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Short communication

Antiaggregatory activity of hypoglycaemic sulphonylureas

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Abstract

Aims/hypothesis. Vascular complications observed in diabetes are often related to altered platelet functions. The most widely used hypoglycaemic drugs for treating Type II (non-insulin-dependent) diabetes mellitus are sulphonylurea derivatives. The purposes of this study were to evaluate the inhibitory effects of hypoglycaemic agents on platelet aggregation, to measure their lipophilicity and identify their structural parameters which assess their antiaggregatory activity.
Methods. An antiaggregatory test in vitro was carried out for 13 sulphonylurea derivatives. Aggregation of platelets, incubated with the agents at concentrations varying from 7.5 to 480 μmol/l, was induced by 10 μmol/l ADP. Drug lipophilicity parameter, log k_w, was measured by gradient HPLC and the agents were subjected to molecular modelling.
Results. The most pronounced inhibition of platelet aggregation was by glimepiride, gliclazide, gliquidone, glibenclamide and compound 2A. The IC_{25} values were 15.9, 18.6, 20.4, 28.5 and 34.7 μmol/l, respectively. Quantitative structure-activity relationships indicate that antiaggregatory activity is mainly affected by electronic and not by lipophilic properties of the agents.
Conclusion/interpretation. Glimepiride appeared to be a more potent ADP-induced platelet aggregation inhibitor in vitro than gliclazide. Antiaggregatory activity was shown for gliquidone and confirmed for glibenclamide. The QSAR analysis supports the hypothesis of a free radical mechanism of action of sulphonylurea derivatives previously suggested for gliclazide. [Diabetologia (2002) 45:1034–1037]

Keywords Hypoglycaemic sulphonylureas, lipophilicity, platelet aggregation, QSAR.

In diabetes secondary vascular complications are often observed and the disease is an independent risk factor for cardiovascular disorders. It has been suggested that one of the factors in the progression of diabetic complications is platelet dysfunction. Platelet malformations concern their structure and function and result in altered membrane lipid dynamics and fluidity, disturbed intermediary metabolism, including arachidonate and prostaglandin pathways, as well as in increased production of free radicals and decreased content of antioxidants [1]. In several investigations, increased platelet adhesiveness, aggregability and TxA_2 release were documented [2]. Moreover, increased platelet sensitivity was reported towards von Willebrand factor (vWF), fibrinogen, immune complexes and glycated low-density lipoproteins as well as enhanced platelet release of intracellular material, like β-thromboglobulin, platelet factor 4 and platelet-derived growth factor, whereas platelet survival and platelet nitric oxide synthase activity decreased [3].

One of the most widely used group of antihyperglycaemic drugs for treating Type II (non-insulin-dependent) diabetes mellitus are sulphonylurea de-

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Abbreviations: ASA, Acetylsalicylic acid; PPP, platelet-poor-plasma; PRP, platelet-rich-plasma; QSAR, quantitative structure-activity relationships; TxA_2, thromboxane A_2.

rivatives. Their auxiliary antiplatelet activity could be useful. An influence of several sulphonylurea derivatives on platelet functions and metabolism in vitro and in vivo has been reported [4, 5] but there has been a lack of a systematic comparison of antiplatelet action of a representative series of sulphonylureas widely used. We therefore examined the antiaggregatory activity of nine commonly used hypoglycaemic drugs (gliclazide, glimepiride, glibenclamide, tolbutamide, gliquidone, glipolamide, glibornuride, chlopropamide, glipizide) and of four synthesised sulphonylurea derivatives of pronounced hypoglycaemic activity in animal tests [6]. We also included torasemide, a loop diuretic with sulphonylurea structure and activity reported as a weak TxA_2 receptor antagonist [7]. Acetylsalicylic acid served as a reference antiaggregatory drug. Quantitative structure-activity relationship (QSAR) studies were carried out to identify structural descriptors of the agents contributing to their antiaggregatory activity and hence clarify the molecular mechanism of their action.

Materials and methods

The procedures related to the study were approved by the Medical University of Gdańsk Bioethics Committee (NKEBN/694/2000). Blood samples were donated at the Blood Donation Centre in Gdańsk by healthy men who gave their informed consent. The donors were 22 to 60 years old, of normal glucose tolerance status, with a BMI in the normal range, with different smoking habits, and they had not taken drugs known to interfere with platelet function for at least 1 week before the venipuncture.

Materials. ADP was purchased from Sigma Chemicals (St Louis Mo., USA); DMSO and sodium citrate from Fluka Chemie (Buchs, Germany). Compounds 2A: N-{4-[2-(pyrazole-1-carbonamide)-ethyl]-benzenesulphonyl}-N′-cyclohexylurea, 5A: N-{4-[2-(4-ethylpyrazole-1-carbonamide)-ethyl]benzenesulphonyl}-N′-cyclohexylurea, 6A: N-{4-[2-(3,5-dimethylpyrazole-1carbonamide)-ethyl]-benzenesulphonyl}-N′-cyclohexylurea, 14A: N-{4-[2-(4-chloro-3,5-dimethyl-pyrazole-1-carbonamide)-ethyl]-benzenesulphonyl}-N′-cyclohexylurea and glipolamide were synthesised according to a procedure reported elsewhere [6]. Glibenclamide, chlorpropamide, tolbutamide and acetylsalicylic acid were gifts from Polpharma (Starogard Gdańsk, Poland), gliclazide from Jelfa (Jelenia Góra, Poland), glimepiride from Hoechst Marion Roussel (Frankfurt, Germany), gliquidone from Boehringer Ingelheim (Biberach, Germany), glibornuride from Grünenthal (Aachen, Germany) glipizide from Pfizer (Groton, Conn., USA) and torasemide from Roche Diagnostics (Mannheim, Germany).

In vitro platelet aggregation. Platelet aggregation was measured by the Born method [8]. Blood was collected by venipuncture into 3.8% sodium citrate solution (volume ratio 9:1) and centrifuged at 150 g for 10 min to obtain platelet-rich plasma (PRP). The remaining material was centrifuged at 2000 g for 15 min to obtain platelet-poor plasma (PPP). Standard platelet count was done and PRP was diluted with PPP to obtain 3×10^8 platelets per ml of plasma. Aggregation was induced by adding 50 μl of ADP solution in Tyrode's buffer to 450 μl of platelet suspension resulting in a final concentration of ADP equal to 10 μmol/l. Percent aggregation was calculated 6 min after adding the aggregating agent and was standardised by assuming that PPP represented 100% and PRP 0% light transmission. The drugs were dissolved in DMSO, which at the concentration used did not alter platelet aggregation. PRP was incubated with solutions of individual agents of fixed concentrations (7.5–480 μmol/l) for 10 min before challenge with an aggregating agent in a dual channel optical aggregometer (Model 490, Chrono-Log, Haverton, Pa., USA) at 37°C under continuous stirring (1000 rpm). Antiaggregatory properties of each compound were studied using blood from at least five donors.

Computer modelling and calculation of physico-chemical properties. As the molecular structure descriptors of the sulphonylureas the net positive charge on the sulphur atom, δ_S, and the energy of the lowest unoccupied molecular orbital, E_{LUMO}, were used in QSAR analysis. The descriptors were calculated by standard CAChe MOPAC 2000 program (Fujitsu FQS, Kraków, Poland). The logarithms of *n*-octanol/water partition coefficients were calculated with the use of ClogP software (BioByte, Claremont, Calif., USA). QSAR models were derived using Statgraphic Plus 4.0 software (Manugistics, Rockville, Md., USA).

Assessment of lipophilicity parameter log k_w by gradient elution. A newly elaborated gradient chromatographic method of lipophilicity assessment [9] was applied. Specificity of the procedure for sulphonylureas consisted in applying a so-called immobilised artificial membrane column IAM.PC.DD 30 mm× 4.6 mm i.d. (Regis, Morton Grove, Ill., USA).

Statistics. The data are expressed as means ± SEM. Differences between control and test were assessed by Student's *t* test; *p* value of less than 0.05 was considered statistically significant.

Results

Effects of sulphonylurea derivatives on platelet aggregation in vitro. Effects of drugs on platelet aggregation in vitro are shown (Fig. 1). The compounds appeared to be relatively weak inhibitors of aggregation. Therefore only the concentrations causing 25% inhibition of aggregation (IC_{25}±SEM, n=8–10) could be assessed for seven of them. The IC_{25} values for 7 out of 14 agents studied are given in Table 1. The IC_{25} for five most potent sulphonylureas: glimepiride, gliclazide, gliquidone, glibenclamide and compound 2A were 15.9, 18.6, 20.4, 28.5, 34.7 μmol/l, respectively. For ASA IC_{25} was 21.9 μmol/l. The IC_{25} values for glibornuride and torasemide were higher than 100 μmol/l and amounted to 123.0 and 316.2 μmol/l, respectively. Chlorpropamide, glipizide, glipolamide, tolbutamide and compounds 5A, 6A and 14A did not show measurable antiplatelet activity at the concentrations attainable.

Relationships between biological activity and chemical structure of sulphonylurea drugs. Drug lipophilicity parameter, *log k_w*, assessed chromatographically

Table 1. Antiaggregatory activity (IC_{25}) and structural parameters, log k_w, ClogP, δ_S, E_{LUMO}, of sulphonylurea derivatives

Agent	IC_{25} (μmol/l)	log k_w	ClogP	δ_S (electrons)	E_{LUMO} (eV)
Glimepiride	15.9	3.74	4.16	2.317	–0.929
Gliclazide	18.6	1.62	1.09	2.317	–0.92
Gliquidone	20.4	3.98	5.06	2.316	–0.866
Glibenclamide	28.5	3.73	4.23	2.316	–0.935
2A	34.7	2.96	2.51	2.318	–1.028
Glibornuride	123.0	2.9	3.18	2.878	–0.995
Torasemide	316.2	1.1	3.36	2.299	–0.98
Chlorpropamide	–	1.68	2.35	2.319	–1.142
Glipolamide	–	1.13	1.24	2.297	–0.896
Tolbutamide	–	1.79	2.50	2.317	–0.918
5A	–	2.7	2.96	2.317	–1.064
6A	–	3.29	2.78	2.317	–1.009
14A	–	3.9	3.50	2.317	–1.036
Acetylsalicylic acid	21.9				

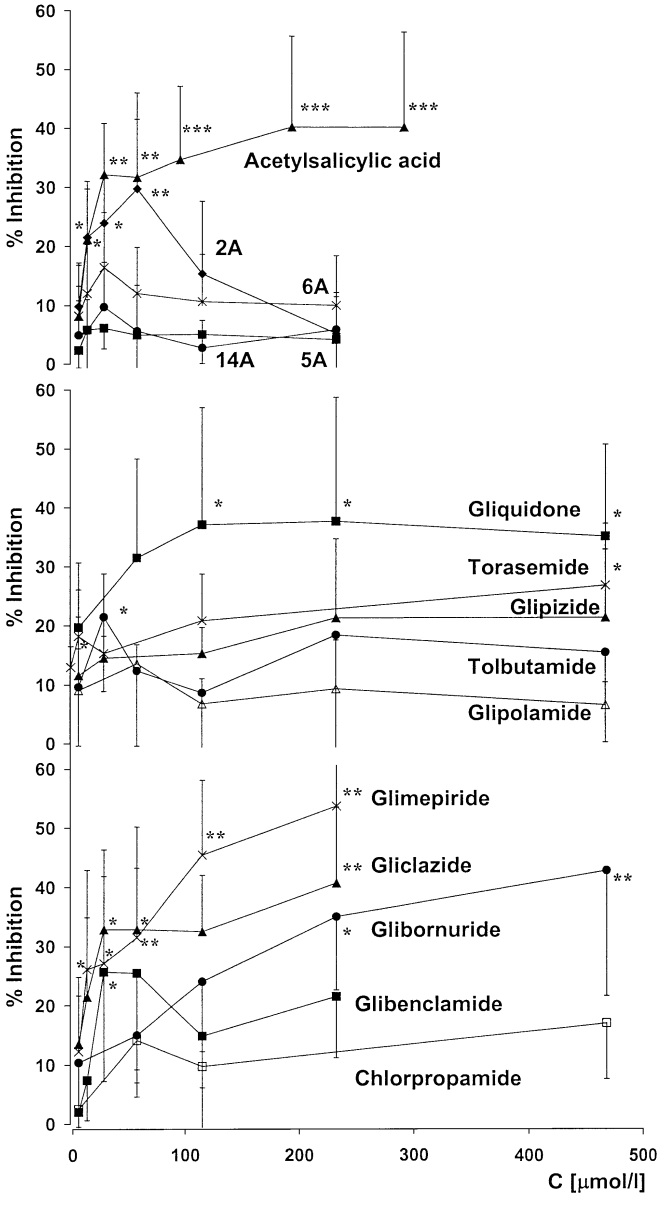

Fig. 1. Inhibition of ADP-induced platelet aggregation in vitro as a function of concentration of sulphonylurea derivatives. Values are means ± SEM for *n*=8–10 experiments; differences from control at 95%, 99% and 99.9% significance level are marked with one, two and three *asterisks*, respectively

was well correlated with the theoretical *ClogP* values (*r*=0.855). However, none of the two lipophilicity descriptors correlated with IC_{25}. QSAR analysis showed that only the descriptors accounting for differences in electronic properties of the agents were related to their inhibitory activity. The following QSAR equation was obtained:

$$\log 1/\mathrm{IC}_{25} = -116.756(\pm 11.2639) + 51.1442(\pm 5.0304)\delta_S + 3.3183(\pm 0.5895)E_{LUMO}$$

$$p = 0.0005 \qquad p = 0.0005 \quad p = 0.0049$$

$$n = 7; \quad R = 0.9817; \quad s = 0.1136 \quad \mathrm{F} = 53,19 \quad p = 0.0013$$

where the dependent variable is the logarithm of reciprocal of IC_{25} and the drug parameters are: δ_S, electron deficiency of the most positively charged atom (sulphur) and E_{LUMO}, energy of the lowest unoccupied molecular orbital. In the equation the values in parentheses indicate 95% confidence intervals. The *F* value represents the statistical significance of the regression model as reflected by the F – test, *n* is the number of agents considered, *s* is the standard error of estimated, *r* is the correlation coefficient and *p* indicates significance levels of individual regression terms and of the whole equation.

Discussion

The prothrombotic and proaggregatory state is strongly connected to pathophysiology of the diabetic com-

plications. Therefore, searching for hypoglycaemic agents with complementary antiaggregatory activity seems to be rational.

The hypoglycaemic agents studied possess a rather weak activity against ADP-induced aggregation. Most potent are: glimepiride, a third generation sulphonylurea drug and gliclazide and gliquidone, the second generation drugs. Activity of these three agents is comparable to that of ASA whereas IC_{50} reported for a specific antagonist, FPL 66096, against aggregation produced by ADP (30 or 100 μmol/l) is 0.0112 μmol/l [10].

The concentration of ADP 10 μmol/l, applied in our work, induces a near maximum aggregatory effect of ADP without unnecessarily overdosing the agonist. A similar concentration (20 μmol/l ADP) has been used by other authors [11] in aggregation inhibition studies in vitro. Our aim was to compare antiaggregatory activity of a representative group of sulphonylurea antidiabetics and for that reason the conditions had to be kept constant for all the drugs. On the other hand these conditions should allow for measurable results for all the 13 sulphonylureas studied. Therefore one agonist in a single concentration was chosen.

As noted by one of the referees the whole blood platelet aggregation methodology applied to the blood of diabetic patients would more likely reveal the clinical importance of the effects here shown with PRP. Such studies require clinical material which is not as readily available as that from normal volunteers and will be undertaken for the most active drugs preselected in this study.

The mechanism of antiaggregatory activity of sulphonylurea derivatives has not been fully explained yet. According to some authors gliclazide is a free radical scavenger [4] whereas glimepiride and glibenclamide exert their inhibitory effect via arachidonic acid metabolism [5]. The QSAR equation here derived comprises only electronic parameters of the agents as the descriptors of their antiaggregatory activity whereas lipophilicity parameters were insignificant in the QSAR analysis. This would support the hypothesis of a free radical mechanism of action of sulphonylurea derivatives which has already been suggested for gliclazide [4].

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