Pharmacodynamic Aspects of Tolbutamide, Glibenclamide, Glibornuride and Glisoxepide I. Dose Response Relations and Repeated Administration in Diabetic Subjects

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Summary. Pharmacodynamic studies were performed using tolbutamide, glibenclamide, glibornuride (Ro 6-4563) and glisoxepide (BS 4231). The four compounds were administered intravenously, and exact dose-response relations were established, calculating the ED 30 of the decrease in blood glucose in healthy volunteers. Accordingly, the dose corresponding to the ED 30 was given twice with a three hour interval to maturity-onset diabetics. Different dynamics of insulin response were observed after tolbutamide, glibornuride and glisoxepide on the one hand, and glibenclamide on the other. In contrast to other reports in the literature, an obvious uniformly weaker degree of insulin secretion was seen after each of the different sulphonylurea compounds when given repeatedly.

Comparaison des actions pharmacodynamiques de nouvelles substances du type des sulfonyles hypoglycémiantes I. Les relations entre les doses des substances et leur efficacité. Applications répétées aux sujets diabétiques

Résumé. Deux types d'expérimentations ont été réalisés: 1. Tolbutamide, glibenclamide, glibornuride (Ro 6-4563) et glisoxepide (BS 4231) ont été appliquées par voie intraveineuse aux sujets normaux pour déterminer les doses exactes, qui diminuent la concentration du glucose du sang de 30% (ED 30). 2. Ces doses (ED 30) ont été appliquées aux diabétiques âgés, deux fois dans un intervalle de trois heures. La deuxième injection de toutes les substances provoquait des concentrations d'insuline inférieures en comparaison avec les résultats initiaux. Ces

effets sont contraires aux résultats des recherches précédentes d'autres auteurs. Les concentrations d'insuline provoquées par les substances injectées sont indiquées et comparées.

Pharmakodynamik von Tolbutamid, Glibenclamid, Glibornurid und Glisoxepid. I. Dosiswirkungsbeziehungen und wiederholte Anwendung bei Diabetikern

Zusammenfassung. Vergleichende klinisch-experimentelle Untersuchungen wurden mit Tolbutamid, Glibenclamid, Glibornurid (Ro 6-4563) und Glisoxepid (BS 4231) durchgeführt. Zunächst wurden exakte Dosiswirkungsbeziehungen aufgestellt, die durch i.v. Gabe der vier Substanzen an Stoffwechselgesunden ermittelt wurden. Aufgrund dieser Untersuchungen wurde die ED 30, bezogen auf den Blutzuckerabfall, errechnet. Daraufhin wurden die vier Sulfonylharnstoffe in der Dosierung der ED 30 Altersdiabetikern zweimal im Abstand von drei Stunden intravenös appliziert. Es zeigte sich ein deutlicher Unterschied in der Dynamik der Insulinsekretion nach Tolbutamid, Glibornurid und Glisoxepid einerseits und Glibenclamid andererseits. Im Gegensatz zu anderen Untersuchungsgruppen wurde bei allen vier Präparaten deutlich niedrigere Insulinspiegel nach der zweiten Stimulation festgestellt als nach der ersten Gabe.

Key words: Tolbutamide, glibenclamide, glibornuride (Ro 6-4563), glisoxepide (BS 4231), i.v. dose response relations, dynamics of insulin secretion, repeated administration of sulphonylurea compounds.

A great number of sulphonylurea compounds has been developed since the introduction of carbutamide and tolbutamide for therapy of diabetes mellitus more than 15 years ago. In 1966 glibenclamide was discovered, a substance with an hitherto unknown blood glucose-lowering potency. The mechanism of action of all these drugs is based on the release of stored pancreatic insulin, though a variety of quantitative [2, 16, 21, 22, 23, 24, 25] and qualitative [7, 8, 10, 11, 13, 18] differences has been described for the action of glibenclamide.

Recently, some new sulphonylureas with a high potency similar to that of glibenclamide have been presented. The main purpose of this study was to find out exact dose-response relations of these compounds compared with tolbutamide. Furthermore, certain differences in the dynamics of insulin secretion were investigated, as already published for tolbutamide and

glibenclamide [14, 20]. When oral hypoglycaemic agents were introduced for therapy of diabetes, one of the most important questions was whether the degree of beta-cell stimulation decreased or increased after application of these substances. Thus, under certain conditions, maturity-onset diabetics have failed to show a satisfactory increase of serum insulin and a subsequent decrease of blood glucose after repeated administration of tolbutamide at 24-h intervals [19]. In contrast, investigations by Raptis *et al.* [20] indicated that two repeated administrations of glibenclamide with a 4-h interval cause an equally high increase in serum insulin and decrease of blood glucose. In respect to these contradictory papers in the literature, the present report will demonstrate the effects of tolbutamide, glibenclamide and the recently developed sulphonylurea compounds on the discussed parameters.

Materials and Methods

Experimental subjects. The subjects studied were normal healthy volunteers, and diabetics with a mild metabolic disorder and fasting blood glucose levels of about 150 mg%. No patient was under any treatment at the time of the study except dietetic regulations. Oral hypoglycaemic agents had been withdrawn for at least 48 h. All persons were of normal weight.

Experimental design. First of all, the dosage causing a 30% decrease of blood glucose from the baseline level (ED 30), as described by Frerichs and Puls [6], was determined after intravenous application of tolbutamide, glibenclamide, glibornuride (Ro 6-4563) and glisoxepide (BS 4231).¹ The four substances were given to the same five healthy volunteers in three different amounts. Samples for determination of blood glucose, free fatty acids and serum insulin were obtained at 0, 5, 10, 20, 40, 60 and 90 min. The ED 30 of each compound was evaluated. Having established

Analytical procedures. Blood glucose was measured by the procedure of Hoffman [12] with a Technicon autoanalyzer; serum insulin by the immunoassay of Meade and Klitgaard [15] in the modified form of Melani *et al.* [17]; and free fatty acids by the method of Dole and Meinertz [5]. Decrease of blood glucose, as well as the decrease of free fatty acids, were expressed in per cent of the starting levels. The results were statistically evaluated according to Student's *t*-test.

Results

Determination of the ED 30: The effect of tolbutamide, glibenclamide, glibornuride and glisoxepide on blood glucose is shown in Fig. 1. Minimum blood glucose levels were usually obtained after 40 min. However, the maximal effect of glibenclamide was found somewhat later when small dosages were given. Using higher doses of this compound, maximal activities for lowering blood glucose were observed earlier.

Fig. 1. Changes in blood glucose in 5 healthy volunteers following i.v. application of different sulphonylureas in various doses. (Mean of starting levels $\pm$ SEM: 95.8 ± 1.07 mg%)

these data, 10 non-obese, maturity-onset diabetics received the four substances over several days. The ED 30 was administered twice with a 3-h interval. Samples were obtained at 0, 5, 10, 20, 40, 60, 90, 120, 180, 185, 190, 200, 240 and 270 min. All tests were carried out starting at 8 a.m. under resting conditions after an overnight fast.

¹ We are indebted to the Hoffmann La-Roche Inc. for the gift of glibornuride (Ro 6-4563) and to the Bayer and Schering Inc. for the gift of glisoxepide (BS 4231).

Fig. 2 demonstrates the insulin response to the four compounds. Two different types of the dynamics of insulin secretion were observed: tolbutamide, glibornuride and glisoxepide caused an immediate sharp peak of serum insulin with effective levels continuing for about 20 min; when glibenclamide was given, however, there was both a delayed output of serum insulin and a plateau-like maximum with effective concentrations lasting for about 60 min. Equipotent doses of these compounds causing a 30% decrease of blood glucose were 7.5 mg tolbutamide,

Fig. 2. Variations in immunoreactive insulin (IRI) in 5 healthy volunteers following i.v. application of various sulphonylureas in different doses

Fig. 3. Variations in blood glucose in 10 maturity-onset diabetics following i.v. administration of two repeated doses of the various sulphonylureas with a 3-hour interval, the doses being the ED 30 evaluated in healthy normals. (Mean of starting levels $\pm$ SEM: 158 ± 4.3 mg%)

Fig. 4. Variation in free fatty acids (FFA) in 10 maturity-onset diabetics following i.v. administration of two repeated doses of the various sulphonylureas with a 3-h interval, the doses being the ED 30 evaluated in healthy normals. (Mean of starting levels $\pm$ SEM: $887 \pm 26.6 \mu\text{E/l}$)

Fig. 5. Variations in immunoreactive insulin (IRI) in 10 maturity-onset diabetics following i.v. administration of two repeated doses of various sulphonylureas with a 3-h interval, the doses being the ED 30 of blood glucose decrease evaluated in healthy normals

0.006 mg glibenclamide, 0.12 mg glibornuride and 0.02 mg glisoxepide per kg body weight.

Response to repeated administration: As Fig. 3 indicates, the four compounds caused an almost linear decrease in the levels of blood glucose. The effect of the four drugs in lowering blood glucose was almost the same, and with no statistical difference using the ED 30. As a matter of fact, the dosages chosen for this part of the experiment seemed to be comparable for diabetic subjects also.

Similar observations were obtained for the levels of free fatty acids (FFA) (Fig. 4). An immediate initial rise, probably due to a short-lasting release of catecholamines, is followed by a distinct decrease of FFA-levels to about 25%. Maximal antilipolytic activities were found after 90 min. The second injection caused a further decrease lasting for one hour only. Depressions of FFA-levels after tolbutamide, glibornuride and glisoxepide were absolutely similar, whereas the decrease after glibenclamide continued for 4.5 h. Measurement of glycerol levels showed almost similar results.

Dynamics of insulin release again indicated the characteristic differences between tolbutamide, glibornuride and glisoxepide on the one hand and glibenclamide on the other (Fig. 5). The first three substances caused maximal serum insulin levels already 5 min after injection. After 10 min, the concentrations had dropped more than 50%. A repeated injection after the 3-h interval caused only a slight increase in serum insulin. Such peaks were significantly lower than those observed after the first stimulation. In accordance with the blood-glucose-lowering activity and the decrease of free fatty acids, the profiles of insulin secretion after tolbutamide, glibornuride and glisoxepide did not show significant differences. In contrast, serum insulin levels after glibenclamide were significantly higher 60 and 90 min after injection ($p < 0.05$) when compared with those after the three other drugs. Apart from the observed differences, glibenclamide and the other compounds were similar in respect of reduced insulin peaks after repeated administration.

Discussion

Our results demonstrate the strong beta-cytotropic activity of all the sulphonylurea compounds investigated in this study. As this is already known for glibenclamide, the new drugs acted in the range of a few milligrams. However, comparing the ED 30 after i.v. administration, glibenclamide was found to be 1250 times more potent than tolbutamide, whereas glibornuride and glisoxepide revealed only a 63 and 375 times higher potency respectively. Since differences in blood-glucose-lowering activities were similar when given to normal subjects and diabetics, these relations may be valid for both groups. Since there are differences in the degree of absorption, these data cannot be transferred

to oral administration. Previous reports [9] and studies performed by Bigler *et al.* [3] and by Christ *et al.* [4] indicated that tolbutamide, glibornuride and glisoxepide have an absorption rate of about 90%, and glibenclamide of only less than 50%. In spite of the different degrees of absorption, examinations concerning the oral ED 30 of tolbutamide and glibenclamide performed by Frerichs and Puls [6], indicated a surprising conformity with the amounts of the ED 30 obtained by our intravenous experiments (i.e. 5.74 mg tolbutamide and 0.0084 mg glibenclamide per kg body-weight). Tolbutamide and glibenclamide were orally administered as solutions or suspensions of the pure substances by these authors, whereas the other studies on the degree of absorption were performed with tablets of quantitatively and qualitatively the same composition as for the normal tablet preparation. Thus, it appears that suspensions of the two sulphonylurea derivatives are absorbed more quickly and obviously to a much higher degree compared with the administration of tablets, thus giving serum concentrations approximately similar to those obtained by intravenous injections. Distinct differences were observed in the dynamics of insulin secretion. Glibornuride and glisoxepide caused a sharp initial increase of serum insulin levels lasting for a relatively short time. In contrast to this tolbutamide-type of stimulation, glibenclamide showed its well-known delayed insulin response with a plateau-like maximum [10, 13, 20]. After administration of tolbutamide, glibornuride or glisoxepide, healthy subjects showed, surprisingly, a much smaller decrease in blood glucose than did diabetics, despite a higher total output of insulin. The maximal decrease in blood glucose was obtained in healthy subjects at 40 min, whereas this was prolonged in diabetics for 3 h. These findings may be explained by even a slight hypoglycaemia in healthy subjects causing glycogenolysis in the liver.

The findings obtained with glibenclamide are quite different. In diabetics, administration of glibenclamide gave an output of insulin that was considerably higher than that given by the other compounds, but with no difference in the lowering of the blood glucose. Data presented by Bänder *et al.* [1] indicated a certain effect of sulphonylurea compounds on the adrenal medulla. It seems possible that glibenclamide, more than other sulphonylurea derivatives, stimulates the adrenal medulla, thus mobilizing liver glycogen by mean of release of catecholamines. This would result in a balance between increased levels of blood glucose on the one hand and increased levels of serum insulin on the other. The extent of antilipolysis would be consistent with this inference.

It is quite surprising that in previous studies an equally high output of insulin was reported after two repeated i.v. administrations of glibenclamide [20]. This effect was seen in both healthy subjects and maturity-onset diabetics. The present study does not confirm these results. Distinctly lower increases in

serum-insulin levels were found following the repeated administration of glibenclamide. The difference in the findings is certainly due to the relatively high dosages used in the other study. It should be pointed out that exact dose-response relations are absolutely necessary if pharmacodynamic aspects of several experimental compounds are to be investigated and discussed.

We should like to conclude that the recent sulphonylurea derivatives glibornuride and glisoxepide have a higher potency for lowering the blood glucose than has tolbutamide. There are no differences, however, in the dynamics of insulin secretion. The four substances gave upon repeated injection a similarly reduced level of insulin.

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