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Antidiabetogenic Effect of Fusidic Acid in Diabetes Prone BB Rats: A Sex-Dependent Organ Accumulation of the Drug is Seen

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Abstract: Fusidic acid and its sodium salt (fusidin) are widely used antistaphylococcal drugs which possesses immunomodulatory properties. This prompted us to investigate whether high concentrations of fusidin could lower the diabetes incidence in diabetes-prone BB (BioBreeding) rats. As fusidin has previously been claimed to be poorly absorbed in rats after oral administration we wanted to measure the activity of the drug in various organs. Three groups of BB rats were used: 63 rats received fusidin dissolved in drinking water; 65 rats received chow containing fusidin; and 72 rats served as controls. The content of fusidin in the organs were examined microbiologically. The incidence of diabetes was significantly lower in the two fusidin-treated groups compared to the control group. The incidence was lower for male than for female rats in both experimental groups while no gender difference was seen in the control group. The female rats had a substantially higher content of fusidin in their organs than the males regardless of the administration way and regardless of diabetes outbreak or not. Interestingly, the fusidin treated non-diabetic rats displayed a lower random blood glucose level than the controls. *In conclusion*, fusidin is well absorbed after oral administration and it significantly reduces the diabetes incidence in BB rats. Fusidin accumulates substantially more in female rats which may be due to the steroid structure of fusidin. Whether the same phenomenon takes place in human beings is not known.

Type 1 diabetes mellitus is an autoimmune disorder characterized by progressive destruction of the pancreatic β-cells. When the diagnosis is made, usually about 10% of the β-cells is left. Hence, an obvious approach in order to prevent the disorder would be intervention directed against the autoimmune process at an early stage of the disease aimed at preserving a critical mass of β-cells for maintenance of normal glucose tolerance. The antibiotic fusidic acid (Godtfredsen *et al.* 1962) might either alone or more likely in combination with one or more drugs represent such an intervention therapy.

Fusidic acid and its sodium salt (fusidin) have steroid primary structures (fig. 1) but without any corticosteroid bioactivity. Fusidin is widely used in clinical practise as an antistaphylococcal antibiotic but it also possesses immunomodulatory properties. It acts on mononuclear cells by reversibly reducing the amount of interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-α) released by activated cells. The production of the T-cell derived cytokines IL-2 and interferon is also suppressed as well as the co-stimulatory activities of IL-1 and IL-6 on T-cells (Bendtzen *et al.* 1990). Fusidin also prevents the inhibitory effect of IL-1β and the stimulatory effect of IL-6 on glucose-induced insulin production *in vitro* (Bendtzen *et al.* 1992). Both IL-1 and IL-6 are thought to be of pathogenetic importance for the development of diabetes (Bendtzen 1989; Castano & Eisenbart 1990). These functions of fusidic acid are strikingly similar to those of cyclosporin A which is a powerful suppressor of immunoinflammatory processes. In contrast to cyclosporin A, side effects observed during the clinical use of fusidic acid are few and relatively harmless and presumably not related to an immunosuppressive function of the drug (Garrod *et al.* 1981). Fusidic acid has already been used with success in various rodent animal models and some human autoimmune diseases such as chronic endoge-

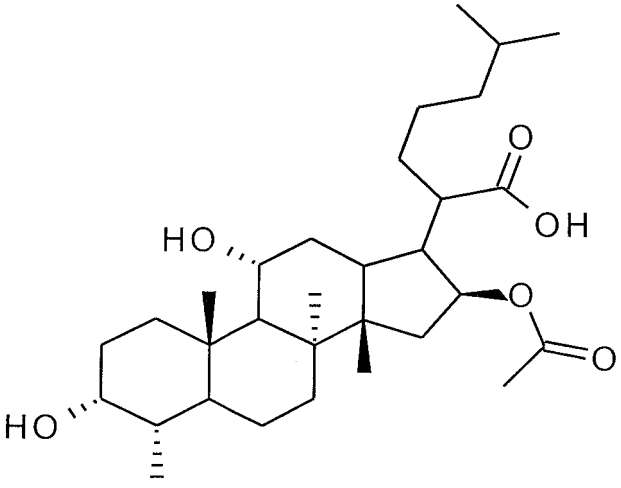

Fig. 1. Structure of fusidic acid.

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nous uveitis (Bendtzen *et al.* 1991), Guillain-Barré syndrome (Nicoletti *et al.* 1998; Di Marco *et al.* 1999), T-cell-dependent hepatic lesions (Nicoletti *et al.* 1997), Crohn's disease (Langholtz *et al.* 1992), Behcet's colitis (Langholtz *et al.* 1991) and multiple sclerosis (Nicoletti *et al.* 1999). There seems to be a positive effect of fusidic acid on the course of diabetes in patients with newly diagnosed disease (Nicoletti *et al.* 1991). This prompted us to examine the effect of fusidin in a well-established animal model of human insulin-dependent diabetes mellitus (Buschard *et al.* 1992). We found fusidin capable of significantly reducing diabetes incidence as also found by Nicoletti *et al.* (1994). The aim of the present study was dual: to see whether higher concentrations of fusidin could lower the diabetes incidence further, using two ways of administration, by drinking water and chow, and measure the activity of the drug in various organs since fusidin has earlier been claimed to be poorly absorbed in rats after oral administration (Findon *et al.* 1991).

Materials and Methods

Fusidin. The water soluble sodium salt of fusidic acid (fusidin), a gift from Leo Pharmaceutical Products (Ballerup, Denmark) was dissolved every day in sterile water at a concentration of 10 mg/ml and given to the rats as drinking water *ad libitum*. This dosage was chosen according to previous pilot studies performed in our laboratory to ensure high concentrations of the antibiotic in various organs (Buschard *et al.* 1992). The average daily intake of water was 20.0 ml for male rats and 16.3 ml for female rats, compared to respectively 27.0 ml and 21.9 ml in the control group.

The fusidin chow was prepared at Brogaarden Feedsupply (Gentofte, Denmark) in a production concentration of 28 mg fusidin/g chow. Samples of the chow was examined at Leo Pharmaceutical Products to determine the actual concentration of fusidin after the production fase at the factory; the concentration was determined microbiologically to be 18.8 mg/g. This dosage was chosen after a pilot study, to ensure that the experimental group and the control group consumed the same amount of chow to avoid the effect of fasting on the diabetes incidence (Pedersen *et al.* 1999). During the study the food intake was measured once weekly. For full grown rats the average daily fusidin chow intake was 18.03 g for male rats and 13.04 g for female rats, compared to respectively 18.53 g and 13.60 g in the control group.

The concentrations of fusidic acid in serum and various organs were determined microbiologically as described elsewhere (Duvold *et al.* 2001). In brief, for the serum samples a turbidimetric method using Staphylococcus aureus as test organism was applied. For the organ samples an agar diffusion method using Corynebacterium Xerosis as test organism was applied. Diethanolaminfusidate was used as standard reference.

BB (BioBreeding) rats (purchased from Møllegaard, Ll. Skensved, Denmark) were paired at the Bartholin Institute. The animals were weaned after 3 weeks, placed randomly in the three groups with free access to chow and drinking water. The study consists of three groups of diabetes prone BB rats, 1) a group of 63 rats receiving fusidin dissolved in their drinking water and normal chow, 2) a group of 65 rats receiving chow containing fusidin and normal drinking water, and 3) a group of 72 rats with free access to as well normal drinking water as chow. The animals were observed twice daily and weighted and examined for glycosuria ("Tes-tape", Lilly, Indianapolis, IN, USA) weekly. Diabetes was diagnosed if at least ++ glycusuria (1/4%) with more than 10% weight loss (compared to previous week) was observed. When this occurred the animals were sacrified, blood glucose values measured and organs (serum, pancreas, liver and kidney) taken out. The caudal part of the pancreas was prepared for histological examination while the rest of

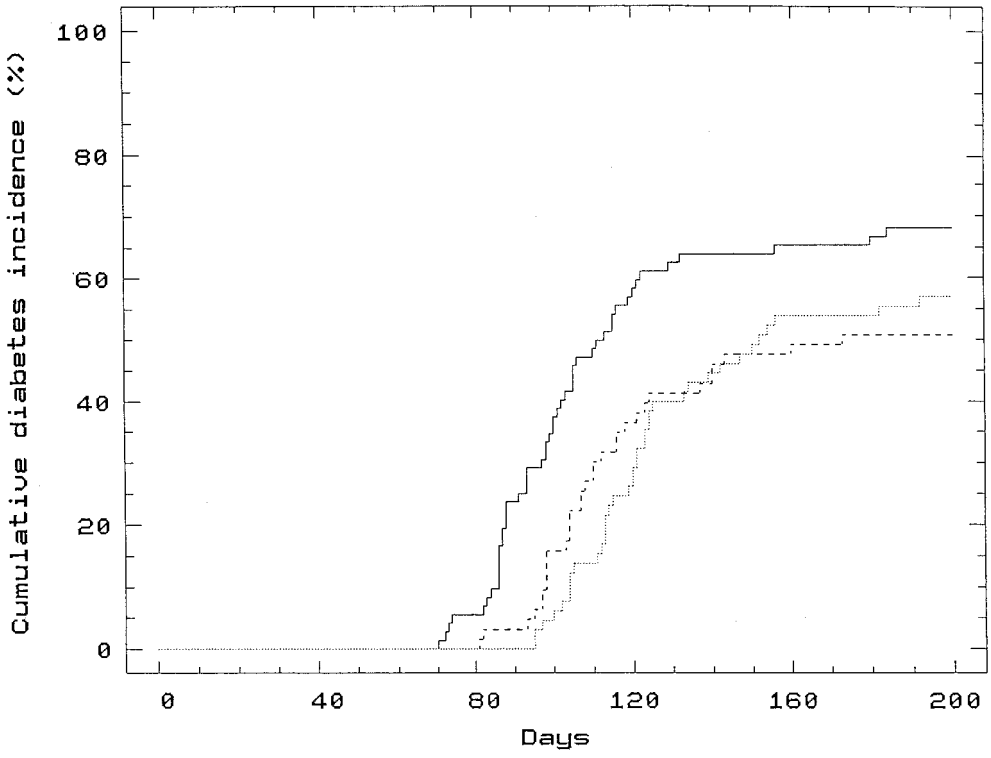

Fig. 2. Diagram showing cumulative diabetes incidense for rats treated with fusidin water (n=63, dashed line), fusidin chow (n=65, dotted line) and control rats (n=72, full line).

Table 1.

Mean age±S.E.M. in days at onset of diabetes. Significance tests refer to the results of the control BB rats.

	Female	Male	Male+female
Control	99±5	106±5	103±3
	(n=25)	(n=24)	(n=49)
Fusidin chow	126±6	123±5	124±4
	(n=18)	(n=19)	(n=37)
	P=0.001	P=0.02	P=0.006
Fusidin water	111±5	116±6	113±4
	(n=21)	(n=11)	(n=32)
	ns	ns	ns

ns=not significant.

the pancreas together with the liver and kidney samples were put in (phosphate buffered saline), homogenated, stored at −20° and, finally, send to Leo Pharmaceutical Products for determination of the content of fusidin. The animals which did not develop diabetes were sacrified at day 200 and the organs treated the same way.

The study complies with the European Community guidelines for the use of experimental animals and was approved by the local ethics committee.

Histological studies. The paraffin-embedded pancreata were cut into 4 μm sections and stained with haematoxylin and eosin. The insulitis was scored semiquantitatively with *Grade 0* denoting morphologically unaffected islets, *Grade 1* a very disperse intra-insular infiltration of mononuclear cells, *Grade 2* a more pronounced intra-insular infiltration, either disperse or small clusters of mononuclear cells and, finally, *Grade 3* denoting islets dominated by infiltrating mononuclear cells. Islets with peri-insular infiltration were placed in group 2 or 3 depending on the intra-insular infiltration of mononuclear cells. Ten islets were scored in each section and the average was calculated and considered the final score of insulitis. Thirty-two rats with free access to fusidin containing drinking water and 31 rats with free access to fusidin containing chow were investigated histologically and then compared with sections from 42 control BB rats. The examination was performed "blindly".

Statistics. Cumulative diabetes incidence was calculated by Kaplan-Meier estimation. Statistical significance was evaluated by the log-rank test. Other differences were estimated by Student's t-tests, except the insulitis data which were evaluated using the Mann-Whitney test. Data are presented as mean±S.E.M.

Results

Fig. 2 shows that the cumulative diabetes incidences of both of the fusidin-treated groups are significantly lower than for the control group. The diabetes incidence of the control group was 68.1%, compared to the incidence being 50.8% (P=0.01) for the animals receiving fusidin in their drinking water and 56.9% (P=0.01) for the rats receiving fusidin chow. The diabetes incidence was generally lower for male than female rats in both experimental groups (52.8% versus 62.1% in the chow group and 39.3% versus 60.0% in the water group), while no sex difference was found in the control group (66.7% versus 69.4%). As seen from table 1 the mean day of debut of the disease was 102 days in the control group versus 124 days in the fusidin chow group (P=0.006) and 113 days in the fusidin water group (ns). According to time of onset there was not found any sex difference.

The daily intake of fusidin in the chow group was 339 mg/day for male rats and 245 mg/day for female rats. In the fusidin water group the daily intake of the drug was 200 mg/day for male rats and 163 mg/day for female rats. The difference in the consumed amount of fusidin depending on the way of administration was reflected in the measurement of antibiotic activity in the various organs, the content of fusidin being higher in the fusidin chow group in all organs (table 2 and 3). What is more surprising, however, is that female rats have a significantly higher content of fusidin in their organs than males, regardless of administration way and regardless of diabetes outbreak or not (table 2 and 3). The content of the drug in the various organs of male rats is significantly higher for the diabetic rats compared to the non-diabetic rats (the liver value of the fusidin chow group is not significant, though). The tendency is the same for the female rats although it is remarkable that the liver values for female rats is significantly higher in the non-diabetic animals, regardless of administration way.

Weight values of the diabetic and non-diabetic rats are seen in table 4. The weights of the non-diabetic fusidin-treated rats are similar to the control rats. The weight values of the diabetic fusidin-treated rats were generally lower than the values of the diabetic control rats, though only significantly lower for the fusidin chow group. This is in good agreement with the clinical observation of a more fulminant diabetes onset in the fusidin-treated group.

The result of the examination of the islets of Langerhans for insulitis is seen in table 5. As seen, the insulitis score tended to be lower in non-diabetic animals compared to

Table 2.

Average antibiotic activity of fusidic acid in sera (μg/ml) and tissue homogenates (μg/g wet weight)±S.E.M. in female rats. Significance tests refer to the results of non-diabetic rats.

	Fusidin chow		Fusidin water	
	non-diabetic (n=7)	diabetic (n=16)	non-diabetic (n=13)	diabetic (n=20)
Serum	11.7±3.3	7.4±1.3	2.6±0.5	5.2±0.8
		ns		P=0.02
Pancreas	10.7±3.1	16.9±5.3	1.1±0.2	8.1±1.7
		ns		P=0.003
Liver	68.0±7.4	44.8±3.9	62.4±5.3	38.9±3.6
		P=0.006		P=0.006
Kidney	7.9±2.0	12.2±2.5	2.0±0.5	6.2±1.2
		ns		P=0.009

Table 3.

Average antibiotic activity of fusidic acid in sera (μg/ml) and tissue homogenates (μg/g wet weight)±S.E.M. in male rats. Significance tests refer to the results of non-diabetic rats.

	Fusidin chow		Fusidin water	
	non-diabetic (n=14)	diabetic (n=14)	non-diabetic (n=15)	diabetic (n=9)
Serum	0.4±0.1	3.8±1.1 P=0.009	0.3±0.1	1.8±0.9 P=0.04
Pancreas	1.7±0.3	11.0±3.8 P=0.03	0.6±0.1	2.9±1.3 P=0.03
Liver	15.4±2.7	19.1±2.4 ns	10.5±1.8	17.7±3.0 P=0.04
Kidney	0.7±0.2	4.4±1.1 P=0.005	0.3±0.1	1.3±0.5 P=0.02

the animals which developed diabetes, applying to all three groups of the study (albeit not significant for the female fusidin water group and the female and male fusidin chow group). The study also showed that the diabetic fusidin-treated rats (chow as well as water) had lower or similar scores of insulitis compared to the control group, the effect being most pronounced for female rats. It should be noted that overall the degree of insulitis was modest and massive insulitis was only rarely seen.

Table 6 shows the average blood glucose values for non-diabetic rats. Interestingly, the fusidin treated non-diabetic animals displayed a lower random blood glucose level than the controls (7.3±0.2 nM versus 8.6±0.4 nM, P=0.002).

Discussion

The present study shows that fusidic acid does reduce diabetes incidence. The method of oral administration of the drug is not of importance. Fusidic acid is well tolerated and contrary to earlier findings (Findon *et al.* 1991), fusidic acid is well absorbed after oral administration. Rather surprisingly, the study also shows that the concentration of fusidic acid in all organs are markedly higher in female than in male rats. Finally, we find that treatment with fusidic acid gives lower blood glucose values in the animals not developing diabetes.

The study confirms earlier studies of fusidic acid having a positive effect on the incidence of diabetes (Buschard *et al.* 1992; Nicoletti *et al.* 1994; Nicoletti *et al.* 1995). The finding of fusidin being able to delay the onset of the disease has not been reported before. The effects are clearly not dose-dependent since the amount of fusidin consumed in the chow group by far exceeds the amount consumed by the fusidin water group without any effect on the incidence parameter. This was not due to the water preparation being more easily absorbed as the organ concentrations were correspondingly higher in the chow group (table 2 and 3). Furthermore, female rats had many times higher values of fusidic acid in their organs without this being reflected on the diabetes incidence.

Overall, the concentrations of fusidic acid in the various organs were higher for the fusidin chow than for the fusidin water-administered animals. This may to a certain degree be due to the fact that the intake of fusidic acid was higher for the fusidin chow-treated rats than for the fusidin water-treated rats. In contrast to the other organs, the liver values of both ways of administration were quite similar. No research elucidating this specific matter seems to have been performed.

As mentioned the animals in the fusidin water group drank less water than the control rats. This is probably because the drug is not very tasty (a fact that can be corrected by using artificial flavouring if to be used in human beings). Nevertheless, the rats continued to thrive and had similar weight gains as the control group (table 4). From tables 2 and 3 it is also seen that diabetic animals generally had

Table 4.

Weight values for diabetic and non-diabetic rats±S.E.M. Significance tests refer to the results of control rats.

	Non-diabetic rats		Diabetic rats	
	female	male	female	male
Control	231±4 (n=9)	388±15 (n=11)	162±8 (n=25)	231±8 (n=24)
Fusidin chow	233±17 (n=7) ns	381±8 (n=14) ns	142±4 (n=18) P=0.05	201±7 (n=19) P=0.009
Fusidin water	227±3 (n=13) ns	403±10 (n=14) ns	140±4 (n=21) ns	225±8 (n=11) ns

Table 5.

Average degree of insulitis±S.E.M. Significance tests refer to the results of control rats.

	Non-diabetic rats		Diabetic rats	
	female	male	female	male
Control	0.7±0.2	0.6±0.1	1.1±0.1	1.1±0.1
	(n=6)	(n=8)	(n=13)	(n=15)
Fusidin chow	0.9±0.2	0.8±0.3	0.9±0.1	1.1±0.1
	(n=6)	(n=6)	(n=11)	(n=8)
	ns	ns	P=0.05	ns
Fusidin water	0.6±0.2	0.4±0.1	0.9±0.1	1.1±0.2
	(n=7)	(n=8)	(n=11)	(n=6)
	ns	ns	ns	ns

significantly higher values of fusidic acid in their organs when compared to non-diabetic animals. This is presumably because of the polydipsia and hyperphagia accompanying diabetes onset. Furthermore, an age-dependent effect is not likely since no correlation was found between fusidic acid concentration in sera and age.

To some surprise we found a marked sex difference of fusidin content in the various organs; the tissue and serum values of the female rats exceeding the male by a factor 2 to 10. To our knowledge this accumulation of fusidic acid in female rats has not been described before. Part of the explanation for this is presumably to be found in the fact that fusidin is fat-soluble (Stewart 1964). However, extra female fat deposits are primarily localized as subcutaneous fat and extra fat content in the organs of female rats would only explain a small part of the striking difference. Fusidic acid is to a very large extent metabolised in the liver and excreted in the bile (Godtfredsen & Vangedal 1966). As the chemical configuration of fusidic acid does have high degree of similarity to the configuration of oestrogen one might hypothesize whether fusidic acid is a competitor to the same enzyme system in the liver as metabolises oestrogens: The metabolism of fusidin is being hindered and accumulations takes place. Whether the same phenomenon is seen in man is not known.

Finally we found fusidin capable of producing lower blood glucose values in non-diabetic animals. That is, even though diabetes does not evolve, fusidin exerts a protective effect, leaving the animals with a presumably greater reserve of β-cells and thereby capacity to resist later stressors that could potentially lead to diabetes. This should further prompt trials of fusidin in humans predisposed to diabetes mellitus.

Thus, in conclusion, the study confirms earlier findings of fusidin as a potent inhibitor of diabetes development but furthermore shows the drug to possess protective abilities even in non-diabetic animals. Regarding the antidiabetogenic mechanism of fusidin the antibiotic effect can not be ruled out. Fusidic acid is well absorbed, well tolerated and the animals were thriving. Nothing emerging from this study contradicts human trials of fusidin as an intervention therapy in predisposed individuals (though presumably most effective as an combination therapy with other drugs). Obviously, the antidiabetogenic effect of fusidic acid is not merely a question of achieving high organ concentration since female rats exhibited the highest concentrations whereas the male rats displayed the lowest diabetes incidence. This fact represents a pharmacologically interesting puzzle which should be addressed in human trials. Prior to more extensive human trials though, it seems most safe to investigate whether the described accumulative effect of fusidin in female rats also apply to human females and whether there are connected side-effects. If connected side-effects emerges this might lead to sex-dependent dosage regimes according to serum levels, which not only would apply to the present study but to other kinds of systemic fusidin treatment.

Table 6.

Average blood glucose values±S.E.M. for non-diabetic female and male rats. Significance tests refer to the results of control rats.

	Female	Male
Control	8.8±0.6	8.5±0.6
	(n=9)	(n=11)
Fusidin chow	8.0±0.4	7.6±0.4
	(n=7)	(n=14)
	ns	ns
Fusidin water	6.6±0.3	7.2±0.4
	(n=13)	(n=14)
	P=0.004	ns

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