Effect of SR58611A, a potent beta-3 adrenoceptor agonist, on cutaneous wound healing in diabetic and obese mice

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Abstract

In diabetic patients, impairment of wound healing is a serious problem which represents a significant health burden. The effect of a highly selective beta-3 adrenoceptor agonist, SR58611A, on wound healing was assessed in animal models of type II diabetes. In db/db diabetic mice, a daily oral treatment with SR58611A (1, 3 and 10 mg/kg/day for two weeks) significantly reduced hyperglycaemia from 3 mg/kg/day onwards. The compound also normalized wound healing, starting from the lowest dose tested (1 mg/kg/day). SR58611A did not affect wound healing of control (lean) mice. An oral anti-diabetic agent, devoid of affinity for beta-3 adrenoceptors, troglitazone (130 mg/kg/day p.o.), normalized glycaemia but did not improve wound healing in db/db mice. Local application of SR58611A (200 μg/day in db/db mice) did not affect wound healing. SR58611A also normalized glucose levels in ob/ob mice, but only slightly improved wound healing in this strain. Moreover, in 17-week old db/db mice (i.e. severely insulin resistant) and in streptozotocin-induced diabetic mice, SR58611A slightly decreased hyperglycaemia and did not affect wound healing. In conclusion, SR58611A improves wound healing in animal models of non-insulin-dependent diabetes. This effect is not related to its effect on glucose levels, but probably implicates systemic effects of the compound.

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1. Introduction

Obesity and type II diabetes (non-insulin-dependent diabetes mellitus, NIDDM) are of growing importance in our industrialized societies. In diabetic patients, impairment of wound healing is a serious problem which represents a significant health burden. For example, ulceration is the most common complication of diabetes that requires hospitalisation (Harding et al., 2002). Wound healing is a complex process which involves an interplay between epidermal and dermal cells, intracellular matrix, angiogenesis, plasma proteins and is governed by the local production of cytokines and growth factors (Singer and Clark, 1999; Harding et al., 2002). It has been suggested that diabetes impairs wound healing through disruption of local cytokine production, notably platelet derived growth factor (PDGF), tumor necrosis factor α (TNFα), interleukin-1β, and vascular endothelial growth factor (VEGF) (Frank et al., 1995; Doxey et al., 1998; Zykov et al., 2000).

Animal models of diabetes are essential in order to determine whether experimental compounds are able to improve various aspects of this pathology. The mutant C57BL/KsJ (db/db) diabetic mouse has emerged as a potentially relevant model of healing impairment (Greenhalgh et al., 1990; Tsuboi et al., 1992). Surgical wounds in these mice exhibit a marked delay as regards exudate inflammation, proliferation of granulation tissue, collagen synthesis and scarring (Greenhalgh et al., 1990). These diabetic mice develop type II diabetes syndrome (with hyperglycaemia, hyperinsulinemia, hypertriglyceridemia,
hypercholesterolemia) up to 20–30 weeks of age, before losing weight (Tuman and Doisy, 1977). The C57BL/6J (ob/ob) obese mouse is primarily an obesity model but also develops many characteristics of type II diabetes with a low expression and functionality of beta-3 adrenoceptors (Begin-Heick, 1996). The genetic basis of these animals is a defect in the ob protein (leptin) receptor gene in the case of db/db mice and a defect in the production of the ob protein in ob/ob mice (for review: Reed and Scribner, 1999).

Previous studies have shown that beta-3 adrenoceptor (β₃-AR) agonists are effective in improving glycaemic control, insulin sensitivity and reducing body weight in obese/diabetic animals (Rochet et al., 1988; Arch and Wilson, 1996). SR58611A is a potent, selective and specific β₃-adrenoceptor agonist (Bianchetti and Manara, 1990), which has been shown to improve glucose tolerance in the mouse (Williams et al., 1999). Since β₃-adrenoceptor agonism has been shown to be associated with anti-inflammatory effects (Anthony, 1996), the release of adipocytokines (Burysek and Houstek, 1997), and an increase in cutaneous blood supply (Berlan et al., 1994), the present studies were undertaken to determine whether the β₃-adrenoceptor agonist SR58611A would be able to improve wound healing in animal models of type II diabetes.

Preliminary results were published in the Proceedings of the Second European Congress of Pharmacology, Budapest, Hungary (Bernat et al., 1999).

2. Methods

2.1. Materials

SR58611A (ethyl(7s)7-((2r)2-(3-chlorophenyl)-2-hydroxy-ethylamino)-5,6,7,8-tetrahydronapht-2-yl)-oxyacetate, hydrochloride) was synthesized at Sanofi-Synthélabo Recherche (Milan, Italy). Streptozotocin was from Sigma (Saint Quentin Fallavier, France). Troglitazone was from Glaxo/Wellcome and was incorporated into standard mouse chow at UAR (Villemoisson, France).

2.2. Animals

Two strains of inbred diabetic mice were used: genetically diabetic C57BL/KsJ-db/db and C57BL/6J-ob/ob mice. SPF outbred OF1 mice were made diabetic by streptozotocin treatment. Male diabetic mice (C57BL/KsJ, db/db) aged either 6–8 weeks or 17 weeks and their normal littermates (db/+ or +/+, lean) and 9-week old C57BL/6J-db/ob/db mice were purchased from the Jackson Laboratory (Bar Harbor, USA). C57BL/KsJ db/db mice develop non-insulin-dependent diabetes mellitus (NIDDM) up to 12 weeks of age. After 12 weeks, the pancreatic β-cells become depleted of insulin and show progressive degranulation leading to collapse of insulin secretion (age >30 weeks) which is characteristic of insulin-dependent diabetes (IDDM). Streptozotocin-treatment (160 mg/kg intra-peritoneally) of male OF1 mice (Iffa-Credo, France) was also used to induce extensive pancreatic destruction, leading to IDDM dia-

betes one week later. Mice were caged individually. All animal experiments were conducted according to the in vivo protocols approved by the Animal Care and Use Committee at Sanofi-Synthélabo Recherche.

2.3. Wound induction

Animals were anaesthetized with sodium pentobarbitone (40 mg/kg i.p.) and their dorsal hair was shaved. A 1×1 cm square area was then delimited with a plug on the midback, the corresponding skin was excised on the mid-dorsum of each mouse and the resulting wound was left exposed to air. Each mouse was housed separately after wound induction. At selected time points, twice a week, the wound area was measured by tracing it onto acetate sheets, which were then cut up and measured by weighing. Results were then expressed as an area (cm²).

2.4. Determination of blood concentrations

Blood was analysed for glucose content using a glucometer (Bayer, Puteaux, France) which allowed glucose levels up to a maximum of 6 g/l to be measured automatically. Blood was obtained from a transection of the end of the tail. Plasma obtained after aortic puncture in pentobarbitone (40 mg/kg, i.p.)-anaesthetized mice was used for the determination of circulating insulin and leptin levels. Insulin concentrations were measured using a radio-immunoassay kit from Amer sham (Les Ulis, France). Leptin concentrations were determined by means of a radio-immunoassay kit from Linco Research Inc. (Saint Charles, Missouri, USA). Plasma triglycerides and cholesterol were measured enzymatically using kits from Sigma (Saint Quentin Fallavier, France). Plasma plasminogen activator inhibitor-1 activity was determined by measuring the appearance of plasmin activity from plasminogen in the presence of tissue plasminogen activator (tPA) using the Chromogenix substrate S-2403 (Coatest PAI, Chromogenix, Molndal, Sweden).

2.5. Treatment schedules

SR58611A was administered orally by gavage for two weeks before wounding and two weeks thereafter until wound healing at the doses of 1, 3 and 10 mg/kg/day. The control animals were similarly treated in parallel, using water instead of drug solution. Troglitazone was incorporated into the chow. The chow was given ad libitum, but the actual chow consumption of 5 g/day was used to determine the mean dose of troglitazone which was around 130 mg/kg/day.

2.6. Histomorphology

Mice were killed 7 days after wound induction by exsanguination after anaesthesia induced by an intra-peritoneal injection of sodium pentobarbitone. Wounds were excised and fixed in 10% buffered formalin solution. After fixation, the tissue was
Effect of SR58611A on various parameters measured after 15 days of treatment in vehicle and SR58611A (10 mg/kg)-treated db/db mice and in vehicle and SR58611A (10 mg/kg)-treated lean mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>SR58611A</th>
<th>Control</th>
<th>SR58611A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>36.0±1.1*</td>
<td>25.9±0.3</td>
<td>25.9±0.3</td>
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</tr>
<tr>
<td>Glucose (g/l)</td>
<td>7.3±0.40</td>
<td>1.06±0.04</td>
<td>1.06±0.05</td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>4.5±0.8</td>
<td>2.2±0.9</td>
<td>1.9±0.5</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>37.9±4.5</td>
<td>1.8±0.2</td>
<td>1.9±0.4</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (g/l)</td>
<td>0.70±0.05</td>
<td>0.45±0.01</td>
<td>0.46±0.01</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (g/l)</td>
<td>1.56±0.11</td>
<td>0.76±0.09</td>
<td>1.06±0.10</td>
<td></td>
</tr>
<tr>
<td>PAI-1 (UA/ml)</td>
<td>24.2±2.6</td>
<td>21.8±2.9</td>
<td>27.0±3.1</td>
<td></td>
</tr>
</tbody>
</table>

* P<0.05 vs. Control db/db. Statistical tests were exclusively carried out between control and treated animals in both db/db and lean mice.

2.7. Statistical analysis

All results are given as mean±S.E.M. Unless stated otherwise, the statistical significance of the effect of treatments was determined by analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test in case of significance of the primary ANOVA.

3. Results

In a first series of experiments (n=13–14/group), the effect of SR58611A (10 mg/kg p.o.) or its vehicle on biological parameters related to obesity and diabetes was assessed (Table 1) in db/db mice. During vehicle treatment, db/db mice (6-weeks old) showed reduced body weight (day 1: 34.9±0.5 g, day 15: 31.2±0.7 g, P<0.05 paired t test) in contrast to the SR58611A-treated db/db mice which maintained their weight (day 1: 36.6±0.8 g, day 15: 36.0±1.1 g). Consequently at the end of the treatment the beta-3 adrenoceptor agonist treated group had a higher body weight (P<0.05, Wilcoxon Test) than control mice (Table 1, left panel). At the same time, the body weight of lean mice treated either with SR58611A or vehicle did not change (Table 1, right panel). In db/db mice, hyperglycaemia was decreased (~29%, P<0.05, t test) by SR58611A, while the same treatment had no effect in lean mice. As expected, insulin and leptin plasma levels were elevated in db/db mice. However, treatment by SR58611A or by the vehicle did not modify the plasma levels of insulin, leptin, cholesterol, triglycerides, as well as plasminogen activator inhibitor-1 (Table 1).

In a second series of experiments (n=7/group), the effect of SR58611A on blood glucose levels was characterized at the end of a fifteen-day treatment period. SR58611A (1, 3, 10 mg/kg/day p.o.) dose-dependently reduced the blood glucose levels (Fig. 1A, B), the lowest dose (1 mg/kg/day) being ineffective. The same maximal anti-hyperglycaemic effect was also obtained with troglitazone (130 mg/kg/day, Fig.

Fig. 1. Effect of SR58611A on blood glucose level in non-insulin dependent diabetic mice. A: Time course of blood glucose level after initiation of treatment in control 6-week old db/db (●) and SR58611A (10 mg/kg)-treated db/db (■), as well as control lean (○) and SR58611A (10 mg/kg)-treated lean mice (□). Blood glucose levels were measured every two days, but for the sake of clarity some intermediate data points have been omitted on the figure. B: Effect of different doses of SR58611A on blood glucose level. Blood glucose level was determined 14 days after treatment in control db/db, in db/db mice treated with SR58611A (1, 3 or 10 mg/kg), in db/db mice treated with rosiglitazone (130 mg/kg) and in control lean mice. C: Time course of blood glucose level after initiation of treatment in control 9-week old ob/ob (●) and SR58611A (10 mg/kg)-treated (■) ob/ob mice, as well as control lean (○) and SR58611A (10 mg/kg)-treated lean mice (□). SR58611A was administered p.o. each day during the whole course of the experiment (*P<0.05 vs. untreated mice, n=7, ANOVA followed by Dunnett’s multiple comparison test).
1B). SR58611A (10 mg/kg p.o.) induced a similar anti-hyperglycaemic effect in 9-week old ob/ob mice (Fig. 1C). Neither the vehicle nor SR58611A modified blood glucose levels in the respective lean strains.

The effect of SR58611A on wound healing was then determined under conditions identical to those used to assess its effect on hyperglycaemia. SR58611A at the highest dose used (10 mg/kg p.o.) strongly reduced the wound areas (Fig. 2A, B) in db/db mice, such that the time course of wound healing in SR58611A-treated db/db mice could be superimposed upon the curve seen in lean mice (Fig. 2A). Furthermore, this effect of SR58611A was observed from the lowest dose (1 mg/kg/day) onwards, contrary to the effect on hyperglycaemia, which became significant only at the higher dose of 3 mg/kg/day. However, the effect of SR58611A was less marked in ob/ob mice (Fig. 2C), although the compound still significantly decreased the wound area as compared to untreated animals. Interestingly, troglitazone did not improve wound healing.

Histological analysis showed some increase in the extent of epithelialization (Fig. 3A,B), although this effect did not reach statistical significance (Table 2), as well as a significant increase in the amount of granulation tissue (Fig. 3C,D). The presence of neovascularisation was indicated by the hemorrhagic infiltration of granulation tissue (Table 2).

However, local application of SR58611A (2×100 μg/day, i.e. 5–10 mg/kg) did not affect wound healing in any mouse strain (data not shown). In 17-week old db/db mice, a model intermediate between type II and type I diabetes, the efficacy of SR58611A to reduce blood glucose levels was slightly reduced (circa 30%) and the effect on wound healing was lost.

Table 2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Lean</th>
<th>Lean</th>
<th>db/db</th>
<th>db/db</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>–</td>
<td>SR58611A</td>
<td>–</td>
<td>SR58611A</td>
</tr>
<tr>
<td>No. of samples evaluated</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Epithelialization</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Granulation tissue</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3b</td>
</tr>
<tr>
<td>Density of mature connective tissue</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Density of capillaries</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cellular density</td>
<td>3</td>
<td>2.5</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Sero-fibrino-leucocytic scab</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Hemorrhagic infiltration of granulation tissue</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4b</td>
</tr>
</tbody>
</table>

The different grades were determined as described under Methods in either lean or db/db mice.

* Median grade.

b P<0.05 vs. untreated animals.

c Incidence.
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...and normalized hyperglycaemia at slightly higher doses (3 mg/kg/day) was able to normalize blood glucose levels, but only slightly improved wound healing, and (iii) SR58611A did not modify either blood glucose levels or wound healing in streptozotocin pre-treated mice. Altogether, the data in db/db and ob/ob mice demonstrate that normalization of blood glucose levels cannot alone explain the effects of SR58611A on wound healing. This conclusion is also consistent with the finding that troglitazone, which was as active as SR58611A on hyperglycaemia, was devoid of any effect on wound healing. Furthermore, it has been shown that the control of hyperglycaemia through diet restriction or insulin did not improve wound healing in diabetic mice, this being partly due to structural changes in the adipose tissue (Goodson and Hunt, 1986).

However, the absence of any effect of SR58611A in streptozotocin-treated mice would tend to suggest that some kind of systemic metabolic or other effect of the compound is necessary to affect wound healing in diabetic animals. This is also emphasized by the inability of locally applied SR58611A to improve wound healing.

Some hints concerning the possible mechanisms mediated by β3-adrenergceptor stimulation and susceptible to improve wound healing can be obtained by observations of the tissue after wound induction. A whole host of studies now shows that the delay in wound healing in diabetic states is related to impaired granulation tissue formation (i.e. neovascularisation) (Greenhalgh et al., 1990; Tsuboi and Rifkin, 1990; Frank et al., 1995; Okumura et al., 1996)). However, it should also be noted that wound healing in diabetes is slowed down during all the different phases of wound closure: delays are noticed in the coagulation process, inflammatory response, epithelialization, formation of granulation tissue, and remodelling of matrix and tissue (Morain and Colen, 1990).

Our data in db/db mice show that wound area in control animals increases shortly after wound induction, as has been reported previously (Okumura et al., 1996), but does not...
expand in either lean or SR58611A-treated animals. Part of the effect of SR58611A may therefore be related to the inhibition of wound expansion, although this effect cannot alone explain all of the effects of the compound. Indeed, the reduction in wound area started as early as 7 to 9 days after wound induction in SR58611A-treated as well as lean animals, and was quite complete 11 days after induction, at which time the wound area only just started to decrease in control db/db mice (Fig. 2A). As wound expansion started very shortly after wound induction, and as this effect was completely avoided in SR58611A-treated mice as well as lean mice, this effect of the compound is probably related to pre-treatment with SR58611A, which normalized the metabolism of db/db mice.

Later during the wound healing process, the treatment with SR58611A induced a small but significant hemorrhagic infiltration of granulation tissue, which is probably due to an increase in blood supply through new vessels (increase in concentration of granulation tissue, which is probably due to an SR58611A-induced small but significant hemorrhagic infiltration which is involved in thermogenesis and probably, in our case, in wound blood supply.

Furthermore, as fibroblasts are key cells in the production of extracellular matrix, it is also possible that the effect of SR58611A on both the blood supply and the release of soluble mediators results in a strong increase in granulation tissue. Thus, selective β3-adrenoceptor agonists have been shown to increase the production of interleukin-6 and interleukin-1 alpha from brown adipocytes (Tonello et al., 1999; Lin et al., 2003) indicating that β3-adrenoceptors play a role in adaptive angiogenesis which is involved in thermogenesis and probably, in our case, in wound blood supply.

In conclusion, SR58611A increased the rate of wound healing in obese hyperglycemic mice. Diabetes 35, 491–495.


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References


