LEADING ARTICLE

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Hepatotoxicity with Thiazolidinediones
Is it a Class Effect?

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Abstract

Decreased insulin sensitivity plays a major role in various human diseases, particularly type 2 diabetes mellitus, and is associated with a higher risk of atherosclerosis and cardiovascular complications. Thiazolidinediones, more commonly termed glitazones, are the first drugs to specifically target muscular insulin resistance. They have proven efficacy for reducing plasma glucose levels in patients with type 2 diabetes mellitus treated with diet alone, sulphonylureas, metformin or insulin. In addition, they are associated with some improvement of the cardiovascular risk profile. However, troglitazone, the first compound approved by the Food and Drug Administration in the US, proved to be hepatotoxic and was withdrawn from the market after the report of several dozen deaths or cases of severe hepatic failure requiring liver transplantation.

It remains unclear whether or not hepatotoxicity is a class effect or is related to unique properties of troglitazone. Rosiglitazone and pioglitazone, two other glitazones, appear to have similar efficacy with regard to blood glucose control in patients with type 2 diabetes mellitus as compared with troglitazone. In controlled clinical trials, the incidence of significant ($\geq 3 \times$ upper limit of normal) increases in liver enzyme levels (ALT in particular) was similar with rosiglitazone or pioglitazone as compared with placebo, whereas troglitazone was associated with a 3-fold greater incidence. In contrast to the numerous case reports of acute liver failure in patients receiving troglitzone, only a few case reports of hepatotoxicity have been reported in patients treated with rosiglitazone until now, with a causal relationship remaining uncertain. Furthermore, no single case of severe hepatotoxicity has been reported yet with pioglitazone. It should be mentioned that troglitazone, unlike pioglitazone and rosiglitazone, induces the cytochrome P450 isoform 3A4, which is partly responsible for its metabolism, and may be prone to drug interactions. Importantly enough, obesity, insulin resistance and type 2 diabetes mellitus are associated with liver abnormalities, especially non-alcoholic steatohepatitis, independent of any pharmacological treatment. This association obviously complicates the selection of patients who are good candidates for a treatment with glitazones as well as the monitoring of liver tests after initiation of therapy with any thiazolidinedione compound. While regular monitoring of liver enzymes is still recommended and more long term data are desirable, current evidence from clinical trials and postmarketing experience in the US supports the conclusion that rosiglitazone and pioglitazone do not share the hepatotoxic profile of troglitazone.

Type 2 diabetes mellitus is a chronic, progressive disease that is associated to a high rate of complications, including cardiovascular disease, renal failure and blindness. It is characterised by reduced insulin sensitivity (better known as insulin resistance), initial compensatory hypersecretion of insulin by pancreatic beta cells, and late beta-cell exhaustion leading to progressive insulin deficiency and a state of chronic hyperglycaemia.[1] Decreased insulin sensitivity is a key defect in patients with type 2 diabetes mellitus, but is also present in many individuals who do not have diabetes mellitus. Insulin resistance (together with compensatory hyperinsulinaemia) is associated with various metabolic disturbances (for example, arterial hypertension and dyslipidaemias). They are important components of the so-called metabolic syndrome or syndrome X, and all contribute to accelerate atherosclerosis and worsen the cardiovascular prognosis of these people.[2]

The cornerstone of therapy for type 2 diabetes mellitus remains diet and exercise, in order to promote bodyweight reduction and improve insulin sensitivity.[3] Oral drug therapy has improved little since the 1950s with the introduction of the sulphonylureas (which act as insulin secretagogues, but cause hypoglycaemia) and biguanides (which have multiple effects, among which is a reduction of hepatic glucose production, but which are associated with lactic acidosis when they are used inappropriately).[4,5] The availability of thiazolidinediones, more commonly termed the glitazones, is therefore an important event, particularly as targeting muscular insulin resistance is a valuable approach to the treatment of type 2 diabetes mellitus.[6-12] Indeed, these new insulin sensitisers may not only improve blood glucose control in patients with type 2 diabetes mellitus,[13] but also reduce other cardiovascular risk factors and possibly improve cardiovascular outcome.[14]

The thiazolidinediones are a new class of oral antidiabetic agents that directly target insulin resistance in the skeletal muscle, one of the principal underlying metabolic defect in type 2 diabetes mellitus.[1,6] Drugs of this class act as ligands for the γ-subtype of the peroxisome proliferator-activated receptor (PPAR-γ), which is directly involved in the regulation of genes controlling glucose homeostasis and lipid metabolism.[6,15] Troglitazone, the first thiazolidinedione to be approved for clinical use, has proven effective in reducing glycaemia in patients with type 2 diabetes mellitus.[15-19] Unfortunately, it has also been associated with hepatotoxicity and the report of several dozen cases of severe liver failure and death[20,21] led to its withdrawal from the US market in March 2000. Rosiglitazone[22] and pioglitazone[23] are two other members of the thiazolidinedione family commercialised in the US in 1999 and approved for use by the European Agency for the Evaluation of Medicinal Products in 2000.[24] Even if no obvious hepatotoxicity has been demonstrated with these two compounds so far,[25,26] careful monitoring of liver tests is recommended at least during the first year of therapy.

The aim of this review is to compare the liver effects of the three main agents of the glitazone family, troglitazone, rosiglitazone and pioglitazone, in order to answer the important question: is hepatotoxicity a class effect concerning all thiazolidinediones or is it specifically related to troglitazone?[27,28] However, before focusing on this key question, a brief overview on the relationship between type 2 diabetes mellitus and liver disease will be presented. Liver abnormalities are indeed more common in patients with type 2 diabetes mellitus,[29] a finding which may make more complex the assessment of drug-related liver toxicity in this population.

1. Diabetes Mellitus and Liver Disease

Non-alcoholic steatohepatitis (NASH) is a disease of increasing recognition, and is now considered as one of the most common liver diseases in the western world.[30-33] It is frequently associated with obesity, especially abdominal adiposity, and is intimately related to various clinical and biological markers of the insulin resistance syndrome.[34] In particular, both the prevalence and the severity of liver steatosis are related to male gender, body mass index, waist circumference, hyperinsulinaemia, hypertriglyceridaemia and impaired glucose

tolerance or type 2 diabetes mellitus. In patients with type 2 diabetes mellitus who are over 60 years old, the prevalence of fatty liver has been reported to be about 45%,[35,36] whereas fatty liver is rare in patients with type 1 diabetes mellitus. Elevated liver enzyme levels, especially levels of ALT, have been found in 20 to 30% of patients with type 2 diabetes mellitus and in 5% of patients with type 1 diabetes mellitus.[37] Using data from the Third National Health and Nutrition Examination Survey (NHANES III) in the US,[38] it has been recently shown that the prevalence of elevations of ALT was 2-fold higher in patients with type 2 diabetes mellitus than in patients without diabetes mellitus. According to several studies, no clear correlation seems to exist between the degree of glucose control or duration of the disease and the fatty infiltration of the liver.[35,36,39] However, in all cases, patients with diabetes mellitus and a fatty liver are remarkably insensitive to insulin, as already suggested by a pioneering work conducted as early as 1950.[40] The role of diabetes mellitus in producing liver pathology has been controversial although 'diabetic hepatitis' has been recognised as a pathological entity.[41] An autopsy study noted a trend toward a higher prevalence of NASH in patients with type 2 diabetes mellitus requiring insulin.[42] Another study reported that the distribution of fatty metamorphosis and fibrosis in the morbidly obese patient correlates in severity with the degree of impaired glycaemic status.[43] However, in a recent series, the presence of diabetes mellitus did not differ among patients with either simple fatty liver or more severe lesions such as steatohepatitis, steatonecrosis or fibrosis.[44] Finally, a higher incidence of hepatitis C has been observed in patients with diabetes mellitus when compared with the general population[45] and type 2 diabetes mellitus occurs more often in persons with hepatitis C virus infection who are older than 40 years.[46]

Most of patients with fatty liver, NASH or hepatitis C typically have mildly elevated aminotransferase enzyme levels which frequently oscillate in and out of the normal range. Consequently, as thiazolidinediones are essentially prescribed in patients with insulin-resistant type 2 diabetes mellitus, it is crucial to have pre-treatment liver tests in order to be able to interpret later liver enzyme abnormalities observed after the initiation of drug treatment. In addition, the observation of a higher prevalence of liver abnormalities in patients with type 2 diabetes mellitus highlights the difficulties in: (i) selecting patients who may safely receive drugs with potential hepatotoxicity such as the glitazones [according to current recommendations, patients with a pre-treatment ALT level of $>1.5 \times$ the upper limit of normal (ULN) should be excluded]; and (ii) interpreting minor elevations in ALT levels during treatment, especially when the $3 \times$ ULN threshold is used as a measure of liver toxicity.

A follow-up study evaluating liver disease in patients treated conventionally for type 2 diabetes mellitus was performed to provide a reference against which reports of liver disease related to novel oral antidiabetic treatments, i.e. glitazones, could be compared.[47] Among 44 406 patients with type 2 diabetes mellitus identified from the UK-based General Practice Research Database, 605 had a computer diagnosis of liver disease with an incidence rate of 53.2/10 000 person-years. Of the 605 patients, a total of 57 cases had liver disturbances that were possibly induced by antidiabetic drugs, with an incidence rate of 5.0/10 000 person-years. Of these 57 cases, 11 were attributed to non-antidiabetic drugs, 8 cases were attributed to fatty liver disease related to diabetes mellitus, and 36 cases were attributed to uncertain causes, mainly transient illness (n = 29). Oral antidiabetic agents as a cause of liver disease could not be ruled out in only 2 cases with an incidence of 0.2/10 000 person-years. Thus, in this particular population, the background incidence of liver disease was high enough to potentially confuse other causes with drug-induced hepatotoxicity secondary to oral antidiabetic agents.

2. Liver Effects of the Thiazolidinediones

The problem of drug-induced hepatic disorders is a major concern in pharmacovigilance studies.[48,49] However, as liver abnormalities are com-

mon in people who are obese, especially in patients with type 2 diabetes mellitus,[29] it is important to use strict criteria for drug-induced liver disorders[50] and to be cautious before considering a causal relationship between abnormal liver tests and previous drug administration.[51] Clinical development of the thiazolidinediones was initially delayed because of insufficient efficacy and/or unacceptable toxicity which led to the discontinuation of ciglitazone and englitazone after the phase II clinical trial.[9] However, three compounds, troglitazone, rosiglitazone, and pioglitazone, appeared to have good efficacy and acceptable toxicity profiles in the clinical trials and were subsequently approved by the Food and Drug Administration (FDA) in the US. The thiazolidine-2-4-dione structure is common to all drugs of this class, the differences lie in their side chains which may modify their pharmacological activity and adverse effect profiles (fig. 1).

We will review the evidence supporting hepatotoxicity of troglitazone and the almost absence of evidence supporting liver toxicity of rosiglitazone and pioglitazone in humans. Another word of caution concerns drug metabolism and potential drug interactions. Troglitazone and pioglitazone, but not rosiglitazone, are partly metabolised by the cytochrome P450 (CYP) isoform 3A4. However, of the three agents, only troglitazone induce the CYP3A4 pathway. Consequently, safety and efficacy could be affected when troglitazone was coadministered with other drugs metabolised via this enzyme or was prescribed in combination with inhibitors of CYP3A4.[52,53]

2.1 Troglitazone

Troglitazone was approved in the US by the FDA in January 1997 for use in combination with insulin, for the treatment of type 2 diabetes mellitus. Subsequently, approval was given for monotherapy and other combination therapies, with sulphonylureas or metformin.[16-19,54] However, severe, and sometimes fatal, hepatotoxicity was observed with

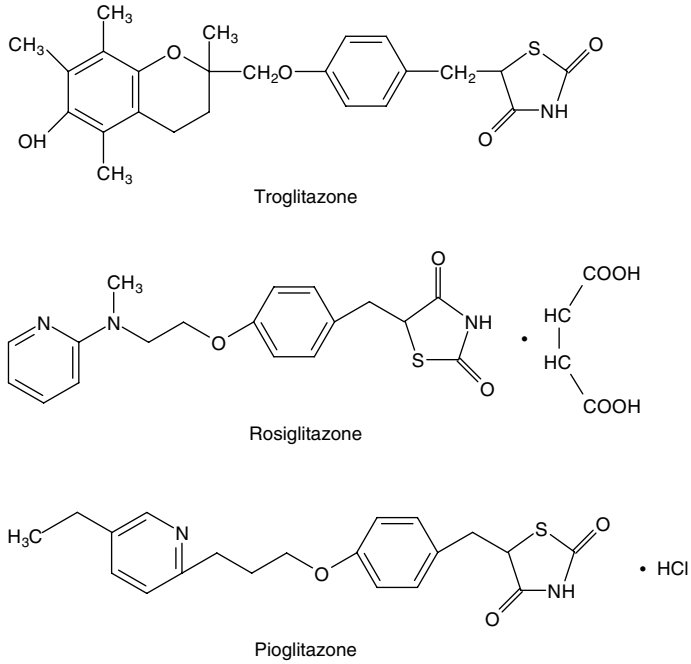

Fig. 1. Structures of troglitazone, rosiglitazone and pioglitazone. There is a thiazolidine-2-4-dione structure common to all, but each has different side-chains.

troglitazone.[20,21] As a result, troglitazone was withdrawn from the market in the UK (in late 1997), only a few weeks after its launch,[55] and this was followed by the abandonment of the overall approval process and all clinical trials in Europe.[56,57] In the US and in Japan, the manufacturer introduced a series of labelling changes for troglitazone recommending close monitoring of liver enzyme levels and for clinical signs of liver dysfunction.[58] Finally, and almost two years after call to ban troglitazone,[59,60] hepatotoxicity led to the withdrawal of the drug from markets in the US and in Japan in March 2000.[61]

2.1.1 Observations from Clinical Trials

In early clinical trials with troglitazone, elevations in serum aminotransferase levels were noted (table I). During the combined US trials of troglitazone, 2510 patients received troglitazone [1134 patients (45%) took the drug for at least 6 months] and 475 received placebo. Serum ALT levels were elevated to ≥3 × ULN in 1.9% of patients (48 of 2510) receiving troglitazone compared with 0.6% of those receiving placebo (3 of 475).[20,28] Increased ALT levels led to discontinuation of treatment in 0.8% of troglitazone-treated patients (20 of 2510) and in no placebo-treated patients. Of these 20 patients, 12 had peak serum ALT levels of ≥10 × ULN while five had levels of ≥20 × ULN. Overall, 18 of these patients were judged by investigators to have significant hepatocellular injury. Liver biopsy performed on two of these patients was consistent with a hepatocellular drug reaction and two other patients had additional cholestatic features. Most of the patients with ALT levels of ≥3 × ULN did not experience symptoms of liver dysfunction and thus were only detected by monitoring during the clinical trials. Two of 2510 patients (0.08%), i.e. two of the 12 patients with ALT levels >10 × ULN, experienced jaundice.[28] The onset of the serum ALT elevations was typically delayed, with only one patient having an elevation during the first month of therapy. In most patients, the peak values occurred between the third and seventh months (mean 147 days; range 1 to 287). In the 20 patients in whom therapy was discontinued, serum ALT levels returned to baseline (mean 55 days; range 8 to 142).[20] In the other 20 patients in whom therapy was continued despite serum ALT levels of ≥3 × ULN (5 of whom had ALT levels of ≥10 × ULN), ALT values also returned to baseline, indicating that in some patients the liver is able to adapt to injury associated with troglitazone.[20]

Table I. Comparison of the incidence of elevated ALT levels in placebo-controlled clinical trials of troglitazone (n = 2510), rosiglitazone (n = 3314) and pioglitazone (n = 1526)

Treatment	ALT level (% of patients)		
	>3 x ULN	>10 x ULN	>30 x ULN
Troglitazone trials			
Placebo	0.6	0	0
Troglitazone	1.9	0.48	0.20
Rosiglitazone trials			
Placebo	0.25	0	0
Rosiglitazone	0.25	0.02	0
Pioglitazone trials			
Placebo	0.25	0	0
Pioglitazone	0.26	0	0

ULN = upper limit of normal range.

2.1.2 Case Reports of Severe Hepatotoxicity

The first cases of acute liver failure due to troglitazone were reported to the FDA within a few months of its approval in the US, and later numerous reports appeared in the literature.[20,21,62-77] By June 1998, the FDA had received 560 reports of troglitazone-related hepatotoxicity, including 24 cases of acute liver failure. At the time of the FDA Advisory Committee Meeting in March 1999, 43 cases of acute liver failure were reported. Nine of these patients received liver transplantation and 28 had died.[28] On average, they had been receiving troglitazone for 116 days (range 4 to 236 days); 69% were taking troglitazone 400 mg/day, 20% were taking 200 mg/day and 11% were taking 600 mg/day. This reflects the usual therapeutic dosage distribution and does not suggest a dose-related effect. At the time of diagnosis, 89% of the patients had jaundice and in 62% of cases it was the first symptom. Histological material was available for several of the patients and showed a consistent pat-

tern of hepatocellular necrosis with bridging necrosis and fibrosis or collapse.[28]

In a systematic analysis of adverse events reported to the FDA, Kohlroser et al.[21] reviewed 46 MedWatch reports considered suspicious for hepatitis. Striking results include the greater than 2 : 1 female to male ratio (suggesting that women may be more susceptible, though it could be that more women than men had taken troglitazone), the marked variability in cumulative drug dose (1200 to 78 000mg), and duration of therapy (6 to 195 days). Most patients had predominantly hepatocellular or mixed heptocellular-cholestatic-type injury, and severity was substantial (it is likely, however, that, among cases reported to the FDA, there was a greater than average severity).[21]

In a series of 35 cases of liver dysfunction from Japan, elevation of ALT levels typically occurred within 2 to 5 months of starting troglitazone therapy.[78] Upon discontinuation of the drug, ALT levels generally declined rapidly, usually to less than half of the ALT peak level within 4 weeks. Interestingly, total bilirubin levels at the time of discontinuation of troglitazone might be a possible prognostic indicator as the levels were less than 85.5 μmol/L (5 mg/dl) in the 31 patients who recovered, but were greater than 136.8 μmol/L (8 mg/dl) in the four patients who subsequently died from liver failure. An investigation by the Ministry of Health and Welfare in Japan on the adverse effects of troglitazone showed that there had been 110 cases of liver dysfunction, seven of which resulted in death by March 1998.[79] In all cases, the symptoms of liver dysfunction developed 2 to 5 months after the patients had first been given troglitazone. A characteristic typical of the patients who died was a rise in the bilirubin level. In a recent paper, it was stated that in Japan 153 patients with diabetes mellitus treated with troglitazone developed severe hepatitis and eight of them died from adverse drug effects.[80]

There is disagreement as to the exact number of 'validated' deaths associated with troglitazone in the US. The incidence of liver-related death or transplant appears to average approximately one case in 50 000 to 60 000 patients.[19,60] Focusing specifically on liver-related deaths, the risk associated with troglitazone appears to have steadily declined from 1 in about 40 000 prior to the inclusion of liver enzyme monitoring in the product labelling (before October 1997) to approximately 1 in 100 000 among patients beginning therapy in 1998, i.e. after the incorporation of a boxed warning and increased monitoring requirements in the product labelling.[19] Thus, even if learning how to more safely use troglitazone was helpful,[81] reason for concern still remained,[82,83] and finally the drug was withdrawn from the US market.[61] By this time, the FDA had received 61 reports of fatal hepatotoxicity 'possibly or probably' associated with the drug and seven cases requiring liver transplantation.

There have been a few reports of fulminant hepatic failure in which pathological examination of the entire liver were made.[63,64,71] Extensive histological studies may be informative about the mechanism of the liver failure attributable to troglitazone. The mechanism of drug-induced liver injury can be classified as intrinsic (direct toxic) and idiosyncratic.[49,84] In less severe cases of troglitazone-induced hepatitis, liver biopsies were performed in two patients (including one with jaundice) and demonstrated the hepatocellular nature of the injury, which was consistent with an idiosyncratic drug reaction.[20] However, the mechanism of troglitazone-induced fulminant hepatitis remains obscure. The first autopsy case of a Japanese patient with diabetes mellitus treated with troglitazone who died from fulminant hepatitis indicated that hypersensitivity may have played an important role in the development of liver damage.[64] This assumption was based upon the positive results of a drug-induced lymphocyte stimulation *in vitro* test and the presence of eosinophilic infiltration. In another autopsy case of fatal subacute hepatic failure after administration of troglitazone, the Japanese authors came to the conclusion that the causative mechanism of liver dysfunction may be 'metabolite aberration, as a result of accumulation of hepatotoxic metabolite(s), in a category of idiosyncratic liver injury'.[71] The idiosyncratic nature of troglitazone-induced hepatotoxicity was a main hurdle to continued use of the

drug. In a recent letter, successful treatment with corticosteroids has been reported (prednisone 20mg twice daily) in a case referred to a transplantation clinic with progressive severe liver dysfunction associated with troglitazone.[76] As a spontaneous recovery has also been described after drug cessation, such improvement should be confirmed in prospective trials before drawing any definite conclusion.

2.1.3 Pharmacokinetics and Metabolism

Troglitazone is an equal mixture of four stereoisomers, as the molecule contains two asymmetric chiral sites. It is not known whether, by separation of these isomers, it would be possible to dissociate the desirable (improvement of insulin sensitivity) from the undesirable (hepatotoxicity) effects of the drug.

Troglitazone undergoes extensive metabolism to three main metabolites: the sulphate conjugate (present at a plasma concentration of approximately 7 to 10 times that of the parent drug), the quinone metabolite (plasma concentration approximately equal to that of troglitazone) and the glucuronide metabolite (low or negligible plasma concentrations).[19,85,86] In patients with moderate to severe hepatic impairment (Pugh-Child score ≥7), the formation of metabolites of troglitazone following a single 400mg dose was not impaired although the capacity to eliminate the metabolites was altered.[87] Because troglitazone and paracetamol (acetaminophen), a widely used proprietary analgesic, have common metabolic pathways and because both of them have been associated with severe hepatic dysfunction, a pharmacokinetics and safety study was performed which showed that the two compounds could be coadministered without adverse clinical consequences.[88]

In animals, a small fraction of troglitazone is metabolised, probably by the CYP system, to a quinone, which then undergoes further phase II conjugation reactions, chiefly sulphation.[89,90] In humans, studies in liver microsomes recently brought evidence for oxidation of troglitazone to a quinone-type metabolite, catalysed by CYP2C8 and 3A4.[91]

Although troglitazone is not extensively metabolised by the CYP3A4 coenzyme pathway (table II), it appears to be a potent inducer of CYP3A4 and to induce drug metabolism by CYP3A4.[92,93] This effect should be considered when troglitazone is prescribed concomitantly with other CYP3A4 substrates [for example, cyclosporin and tacrolimus, ethinylestradiol and norethindrone, some 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors].[19,53]

2.1.4 Cause of Troglitazone Hepatotoxicity

The proximate cause of troglitazone hepatotoxicity is not known yet, nor have clinical observations so far revealed any clear ways to predict which patients are at increased risk for the development of such toxicity.[20,81,82] The patients with elevated ALT values did not have fever, rash, or eosinophilia, making a classic immune mechanism unlikely.[20] An analysis of treatment modalities in all cases of hepatic failure related to troglitazone does not suggest a dose-related effect, considering either the daily dosage (200, 400 or 600 mg/day) or the total duration exposure (from a few days to several months). In a retrospective study among 291 patients with type 2 diabetes mellitus receiving troglitazone, age and concurrent therapy with HMG-CoA reductase inhibitors were the only significant predictors of 3-fold ULN elevations in liver enzymes.[94]

There appeared to be two patterns in the development of liver failure in troglitazone-treated patients: 'rapid ALT risers' in whom the course from onset of symptoms to liver failure took only a few days and another group in which the course was more prolonged and largely unknown.[28] Whether

Table II. Liver metabolism via cytochrome P450 (CYP) system for troglitazone, rosiglitazone and pioglitazone

Troglitazone[a]
Quinone-type metabolite catalysed by cytochromes CYP2C8 and 3A4
Rosiglitazone
Primary pathway via CYP2C8
Secondary pathway via CYP2C9
Pioglitazone
Primary pathway via CYP2C8
Secondary pathways via CYP3A4, CYP2C9, CYP1A1/2

a Troglitazone can also induce CYP3A4.

these two clinical and biological patterns correspond to different underlying mechanisms responsible for hepatotoxicity remains unknown.

The hepatotoxicity observed with troglitazone might be related to its α-tocopherol side chain, although this remains controversial.[95] The α-tocopherol moiety has been shown to scavenge free radicals *in vitro* and may have protective properties against oxidant stress.[96] It is known that the basic quinone structure of α-tocopherol is common to other drugs (such paracetamol) which are subject to CYP2E1-mediated oxidation reactions forming free radicals which are hepatotoxic.[28] It is not known whether patients at risk for troglitazone hepatotoxicity have a polymorphism in CYP or other metabolising enzyme expression that produces more of a toxic, highly reactive intermediate. It has been suggested that patients receiving drugs interfering with the CYP pathway (such as the HMG-CoA reductase inhibitors) might be at greater risk of troglitazone-associated liver abnormalities.[97]

Use of hepatocyte cultures may be helpful for studying the hepatotoxicity of compounds.[98] Studies in human and porcine hepatocyte cultures suggested that inhibition of troglitazone sulphation may result in increased hepatotoxicity due to exposure to parent drug, or increased metabolism by alternate pathways.[99] Interestingly, studies in rat hepatocyte cultures showed that troglitazone, but not rosiglitazone, is hepatotoxic, an observation which is in accordance with clinical experience in humans.[100] Troglitazone metabolites were concentrated in the liver manyfold compared with rosiglitazone.

The cholestatic potential of troglitazone as a possible factor contributing to troglitazone-induced hepatotoxicity was tested in animals.[101] The results suggested that mainly the high concentrations of troglitazone sulfate, a conjugated metabolite, were responsible for an interaction with the hepatobiliary export of bile acids at the level of the canalicular bile salt export pump in rats. According to the authors, such an interaction might lead to troglitazone-induced intrahepatic cholestasis in humans as well, contributing to the formation of troglitazone-induced liver toxicity.

Finally, recent information suggested that PPAR-γ receptors may be important in controlling the activation state of hepatic stellate cells (HSCs), and their repression or inactivation may predispose to hepatic fibrosis.[102] Indeed, HSCs represent the key cellular elements in liver wound healing and the development of hepatic fibrosis. Upon liver injury, HSCs acquire the ability to proliferate and migrate toward the damaged areas and increase the production of extracellular matrix components. In addition, activated HSCs regulate the recruitment of inflammatory cells via secretion of chemotactic factors, including chemokines, and immunomodulatory cytokines such as interleukin (IL)-10. Activation of PPAR-γ such as is obtained with troglitazone has been shown to modulate the profibrinogenic and proinflammatory actions in HSCs.[103] Although troglitazone-associated hepatotoxicity is likely to represent an idiosyncratic reaction in most cases, the medical community will need to be alert to the possibility that interference with these receptors may cause hepatic dysfunction.[102]

2.1.5 Troglitazone and Non-Alcoholic Steatohepatitis

A recent study showed that troglitazone prevents fatty changes of the liver in obese diabetic rats, presumably by reducing insulin resistance and improving the metabolic profile.[104] These results in an animal model are in agreement with the observation that the average ALT level in the glitazone clinical trial data set was lowered with therapy.[28,105]

However, in a pilot study involving 10 women (all but two of whom were obese) with nonalcoholic steatohepatitis, normal ALT levels were seen in 70% of patients at the end of a treatment with troglitazone at a dose of 400 mg/day for ≥6 months.[106] Nevertheless, this biochemical response was associated with only mild histological improvement. Therefore, the authors concluded that normalisation of the liver enzymes in patients with NASH who are treated with thiazolidinediones should be viewed with reservation and that follow-up biop-

sies are essential to evaluate the efficacy of these agents.

2.2 Rosiglitazone

Rosiglitazone was approved by the FDA and launched in the US in May 1999. The European Agency for the Evaluation of Medicinal Products gave its approval in March 2000, although with severe restrictions of clinical use: monotherapy and combination with insulin were excluded and only the combination with sulphonylurea or metformin was approved.[24,107] The pharmacological and clinical characteristics of rosiglitazone have recently been extensively reviewed.[22,24,108,109] Controlled clinical trials have proven its efficacy in improving blood glucose control in patients with type 2 diabetes mellitus treated with diet alone,[110-112] sulphonylurea,[113] metformin[114] or insulin.[22]

2.2.1 Observations from Clinical Trials

In a total of 4598 patients with diabetes mellitus who have received rosiglitazone in clinical trials (3314 of them for 6 months or more, for a total of 3673 patient-years of exposure), the incidence of liver abnormalities (defined as any ALT level elevation of ≥3 × ULN) was low (0.25%), and similar to that observed in placebo-treated patients (0.25%)[115] (table I). Only one patient receiving rosiglitazone (0.02%) had a ≥10 × ULN elevation in ALT level.[28]

In November 1999, exposure to rosiglitazone in clinical trials had substantially increased and comprised over 5000 patient years including more than 1000 patients treated for ≥2 years.[116] For all rosiglitazone-treated patients (including monotherapy and in combination with sulphonylurea or metformin), the rate of ALT level elevations of ≥3 × ULN is 0.30 cases per 100 patient years, compared to 0.59 cases per 100 patient years for placebo-treated patients and 0.73 cases per 100 patient years for sulphonylurea- or metformin-treated patients. Thus, the current clinical trial experience with rosiglitazone indicates no evidence of troglitazone-like hepatotoxicity. On the contrary, recent data showed a trend to lower incidence of elevated ALT levels in patients with type 2 diabetes mellitus receiving rosiglitazone than in those treated with placebo or other oral antidiabetic agents. This favourable tendency should be confirmed in further studies, but may be explained by the rosiglitazone-induced reduction of insulin resistance, a metabolic state which has been shown to be associated with fatty liver and NASH (see section 1). Indeed, rosiglitazone was shown to rapidly and durably reverse hepatic steatosis and hepatomegaly in Zucker fatty rats. This effect occurred with improved hyperinsulinaemia but was seemingly independent of plasma lipids, perhaps suggesting a direct mobilising effect of rosiglitazone on liver fat.[117]

2.2.2 Case Reports of Hepatotoxicity

Three reports of acute hepatotoxicity attributed to rosiglitazone have appeared in the literature,[118,119] one of which was challenged by the manufacturer.[120] As these three reports are unique, they deserve further consideration.

In the first report, a 69-year-old man developed severe hepatic failure after 21 days of rosiglitazone therapy at a daily dose of 4mg, and the patient became comatose.[118] The authors reported that the patient developed nonspecific symptoms during rosiglitazone treatment that in retrospect probably reflected acute liver injury within 1 week of the start of rosiglitazone therapy. The patient was managed with intensive medical care and he gradually improved over the subsequent 2 weeks after rosiglitazone cessation. Other causes of hepatic failure, such as viruses and toxins, were excluded. This patient was also taking verapamil and pravastatin, both of which can cause hepatitis, but he had been receiving these drugs for more than 1 year without any problem. A liver biopsy was not performed. It was possible that ischaemic hepatitis ('shock liver') played a superimposed role in this patient's hepatic dysfunction. However, according to the authors, ischaemia alone was unlikely to explain the patient's initial clinical picture and the decrease in the serum albumin level which was associated with the patient's illness is not typically associated with shock liver.[118] However, this interpretation was challenged by three independent hepatologists whose opinion was solicited by

SmithKline Beecham Pharmaceuticals.[120] They concluded that this patient's liver injury was probably the result of ischaemia and not rosiglitazone. Indeed, the pattern and time course of biochemical abnormalities were characteristic of ischaemic hepatitis, particularly the decrease in serum aspartate aminotransferase from greater than 11 000 U/L to normal within 9 days. Such high and rapidly normalising serum aminotransferase levels are unusual for most cases of drug-induced liver disease and were not characteristic of troglitazone-induced liver dysfunction.[20]

In a second report, a 61-year-old man receiving rosiglitazone 4 mg/day for 2 weeks presented with anorexia, vomiting, and abdominal pain.[119] The patient noted the onset of his symptoms 8 days after starting rosiglitazone therapy. On admission, liver function tests revealed severe hepatocellular injury. ALT levels peaked at day 1 (1706 U/L; normal range 0 to 40). Total bilirubin levels remained within the normal range while direct bilirubin levels were mildly and transiently elevated. Serum albumin levels were markedly decreased (minimum value: 2.3 g/L). However, at no time were signs or symptoms of hepatic failure observed. Discontinuation of rosiglitazone led to rapid improvement of liver function (ALT levels were 1251 U/L on day 8, 558 U/L on day 14, 133 U/L on day 21 and 41 U/L on day 52) and resolution of symptoms. Serological tests excluded viral hepatitis. The patient's medical history included chronic obstructive pulmonary disease, a remote history of alcoholism and intermittent headache. He reported no recent alcohol intake, but he regularly used paracetamol at a dose of three to four tablets daily before admission. However, his paracetamol concentration on admission was within the therapeutic range, which made the diagnosis of paracetamol toxicity unlikely. The authors concluded that liver injury was caused by rosiglitazone in this patient and probably involved an idiosyncratic process. They proposed that patients receiving rosiglitazone should have liver enzyme levels monitored earlier and more frequently than initially recommended. However, as pointed out later on, this case was not only clouded by a history of alcohol abuse and paracetamol use, but also by concomitant administration of zafirlukast, a compound which may provoke hepatitis and hyperbilirubinemia and, in rare cases, hepatic failure and inhibits one of the metabolic pathways for the clearance of rosiglitazone, the CYP2C9 pathway.[121]

A third case was briefly described in a letter.[122] A 58-year-old woman with long-standing type 2 diabetes mellitus received, in addition to previously prescribed glibenclamide (glyburide), rosiglitazone at a dosage of 4 mg/day. Two weeks after the initiation of rosiglitazone, she felt ill and 1 week later, she was jaundiced and admitted to hospital. Although liver function tests were normal before rosiglitazone, her AST and ALT levels peaked at 5.23 μkat/L and 4183 nkat/L, respectively. Her bilirubin level peaked at 41 μmol/L (2.4 mg/dL). There was no evidence of liver failure. Three days after rosiglitazone therapy was discontinued, her AST and ALT levels decreased to 1.96 μkat/L and 1083 nkat/L, respectively, and 4 weeks later they returned to normal. Viral serologic studies and ultrasonography of the liver revealed no other cause for the liver injury. The woman did not take any other medications, except lisinopril and hydrochlorothiazide whose doses had remained unchanged for at least 1 year. After having applied the criteria for determining the likelihood of a drug being responsible for the adverse reaction, the authors considered that rosiglitazone was a highly likely cause of the patient's hepatocellular injury.

Finally, a Canadian report briefly mentioned the case of a 51-year-old man, who was positive for hepatitis B surface antigen (but had relatively normal baseline liver enzymes before taking rosiglitazone) who showed a marked increase in liver enzymes after a 6-month treatment period with rosiglitazone.[123] The drug was discontinued, but the patient later died from liver failure. In 10 cases of liver and biliary disorders cited in the same report, all of the patients, who were on treatment with rosiglitazone for between a few weeks and 6 months, showed elevated liver enzymes. However, in most of these cases, there were not enough data to pro-

vide a meaningful assessment of causality and at least three patients had known hepatic disorders before receiving rosiglitazone.[123]

These few reports, even if alternative causes for hepatic failure have been suggested[120,121] underline the need for further investigations into the potential hepatotoxicity of rosiglitazone and other drugs of the glitazone family. However, after more than 2 years of commercialisation of rosiglitazone in the US, the number of case reports of rosiglitazone-related hepatotoxicity remains extremely low, and indeed much lower than the corresponding one of troglitazone-induced cases of liver dysfunction after the same time interval following launch. It remains unclear whether rosiglitazone *per se* may be hepatotoxic in very rare cases or not. However, if anorexia, fatigue, abdominal pain, nausea, or jaundice occur with rosiglitazone (especially during the first few weeks after the initiation of treatment), it is wise to stop therapy with this agent and to monitor for hepatic dysfunction. Of course, rosiglitazone therapy should be discontinued immediately if liver enzyme levels become elevated.

2.2.3 Pharmacokinetics and Metabolism

The major routes of rosiglitazone metabolism were *N*-demethylation and hydroxylation with subsequent conjugation. The major metabolites, those of intermediate importance, and nearly all of the trace metabolites in human have been identified.[124] Unlike troglitazone, rosiglitazone is not metabolised by the CYP3A4 metabolic enzyme system, and has shown only negligible effects on this system *in vitro* (table II).[125,126] Unlike troglitazone, in healthy adults rosiglitazone has no significant effect on the pharmacokinetics of drugs that are metabolised via liver CYP enzymes, especially CYP3A4, such as nifedipine and oral contraceptives.[108,127]

Although rosiglitazone is contraindicated in patients with clinical or biological evidence of active liver disease, acute pharmacokinetics has been studied in patients with hepatic impairment (Child-Pugh class B/C).[128] Unbound oral clearance of rosiglitazone was significantly lower, resulting in 2-fold higher maximum plasma concentrations and an elimination half-life approximately 2 hours longer than in healthy individuals.

2.3 Pioglitazone

Pioglitazone was approved by the FDA and commercialised in the US in July 1999. The European Agency for the Evaluation of Medicinal Products gave its approval in October 2000 with the same limitations as for rosiglitazone.[23,24,107,129] Well-controlled studies demonstrated that pioglitazone improves blood glucose profile of patients with type 2 diabetes mellitus receiving only diet therapy[130] and of patients already receiving sulphonylurea,[23] metformin[131] or insulin.[23,24]

2.3.1 Observations from Clinical Trials

Results from the US placebo-controlled study programme showed a total of 4 reports of elevated ($\geq 3 \times$ ULN) serum levels of ALT after pioglitazone treatment in 1526 evaluable patients (table I).[132] This 0.26% incidence was similar to that observed with placebo, as two of 793 placebo-treated patients (0.25%) also had elevated serum ALT levels. The proportion of patients withdrawn from US clinical studies because of abnormal liver function test results was below 0.12%.[23] All patients with follow-up values had reversible elevations of ALT. No patient receiving pioglitazone had a $\geq 10 \times$ ULN elevation in ALT level (table I).

The uncertainty about relationship between ALT level increases and fatty liver or NASH in patients with type 2 diabetes mellitus is underscored by more detailed examination of what happened to patients in all pioglitazone trials who had elevations $\geq 3 \times$ ULN.[28] Among 10 patients with ALT $\geq 3 \times$ ULN, five had definite causes other than drug-associated liver disturbances and two were probably due to drugs other than pioglitazone and indeterminate causes (in one the temporal relationship was unknown, in another there was a chronic elevation of alkaline phosphatase). So, it is not yet known if any hepatotoxicity occurs with pioglitazone.

2.3.2 Case Reports of Hepatotoxicity

To date, no single case of severe liver toxicity has been reported in the literature with pioglitazone despite extensive use in the US for over 2 years.[133]

The findings of one animal study suggested that pioglitazone acts as an insulin sensitiser in rat hepatoma cells, increasing basal and insulin-stimulated DNA synthesis, and stimulating fat synthesis and liver hypertrophy in diabetic KKA^y mice.[134] Indeed, the hepatocytes of the pioglitazone-treated KKA^y mice were markedly distended with evidence of severe lipid generation, but without any signs of hepatocellular necrosis. The possible physiological or toxicological significance of this finding remains to be determined.

2.3.3 Pharmacokinetics and Metabolism

Pioglitazone undergoes extensive hepatic metabolism, predominantly via the CYP2C8 system (39%).[53,135,136] Secondary pathways include CYP3A4 (17%), CYP2C9 and CYP1A1/2 (table 2). Since multiple CYP isozymes are involved in the metabolism of pioglitazone, the potential for other drugs to inhibit the metabolism of pioglitazone to a clinically significant extent must be very small.[135,137]

Lack of induction or inhibition of hepatic enzyme systems (CYP3A4, 1A1/2 or 2C9) was indicated by data showing no statistically or clinically significant effect of pioglitazone on the pharmacokinetics of warfarin, oral contraceptives or hormone replacement therapy.[127,135,138] These *in vivo* results confirm *in vitro* observations which showed that pioglitazone, in contrast to troglitazone, does not inhibit the CYP isozymes involved in drug metabolism.[23] In addition, a study comparing the effects of troglitazone and pioglitazone in corticosteroid-induced diabetes in humans demonstrated that troglitazone, but not pioglitazone, reduces the elimination half life of prednisolone and increases urinary excretion of 6-beta-hydroxycortisol. These observations were confirmed in healthy volunteers in whom pioglitazone does not modify the 6-beta-hydroxycortisol : free cortisol urinary ratio, commonly used as a specific marker of CYP3A4 induction. Thus, there is no evidence to date that pioglitazone induces the hepatic CYP3A4 system.[136]

The single-dose pharmacokinetics of an oral dose of 30mg pioglitazone were evaluated in a study of patients with chronic liver insufficiency (Child-Pugh class B or C) compared with healthy individuals.[135] The mean area under the curve serum concentrations and total clearance were similar in the two groups. However, volume of distribution of pioglitazone was increased by 55% in the patients with hepatic insufficiency, and mean maximum plasma drug concentration of pioglitazone in these patients was approximately 57% of that reported in controls. This is consistent with a decreased degree of plasma protein binding, a condition which should result in a significant increase in the free fraction of pioglitazone. Finally, the comparison of various metabolite serum concentrations suggests impaired oxidative biotransformation of pioglitazone in patients with hepatic insufficiency.[135]

3. Conclusions

By offering a new mechanism to improve insulin sensitivity, glitazones raised expectations for therapy directed against the underlying insulin resistance of type 2 diabetes mellitus. However, a major issue is whether hepatotoxicity is a characteristic of all thiazolidinediones related at least partly to the activation of PPAR-γ receptors or whether it is unique to troglitazone. The three glitazones do not share the same metabolic pathways and only troglitazone can induce CYP3A4. In the case of troglitazone, the incidence of an ALT level of $\geq 3 \times$ ULN in controlled clinical trials was approximately 3-times greater than that of placebo, while in the case of rosiglitazone and pioglitazone it was equal to placebo. Furthermore, a 10-fold elevation of ALT was exceptional with rosiglitazone (1 patient) or was never observed with pioglitazone, contrasting with the 12 cases reported with troglitazone.[28] From data on troglitazone- and other drug-induced hepatocellular disease, approximately 10% of those patients with an ALT level of $>10 \times$ ULN will develop jaundice, and about 10% of those developing jaundice could be expected to die from hepatic insufficiency. If this is true, acute liver failure would be very rare with pioglitazone and rosiglitazone. This

is confirmed by the clinical observations available for over 2 years of commercialisation of these two glitazones in the US. Only a few case reports of hepatotoxicity with rosiglitazone (with controversial causal relationship) and none with pioglitazone have been reported in the literature while more than 1 million of patients have been treated with each of these new glitazones. Thus, several convincing arguments suggest that hepatotoxicity reported with troglitazone, which led to the withdrawal of this compound from the market, is probably specifically related to specific properties of the molecule rather than to the common thiazolidine-2-4-dione structure shared by rosiglitazone and pioglitazone. However, pre-existing liver disease should be a contraindication and liver enzyme monitoring is recommended before initiation of therapy with either new glitazone, and periodically thereafter. While more long term data are desirable, current evidence from clinical trials and post-marketing experience supports the conclusion that rosiglitazone and pioglitazone do not share the hepatotoxic profile of troglitazone, which may indicate that hepatotoxicity is not a class effect of the thiazolidinediones.

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