to include variables not routinely captured in a claim database, such as patients' smoking history, family history of cardiovascular diseases, obesity or laboratory data. Fourth, our data did not link to a mortality database and mortality could not be included in our analysis and, finally, our sample included a relatively small number of pioglitazone users who were observed for a relatively short period of time, which may have resulted in an underestimation of risk, especially the long-term risk, of pioglitazone use. However, in contrast to an earlier study[21], our study has included a population of patients receiving pioglitazone as an add-on therapy and, to our knowledge, this is the first study to investigate this.

Conclusions

Despite limitations, our study of a nationwide diabetic population offers additional insight into the use of TZDs and risk of cardiovascular events. Rosiglitazone monotherapy was associated with an increased risk of any cardiovascular event, MI, angina pectoris and TIA. Overall, the cardiovascular risk of adding either rosiglitazone or pioglitazone to other oral antihyperglycaemic agents was comparable in our study. The point estimate, however, suggests a non-significant association towards a more favourable effect in pioglitazone users. This indicates an area where further research is needed. Our findings extend the evidence from current literature to a real-world setting and support data from clinical trials that the disadvantages or harm caused by TZDs, especially rosiglitazone, may outweigh their benefits in patients with type 2 diabetes.

Acknowledgements

We would like to thank Taiwan's National Science Council (NSC) Grant for providing a research grant (NSC-96-N-6805). We also thank the Bureau of National Health Insurance (BNHI) and National Health Research Institutes (NHRI) for making available their databases for this study. The content of this article, however, in no way represents any official position of the BNHI or NHRI. The authors accept all responsibility for the results and their interpretation. We would like to thank Dr C. Daniel Mullins for providing consultation and assistance with the revised manuscript. We would also like to thank Mr James Steed for his assistance in editing this manuscript.

This study is a component of a research grant by Taiwan's National Science Council for which Dr W.F. Huang served as the principal investigator. The authors have no conflicts of interest to declare.

References

1. Diamant M, Heine RJ. Thiazolidinediones in type 2 diabetes mellitus: current clinical evidence. Drugs 2003; 63: 1373-405
2. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 2007; 356: 2457-71
3. Home PD, Pocock SJ, Beck-Nielsen H, et al., on behalf of the RECORD Study Group. Rosiglitazone evaluated for cardiovascular outcomes-an interim analysis. N Engl J Med 2007; 357: 28-38
4. US Food and Drug Administration. Rosiglitazone maleate (marketed as Avandia, Avandamet, and Avandaryl) information, 2007 [online]. Available from URL: http://www.fda.gov/cder/drug/infopage/rosiglitazone/default.htm. [Accessed 2008 Apr 2]
5. US Food and Drug Administration. Pioglitazone HCl (marketed as Actos, Actoplus Met, and Duetact) information, 2007 [online]. Available from URL: http://www.fda.gov/cder/drug/infopage/pioglitazone/default.htm. [Accessed 2008 Apr 2]
6. Roper NA, Bilous RW, Kelly WF, et al. Excess mortality in a population with diabetes and the impact of material deprivation: longitudinal, population-based study. BMJ 2001; 322: 1389-93
7. US Food and Drug Administration. Summary for Actos and Avandia, 2002 [online]. Available from URL: http://www.fda.gov/medwatch/safety/2002/summary-actos-avandia.pdf [Accessed 2008 Apr 2]
8. Takeda Pharmaceuticals. Actos prescribing information, 2002 [online]. Available from URL: http://www.actos.com/pi.pdf [Accessed 2008 Apr 2]
9. GlaxoSmithKline Pharmaceuticals. Avandia prescribing information, 2002 [online]. Available from URL: http://us.gsk.com/products/assets/us_avandia.pdf [Accessed 2008 Apr 2]
10. Nesto RW, Bell D, Bonow RO, et al., on behalf of the American Heart Association, American Diabetes Association. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and the American Diabetes Association. Circulation 2003; 108: 2941-8
11. Lago RM, Singh PP, Nesto RW. Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. Lancet 2007; 370: 1129-36
12. Rajagopalan R, Rosenson RS, Fernandes AW, et al. Association between congestive heart failure and hospitalization in patients with type 2 diabetes mellitus receiving treatment with insulin or pioglitazone: a retrospective data analysis. Clin Ther 2004; 26: 1400-10
13. Karter AJ, Ahmed AT, Liu J, et al. Pioglitazone initiation and subsequent hospitalization for congestive heart failure. Diabet Med 2005; 22: 986-93
14. Maru S, Koch GG, Stender M, et al. Antidiabetic drugs and heart failure risk in patients with type 2 diabetes in the U.K. primary care setting. Diabetes Car 2005; 28: 20-6
15. Masoudi FA, Inzucchi SE, Wang Y, et al. Thiazolidinediones, metformin, and outcomes in older patients with diabetes and heart failure: an observational study. Circulation 2005; 111: 583-90
16. Singh S, Loke YK, Fuberg CD. Long-term risk of cardiovascular events with rosiglitazone: a meta-analysis. JAMA 2007; 298: 1189-95
17. Psaty BM, Furberg CD. The record of rosiglitazone and the risk of myocardial infarction. N Engl J Med 2007; 357: 67-9
18. Lincoff AM, Wolski K, Nicholls SJ, et al. Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials. JAMA 2007; 298: 1180-8
19. Hernandez AV, Walker E, Ioannidis JP, et al. Challenges in meta-analysis of randomized clinical trials for rare harmful cardiovascular events: the case of rosiglitazone. Am Heart J 2008; 156: 23-30
20. Diamond GA, Bax L, Kaul S. Uncertain effects of rosiglitazone on the risk for myocardial infarction and cardiovascular death. Ann Intern Med 2007; 16 147: 578-81
21. Lipscombe LL, Gomes T, Lévesque LE, et al. Thiazolidinediones and cardiovascular outcomes in older patients with diabetes. JAMA 2007; 298: 2634-43
22. Winkelmayer WC, Setoguchi S, Levin R, et al. Comparison of cardiovascular outcomes in elderly patients with diabetes who initiated rosiglitazone vs pioglitazone therapy. Arch Intern Med 2008; 168: 2368-75
23. WHO Collaborating Centre for Drug Statistics Methodology. The ATC and DDD system 2008 [online]. Available from URL: http://www.whocc.no/atcddd/ [Accessed 2008 Sep 2]
24. Gerrits CM, Bhattacharya M, Manthena S, et al. A comparison of pioglitazone and rosiglitazone for hospitalization for acute myocardial infarction in type 2 diabetes. Pharmacoepidemiol Drug Saf 2007; 16: 1065-71
25. Johannes CB, Koro CE, Quinn SG, et al. The risk of coronary heart disease in type 2 diabetic patients exposed to thiazolidinediones compared to metformin and sulfonylurea therapy. Pharmacoepidemiol Drug Saf 2007; 16: 504-12

26. McAfee AT, Koro C, Landon J, et al. Coronary heart disease outcomes in patients receiving antidiabetic agents. Pharmacoepidemiol Drug Saf 2007; 16: 711-25
27. Selvin E, Bolen S, Yeh HC, et al. Cardiovascular outcomes in trials of oral diabetes medications: a systematic review. Arch Intern Med 2008; 168: 2070-80
28. Erdmann E, Dormandy JA, Charbonnel B, et al. The effect of pioglitazone on recurrent myocardial infarction in 2, 445 patients with type 2 diabetes and previous myocardial infarction: results from the PROactive (PROactive 05). Study. PROactive Investigators. J Am Coll Cardiol 2007; 49: 1772-80

Correspondence: Dr *Yi-Wen Tsai*, Center for Health Policy Research and Development, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 350, Taiwan.
E-mail: ivytsai@nhri.org.tw