Medical University of Graz

Premature termination of a Clinical Trial

Full title of the clinical Trial:

Pilot study of the incretin effect on the immunological phenotype in healthy subjects and subjects with type 1 diabetes: Step 4 of the Austrian Diabetes Prevention Programme (ADPP-004).

EudraCT Number: 2011-006300-12

Sponsor: Medical University of Graz
Represented by (name): Dr. Gerlies Treiber

Reason for premature termination of the clinical trial:

Investigation of incretin effect on the immunological phenotype in subjects with type 1 diabets (second part of protocol) was not performed. After evaluation of available resources (laboratory appointments, budget and volunteers) and prioritization of immunophenotyping slots and recruitement of type 1 diabetes patients for other clinical trials, the ADPP004 study was not continued.

Study results (if available):

Results ADPP004 – Healthy

Data published as part of the PhD-Thesis from Barbara Prietl (2016)

The aim of the Austrian Diabetes Prevention Program 4 study was to assess the short term effects of the treatment with GLP-1R agonists or DPP4-inhibitors on peripheral immune cells in healthy volunteers. The study was designed as monocentric, blinded and randomized. Healthy adults, aged at least 18 years were enrolled and the study was registered at ClinicalTrials.gov (NCT01782261). Exclusion criteria were preexisting inflammatory systemic diseases, disorders in calcium metabolism, kidney diseases, a family history in autoimmune diseases, allergic predisposition and pregnancy. All individuals were randomized into two study

groups (liraglutide and saxagliptin group) using the Randomizer program (http://www.randomizer.at). Study medication was either liraglutide (Victoza®, Novo Nordisk, Denmark) to be applied as subcutaneous self-injection by the study participant or saxagliptin (Onglyza®, BMS, USA) to be administered orally. Liraglutide was initially applied in a starting dose (0.6 mg/day) for 1 week followed by self-injection of 1.2 mg/day for 3 weeks. Saxagliptin was taken orally in a constant dose (5 mg/day) for the whole duration of 4 weeks. 4 visits were performed within 8 weeks for each study participant. After the baseline visit, patients were seen after 2, 4 and 8 weeks. Safety evaluation, study medication supply and venous blood draw were performed at each visit after a 12h overnight fast. In total 65 ml of blood were drawn per visit. Demographic baseline data are shown in Tab. 1.
In total 15 healthy adults were included in this randomized trial. All participants were randomly chosen for liraglutide (Victoza®, Lira group) or for the saxagliptin (Onglyza®, Saxa group) treatment. Because of the different application mode of the two investigated treatments (liraglutide as daily self-injection versus DPP-4 inhibitor as daily oral intake) it was not possible to conduct this study in a double-blinded way. Nevertheless the whole laboratory staff was blinded for the group allocation of the participants. Liraglutide was initially applied in a starting dose (0.6 mg/day) for 1 week followed by self-injection of 1.2 mg/day for 3 weeks. Saxagliptin was taken orally in a constant dose (5 mg/day) for the whole duration of 4 weeks. In total 4 visits were conducted at baseline, after 2, after 4 and after 8 weeks (follow up visit). Data from one volunteer in the Lira group were excluded due to false statement about smoking status and recent infections. Finally, data from 6 participants in the liraglutide group and from 8 participants in the saxagliptin group were analyzed.

1) Treatment with liraglutide but not saxagliptin significantly changes the percentage of CD4pos Tregs in the peripheral blood of healthy participants

The percentage of regulatory T cells (%Treg) within CD4pos T cells increased significantly from a median baseline level of 3.4% (IQR: 3.3-3.8%) to 4.1% (IQR: 3.6-4.9%) after 4 weeks of subcutaneous self-injection of liraglutide (0.6 mg/ day in the first week, followed by 1.2 mg/day for 3 weeks). Wilcoxon analysis showed a significant increase of %Treg resulting in a p-value of 0.031 (Fig. 53 and table 13). In contrast to this, oral intake of DPP-4 inhibitors (Saxagliptin) for 4 weeks, did not result in significant changes of %Tregs ($p = 0.875$). After termination of liraglutide administration, the elevated levels of Tregs were stable until the follow-up visit after 8 weeks (Fig. 1).

No significant changes were found in the percentage of Tregs expressing the transcription factor Helios in any of the investigated groups during the whole study period, although there was a trend towards elevated Helios expression during the administration of liraglutide followed by a decreased expression of Helios after termination of liraglutide administration in the lira group (Fig. 1; median values: BL: 42% (35-60%), 4 weeks: 58% (55-59%), 8 weeks: 49% (23-61%)).

The percentage of Tregs within CD4pos T cells at baseline was higher in the saxa group compared to the lira group, but this difference was not significant (table 1). In contrast, the percentage of Heliospos cells within Tregs was significantly higher in the saxa group compared to the lira group at baseline (68% (57-71%) vs 42% (35-60%), $p = 0.043$) but not after 8 weeks ($p = 0.107$, Fig. 1).

1) No effects of liraglutide or saxagliptin administration on human peripheral cells of the innate and adaptive immune system

In both investigated groups, treated with liraglutide (lira group) or saxagliptin (saxa group), the percentage of T cell subtypes, such as CD4pos and CD8pos cells or Th1, Th2 and Th17 cells did not change significantly. Also, liraglutide or saxagliptin administration did not influence the percentage of peripheral NK cells, NKT and iNKT cells, myeloid and plasmacytoid dendritic cells (table 2).

ADPP004 Baseline	lira group	saxa group	p value
n	6	8	
Gender (% female)	67%	63%	
BMI (kg/m²)	24.81 ± 4.24	24.17 ± 4.88	0.295
Age (years)	26.5 (23.5 - 31)	22.5 (20 - 29)	0.741
NLR	2.03 ± 1.30	1.44 ± 0.58	0.652
CD4/CD8 ratio	2.5 (1.9-3.2)	2.5 (1.5-3.4)	0.181
Tregs in CD4 (%)	3.4 (3.3-3.8)	4.1 (3.6-4.9)	0.076

Table 1: Demographic data of our studied populations at time of recruitment. Data are presented as mean ± SD when normally distributed or median + (IQR) when not normally distributed. NLR = neutrophile to lymphocyte ratio.

Figure 1: Changes in % Tregs and expression of the transcription factor Helios upon *in vivo* liraglutide- or saxagliptin treatment of healthy participants. **A.** Changes in % Tregs within CD4pos T cells in the liraglutide treated group (n=6). Liraglutide was initially applied in a starting dose (0.6 mg/day) for 1 week followed by self-injection of 1.2 mg/day for 3 weeks. After additional 4 weeks a follow up visit (week 8) was performed. **B.** Changes in % Tregs within CD4pos T cells in the saxagliptin treated group (n=8). Saxagliptin was taken orally in a constant dose (5 mg/day) for the whole duration of 4 weeks. After additional 4 weeks a follow up visit (week 8) was performed. **C.** Changes in the % Heliospos cells within Tregs in the liraglutide treated group and the saxagliptin treated group. The medication was given for 4 weeks and a follow up visit was performed at week 8. Normally distributed data are given in mean ± SD, not normally distributed data are given in boxplots with median + IQR, whiskers indicate the minimum and maximum values.

	Liraglutide			Saxagliptin		
Cell type	Baseline	4 weeks	p-value	Baseline	4 weeks	p-value
CD4 (% of lympho)	54 (45-56)	56 (40-57)	0.313	48 (36-55)	47 (35-52)	0.297
CD8 (% of lympho)	21 (18-29)	22 (16-36)	0.625	26 (24-29)	28 (24-31)	0.219
Treg (% of CD4)	3.4 (3.3-3.8)	4.3 (4.0-4.7)	**0.031**	4.1 (3.6-4.9)	5.0 (3.6-5.5)	0.875
Helios (% of Treg)	42 (35-60)	58 (55-59)	0.156	68 (57-71)	62 (60-67)	0.688
Th1 (% of CD4)	5.7 (2.6-8.0)	6.7 (4.0-11)	0.563	5.4 (2.6-8.3)	5.9 (3.9-9.4)	0.578
Th2 (% of CD4)	0.55 (0.10-1.20)	0.85 (0.4-1.5)	0.531	1.3 (0.43-2.0)	0.70 (0.60-2.9)	0.578
Th17 (% of CD4)	0.60 (0.35-1.0)	0.65 (0.5-0.98)	0.375	1.1 (0.28-3.1)	1.0 (0.60-4.50)	0.313
NK (% of lympho)	8.2 (7.7-11)	6.9 (6.1-13)	0.313	11.0 (2.4-15.0)	9.6 (4.6-18.0)	0.936
NKT (% of lympho)	3.8 (2.6-6.3)	3.4 (2.5-8.9)	0.999	3.6 (2.0-5.4)	2.9 (2.9-4.4)	0.468
iNKT (% of lympho)	0.13 (0.01-0.21)	0.20 (0.06-0.26)	0.375	0.07 (0.02-0.16)	0.03 (0.01-0.16)	0.999
pDC (% of lympho)	28 (17-38)	34 (20-37)	0.813	28 (18-40)	30 (22-42)	0.813
mDC (% of lympho)	57 (47-65)	59 (53-69)	0.999	52 (38-62)	49 (44-67)	0.375

Table 2: Overview for the changes in the % of peripheral immune cell subtypes after a 4 week liraglutide or saxagliptin intervention. Data are given in median + IQR. Wilcoxon-test was used for the calculation of significant changes.

Date and Signature of Sponsor representative:

30.11.2020