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Analgesic and anti-inflammatory effectiveness of sitagliptin and vildagliptin in mice

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ABSTRACT

To validate the potential anti-inflammatory and analgesic role of sita- and vildagliptin, five different experimental models were used in mice: i) mustard oil-induced ear edema, ii) neutrophil accumulation, iii) mechanical and iv) thermal touch sensitivity in complete Freund's adjuvant-induced arthritis and v) capsaicin-induced plasma extravasation in the urinary bladder. For the complete examination period in i) the dose of 10 mg sitagliptin as well as 1–10 mg vildagliptin was found to significantly decrease ear edema as compared to positive control ($p < 0.05$, $n = 8$/group). All doses of sitagliptin provided an anti-inflammatory effect $p < 0.005$ ($n = 10$/group) in test ii) and an analgesic effect in iii) except 3 mg. Vildagliptin was similarly effective in test ii) ($p < 0.005$, $n = 10$/group) as sitagliptin, but it failed to affect mechanical touch sensitivity. Unlike mechanical touch sensitivity, both gliptins could beneficially act on the thermal threshold ($p < 0.05$, $n = 10$/group). And only in tests v) could both gliptins reverse inflammation. Further studies are needed to support the suggestion that the utilization of these beneficial effects of gliptins may be considered in the treatment of Type 2 diabetic patients.

1. Introduction

Chronic inflammation and pain can be highly debilitating. To reduce the inflammation itself or to relieve the related pain is a justifiable expectation of the patients. Anti-inflammatory and analgesic drugs are commonly prescribed for the symptomatic treatment of different diseases and the range of chemical classes of available drugs is quite broad. The most frequently used drugs are the non-steroidal anti-inflammatory drugs, although the application of steroid compounds in serious cases is also widely accepted. The conditions when these drugs are applied are mostly immune-driven diseases like multiple sclerosis, inflammatory bowel disease, or rheumatoid arthritis. Moreover, diabetes related pain such as diabetic neuropathy or painful diabetic neuritis afflicts a majority of diabetic patients especially, if the diabetes is not treated adequately.

Since diabetes (especially type-2 diabetes) has a growing prevalence worldwide, novel treatments of the disease are in the focus of scientific interest. The two most recently accepted incretin mechanisms involving drug categories are the degradation-resistant glucagon-like peptide-1 (GLP-1) receptor agonists (incretin mimetics) and the inhibitors of dipeptidyl peptidase-4 (DPP-4) activity (incretin enhancers) [1]. The pharmacological actions of GLP-1 analogues and DPP-4 inhibitors have been reviewed recently [2].

There are intestinal hormones released after the oral administration of glucose. These hormones are released in a glucose-dependent manner and are responsible for augmenting insulin secretion, promoting ß cell proliferation and reducing apoptosis. This is defined as the incretin effect. The two most important hormones involved in the incretin mechanism are the glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 [1,3]. Both GIP and GLP-1 are rapidly inactivated after their release; the half-life of active GLP-1 being less than 2 minutes. The inactivation is caused by a truncation of the peptides by the removal of the N-terminal peptide end. This process is executed by the enzyme dipeptidyl peptidase-4 (DPP-4) [4]. DPP-4 is a 110-kDa type-II integral membrane glycoprotein with ubiquitous expression and whose enzyme activity has been recorded in rats, mice and humans. It is present in the epithelial cells of the intestine, kidney, liver, lung, thymus, lymph node, spleen, prostate and in adipocytes, as well as on activated lymphocytes and monocytes [5]. Besides the incretin hormones, a number of bioactive peptides are potential substrates for DPP-4. These include neuropeptide Y, peptide YY, gastrin-releasing polypeptide, pituitary

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adenylate-cyclase-activating polypeptide, insulin-like growth factor-1, substance P and various chemokines [6]. DPP-4 is also known as the cell surface antigen CD26 and it can associate with the lymphocyte cell-surface molecules CD45 and adenosine deaminase (ADA) to have a co-stimulatory function in the immune response [5]. An interesting observation is the increase in the plasma concentration of DPP-4 as a soluble protein during continuous treatment of humans by sitagliptin (100 mg/day). This might originate from shedding of CD26 proteins from mononuclear cells evoked by sitagliptin [8].

Dipeptidyl peptidase-4 inhibitors, like sitagliptin and vildagliptin, have been already introduced to the market since 2006 and are used for the treatment of type-2 diabetes. Gliptins are found to improve the vascular endothelial function, thus performing pleiotropic cardiovascular actions [7]. The safety of the gliptin family was questioned recently, but in two long-term cardiovascular outcome trials, Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus-THrombolysis in Myocardial Infarction 53 (SAVOR-TIMI 53), it has been proven that saxagliptin is safe from the cardiovascular point of view. It was shown that the primary endpoints of the study (a composite of cardiovascular death, non-fatal myocardial infarction or non-fatal ischemic stroke) occurred in 7.3% of the saxagliptin group compared with 7.2% of the placebo group (ClinicalTrials.gov Identifier: NCT01107886). The conclusion of Cardiovascular Outcomes Study of Alogliptin in Patients With Type 2 Diabetes and Acute Coronary Syndrome (EXAMINE) study (ClinicalTrials.gov Identifier: NCT00968708) was that in type-2 diabetic patients with recent acute coronary syndrome, major cardiovascular event rates for alogliptin were not increased compared to placebo. In this trial acute pancreatitis development as a serious adverse event was only 0.07% compared to placebo (0.15 %), thus it is valid to state that alogliptin is free from this side effect.

Both incretins, GIP and GLP-1 stimulate insulin secretion in a glucose dependent manner and consequently, DPP-4 inhibitor treatment does not increase the risk of hypoglycaemia. Not only was the occurrence of hypoglycaemic events incidentally similar or lower when comparing groups treated with DPP-4 inhibitor (either monotherapy or in combination) with placebo treated groups in different studies, but the number of reported adverse events did not differ from the actively treated groups. [4]. It has been demonstrated in animal studies that toxicity may be caused by the inhibition of other enzymes in this family, like DPP-8 and DPP-9 [9], so the selectivity of inhibitors to DPP-4 is crucially important to ensure an optimal safety profile. Since both sitagliptin and vildagliptin show a higher relative selectivity for DPP-4, the risk of development of adverse effects due to inhibition of other enzymes is minimized [4,10]. However, it did turn out that during the post marketing period of gliptins these DPP-4 inhibitors increased the rate of infections such as nasopharyngitis and urinary tract infections [11]. In addition, pancreatitis was reported mainly associated with the use of sitagliptin and linagliptin [12], although a recent meta-analysis could not find differences between DPP-4 inhibitors [13]. In spite of the increased risk of infections, sitagliptin and vildagliptin are well tolerated in general. Besides the primary targeted therapeutic area, *in vitro* and *in vivo* studies showed anti-inflammatory properties of DPP-4 inhibitors that could lead to a novel drug class for anti-inflammatory disorders [14]. Altered circulating peptidase activity and membrane DPP-4 expression have been demonstrated in a number of human inflammatory diseases [15]. DPP-4 is responsible for the modification of a number of regulatory factors, such as peptides or chemokines and affects the signaling functions. This suggests that DPP-4 is involved in determining immune response and procession of inflammatory disorders as well. As mentioned previously, DPP-4 is also known as the cell surface antigen CD26, which signals T-cells to proliferate. However, this mechanism cannot be attributed to the DPP-4 inhibition [16] because the T-cell activation seems to be independent of the DPP-4 enzyme activity and the ADA-binding capability [16,17]. Moreover, reversible DPP-4 inhibitor Lys[Z(NO_2)]-pyrrolidide was shown to suppress autoimmune encephalomyelitis and upregulated TGF-β1 secretion *in vivo* [18].

The possible anti-inflammatory property of the gliptin group can be considered as an additional value of these drugs in diabetic patients with neuritis or diabetic neuropathy, or patients with atherosclerosis considering that these diseases are driven by inflammatory processes [19]. Moreover, the reduction in plasma C-reactive protein concentration and systolic blood pressure have been described for exenatide [20]. The anti-inflammatory action of sitagliptin [8] and exenatide [19] are proven biochemically in humans, thus in the present series of experiments we aimed to examine the possible anti-inflammatory effect of two potent DPP-4 inhibitors, sitagliptin and vildagliptin. They were applied in *in vivo* inflammation and analgesic models in mice.

2. Materials and methods

2.1. Animals and ethics

Experiments were performed on 25–35 g CD1 male mice (Charles River, Gödöllő, Hungary), kept under standard pathogen-free conditions at 24–25 °C and provided with standard rodent chow and water *ad libitum*. The light/dark cycle was 12 h/12 h. Animal procedures were approved by the local animal ethics committee and National Food Chain Safety Office Animal Health and Animal Welfare Directorate under the number 26/2007/DE MÁB in accordance with the European Communities Council Directives (86/609/ECC) and the Hungarian Act for the Protection of Animals in Research (XXVIII tv. 32§) and complied with the recommendations of the International Association for the Study of Pain [21] and the Helsinki Declaration. The design of the study was carried out in a manner in which to minimize the number of animals used and their suffering.

2.2. Substances and their application

Mice were dosed with 1, 3 or 10 mg/kg sitagliptin or vildagliptin (Nanjing Ange Pharmaceuticals, Nanjing, Jiangsu, China) dissolved in saline by oral gavage (1 ml/100 g). Control groups were given the vehicle in the same amount and way. A single application was used in the case of one-day experiments, while daily application was used in the 21 day long experiments, as suggested by Thomas et al. [22]. Treatments and measurements were implemented 30 min after the oral gavage in every case.

2.3. Allyl-isothiocyanate (AITC)-induced inflammation model

Anesthesia was induced by thiopental (Trapanal, Sandoz, Basle, Switzerland) in an amount of 50 mg/kg intraperitoneally (i.p.), repeated as required. The inner and outer surface of the right ear was then smeared with 1% allyl-isothiocyanate (AITC) (Sigma-Aldrich, Budapest, Hungary) dissolved in paraffin oil, using a cotton-wool stick. This treatment was applied 30 min after the oral gavage (substances dissolved in saline or vehicle in the control group) and the procedure was repeated 45 min after the first application following the instructions of Bánvölgyi [23] and Inoue et al. [24]. Thus the oral administration of gliptins was performed firstly and the induction of inflammation was carried out secondly.

At the end of the experiment the animals were sacrificed by cervical dislocation and ears were stored on -20 °C for the neutrophil accumulation assay.

2.4. Measurement of ear edema

Ear thickness was measured by a micrometer caliper (Oxford Precision, Leicester, England) with 0.1 mm accuracy before the AITC treatment, 15 min after the first AITC application, then by each hour during a 6 hour period after each AITC treatment according to Inoue et al. [24] with slight modifications. Gliptin treatment was performed 30 minutes