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European Journal of Pharmacology 558 (2007) 167–171
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Sulodexide induces hepatocyte growth factor release in humans

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Received 9 October 2006; received in revised form 3 December 2006; accepted 6 December 2006
Available online 29 December 2006

Abstract

Heparin influences numerous pleiotropic growth factors, including hepatocyte growth factor (HGF), partially by their release from endothelial and extracellular matrix stores. The effects of sulodexide, a heparin-like glycosaminoglycan medication of growing importance in medicine, on HGF liberation are not known. We performed a 2-week open-label sulodexide trial in healthy male volunteers. The drug was initially administered intravenously (i.v.) in a single dose of 1200 Lipoprotein Lipase Releasing Units (LRU), then – orally for 12 days (500 LRU twice a day), and – again by i.v. route (1200 LRU) on day 14. Intravenous sulodexide injections were repeatedly found to induce marked and reproducible increases in immunoreactive plasma HGF levels (more than 3500% vs baseline after 10 min, and more than 1200% after 120 min), and remained unchanged when measured 120 min following oral sulodexide administration. The percentage increments in plasma HGF evoked by i.v. sulodexide at both time points and on both days inversely correlated with baseline levels of the growth factor. On day 14, the HGF levels after 120 min and their percentage increase vs baseline were strongly and directly dependent on i.v. sulodexide dose *per* kg of body weight. This study shows that sulodexide has a novel, remarkable and plausibly biologically important stimulating effect on the release of pleiotropic hepatocyte growth factor in humans.

Keywords: Glycosaminoglycan; Human study; Heparin; Hepatocyte growth factor; Sulodexide

1. Introduction

Sulodexide is a purified anionic glycosaminoglycan compound consisting of 80% fast-moving heparin fraction (containing heparan sulfate) and 20% dermatan sulfate (also known as chondroitin sulfate-B). This heparin-like drug is available in both oral and parenteral form, and has reduced effects on global coagulation and bleeding events compared with those exerted by unfractionated heparin (Harenberg, 1998; Ofosu, 1998). Sulodexide is useful in the treatment and/or secondary prevention of ischemic arterial cardiovascular events (Condorelli et al., 1994; Coccheri et al., 2002b), deep vein thrombosis (Coccheri et al., 2002a; Errichi et al., 2004; Cirujeda and Granado, 2006), cerebrovascular dementia (Parnetti et al., 1997) and chronic kidney disease (Funakoshi and Nakamura, 2003; Liu, 2004; Abaterusso and Gambaro, 2006). It also has been reported to protect endothelial cells (Kristová et al., 2000), blunt systemic and local inflammation (Fracasso et al., 2003; Lauver et al., 2005), and prevent neointimal proliferation and restenosis of injured carotid artery (Park et al., 1997). These intriguing effects of sulodexide may only partially be ascribed to its recognized anticoagulant actions.

Pleiotropic effects of heparin result mainly from its emerging impact on numerous growth factors, cytokines, chemokines, enzymes and receptors regulating vital functions of the human body and a course of numerous diseases (Elsayed and Becker, 2003; Norrby, 2006). In the growing family of heparin-binding proteins, the essential and relatively well recognized one is hepatocyte growth factor (HGF, also known as scatter factor) — multipotential cytokine involved in embryonic development, and the repair, regeneration and protection of various organs from injuries (Funakoshi and Nakamura, 2003). HGF exhibits mitogenic and anti-apoptotic activities, enhances motility of epithelial cells, and promotes angiogenesis. Following tissue damage, HGF is expressed in mesenchymal cells, while its receptor c-*Met* is expressed by almost all epithelial cells, vascular endothelium and hematopoietic progenitor cells. Then, the activated HGF acts in a complex auto-, para- and, remarkably,

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doi:10.1016/j.ejphar.2006.12.009

endocrine- and concentration-dependent manner to limit the extent of local and even distant tissue injury. Large amounts of HGF are lodged on endothelial cells and remain assembled with glycosaminoglycans in the extracellular matrix of the vascular wall (Lyon et al., 2002; Elsayed and Becker, 2003; Norrby, 2006). HGF can be easily released from these stores by endogenous (Kinoshita et al., 2002) as well as exogenous heparin (Seidel et al., 1999; Salbach et al., 2000; Borawski et al., 2003). The latter effect is particularly striking, induced by both unfractionated heparin and low-molecular-weight heparin, and directly dependent on the dose of the heparins. So far, no study addressed the issue of HGF release by sulodexide. This may constitute a new pharmacological effect of the drug.

The aim of the present study was to investigate the effects of intravenous (i.v.) and oral (p.o.) sulodexide administration on plasma HGF levels in healthy volunteers during a 2-week open-label pilot trial.

2. Subjects and methods

2.1. Subjects

The study conformed to the Declaration of Helsinki and was approved by our institutional ethical board. Eleven healthy males aged 30.4±1.16 years, of a mean weight of 84.5±11.1 kg, height of 1.81±0.05 m and body mass index of 25.7±2.44 kg/m^2 were enrolled. They were non-smokers and no drug or alcohol abusers, with commitment to no occasional substance use during the study. Their health status was ascertained by history, physical examination and selected laboratory tests. Special attention was given to arterial blood pressure, mental disorders, history of drug hypersensitivity, bleeding and thrombotic disorders, gastroduodenal disease, complete blood counts, urinalysis, serum C-reactive protein, creatinine, liver enzymes, viral hepatitis B/C and HIV infection, blood glucose and routine hemostatic tests. None of the participants experienced major trauma, surgery or infection in the preceding month and was not receiving any medications within the past 2 weeks.

2.2. Sulodexide administration and blood sampling

At study initiation (day 1), fasted blood samples (T_0) were obtained from the antecubital vein (punctured with an 18-gauge needle without the use of tourniquet) into ethylenediamine tetraacetate coated vacutainers. Then, sulodexide (Vessel Due F, Alfa Wassermann SpA, Bologna, Italy) was injected i.v. at a dose of 1200 Lipoprotein Lipase Releasing Units (a single 2 ml ampoule contains 600 LRU=60 mg) and pushed with 10 ml of normal saline. Afterwards, the patients remained ambulatory within the clinic and were allowed habitual water intake; the consecutive blood samples were drawn after 10 min (T_{10}) and 120 min (T_{120}). Next, the participants were asked to regularly take oral sulodexide at a daily dose of 1000 LRU (2 capsules of 250 LRU every 12 h).

On day 7, fasting blood samples were obtained at T_0; then the participants took the previously prescribed dose of p.o. sulodexide (500 LRU) and the sampling was repeated at T_{120}.

On day 14, no usual morning oral sulodexide was administered and 1200 LRU of the drug was again injected i.v.; further procedures strictly followed those performed on day 1. Then, the study was terminated.

The daily oral sulodexide dose of 1000 LRU (equivalent to 100 mg) was chosen based on those employed in a majority of previous clinical trials (Condorelli et al., 1994; Parnetti et al., 1997; Coccheri et al., 2002a,b; Gambaro et al., 2002; Fracasso et al., 2003). The i.v. dose of 1200 LRU was selected as a part of a phase I study. Compliance to the oral drug was evaluated by specifically questioning the volunteers and counting the capsules. All the participants completed the study and the follow-up period was uneventful; no delayed adverse effects of sulodexide were noted on medical examination performed 1 month after study termination.

2.3. Hepatocyte growth factor measurements

Plasma for HGF quantification was prepared traditionally, aliquoted and stored at −70 °C until batch analysis. Enzyme-linked immunosorbent assay kits from R&D Systems, Inc., Minneapolis, MN, USA (Quantikine®; DHG00, minimum detection limit 0.04 ng/ml) were used. The measurements were performed in duplicate using a 400 SFC microplate reader (SLT-Labinstruments, Gröding/Salzburg, Austria), and calibrated using provided recombinant human reference samples and standards. For calculations of the results, a computer and a curve-fitting program were used. The within- and between-assay coefficients of variations were <7%.

2.4. Statistical analysis

All data were normally distributed as provided by Shapiro–Wilk W test, and expressed as mean±1 S.D. Differences in plasma HGF levels following i.v. sulodexide injection on days 1 and 14, as well as between the T_0 HGF concentrations on days 1, 7 and 14 (three time points) were assessed using the analysis of variance for repeated measures; the *post-hoc* Newman–Keuls test was used for multiple comparisons. The values measured at two time points (day 7) were compared with paired Student *t*-test. Bivariate correlations were assessed with Spearman regression analysis. The tests were two-sided, and P values<0.05 were considered significant. Statistica 6.0 PL for Windows (StatSoft, Tulsa, OK, USA) data analysis package was used.

3. Results

3.1. Effects of intravenous and oral sulodexide on plasma hepatocyte growth factor

On day 1, HGF levels significantly changed following i.v. sulodexide administration (F=854, P<0.0001) (Fig. 1A). They increased by 3557±802% — from 0.51±0.11 ng/ml at T_0 to 18.2±1.62 ng/ml at T_{10} (P<0.0001). At T_{120}, HGF levels were 6.63±1.54 ng/ml, lower than those at T_{10} (P< 0.0001) but still elevated by 1249±448% compared with the baseline

values ($P<0.0001$). Following oral sulodexide administration on day 7 (Fig. 1B), plasma HGF values at T_{120} were not different from those at T_0 (0.48±0.08 ng/ml vs 0.49±0.07 ng/ml, $P=0.740$). On day 14, the i.v. sulodexide injection resulted in the striking and statistically similar increase in plasma HGF as that observed on day 1 ($F=786$, $P<0.0001$) (Fig. 1C). The HGF levels were: T_0 0.49±0.08 ng/ml, T_{10} 17.8±1.66 ng/ml and T_{120} 7.22±1.33 ng/ml; the percentage increments in plasma HGF were: T_{10} vs T_0 3616±483% and T_{120} vs T_0 1409±326%. The HGF values at the particular time points on day 14, as well as the magnitude of its increments were remarkably similar to those on day 1 (all $P>0.464$). The T_0 HGF levels measured on days 1, 7 and 14 did not differ one from another ($F=0.277$, $P=0.761$).

3.2. Variables associated with sulodexide-induced hepatocyte growth factor release

On day 1, the T_{120} HGF levels tended to be associated directly with i.v. sulodexide dose *per* kg of body weight ($r=0.598$, $P=0.052$, Fig. 2A). The relation between the percentage of HGF increase and this sulodexide dose was also direct but not significant at that time ($r=0.472$, $P=0.143$, Fig. 2B). The percentage increment in plasma HGF (ΔHGF) at both T_{10} ($r=-0.869$, $P=0.001$, Fig. 2C) and T_{120} ($r=-0.648$, $P=0.031$, Fig. 2D) inversely correlated with the baseline levels of the growth factor. On day 14, the direct relation between HGF levels at T_{120} and i.v. sulodexide dose became statistically significant ($r=0.781$, $P=0.005$, Fig. 2E), and the percentage increase in HGF at T_{120} vs baseline became remarkably dependent on the dose of the drug ($r=0.810$, $P=0.002$, Fig. 2F). The inverse correlations between the post-sulodexide HGF increments and baseline HGF levels on day 14 (ΔHGF $T_{10}-T_0$ vs HGF T_0 $r=-0.827$, $P=0.002$; and ΔHGF $T_{120}-T_0$ vs HGF T_0 $r=-0.671$, $P=0.022$) were statistically similar to those found on day 1.

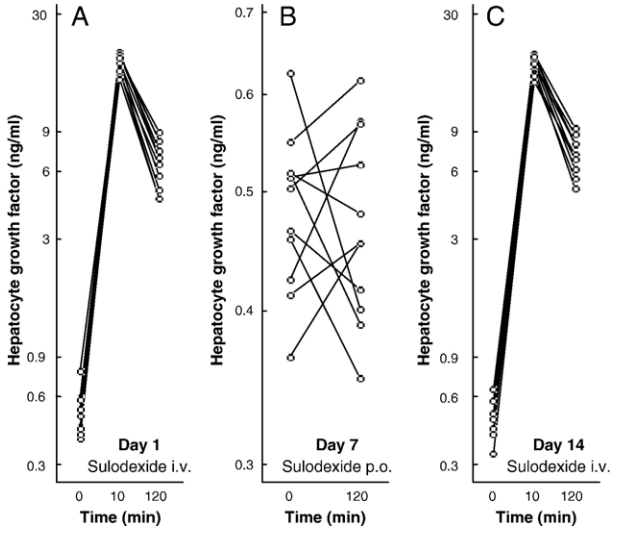

Fig. 1. Case profiles of plasma hepatocyte growth factor levels following sulodexide administration in healthy humans. Panel A — effects of initial intravenous (i.v.) injection; Panel B — effects of oral (p.o.) administration; Panel C — effects of i.v. injection after 12 days of oral administration.

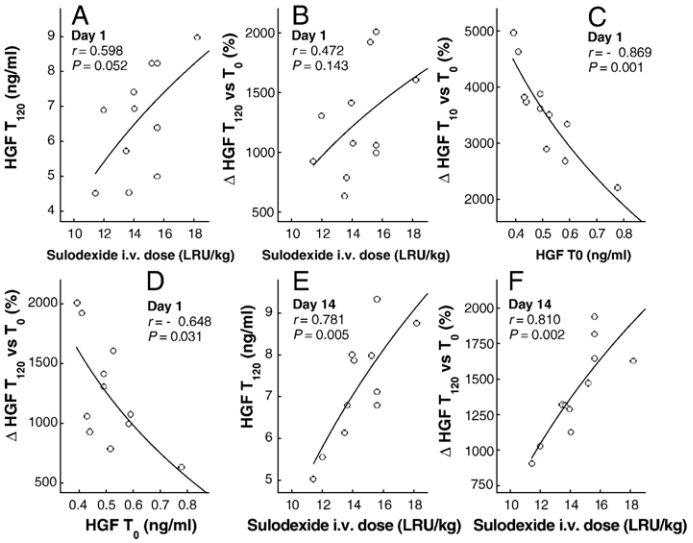

Fig. 2. Variables influencing hepatocyte growth factor (HGF) release by sulodexide in healthy humans. The Δ symbol denotes the change in plasma HGF levels at 120 min (T_{120}) or 10 min (T_{10}) after intravenous sulodexide administration vs baseline HGF concentrations (T_0). LRU — Lipoprotein Lipase Releasing Unit. The regression lines represent the subjective best-fit model for the non-linear Spearman correlation analysis.

4. Discussion

The main finding of this study is that i.v. sulodexide administration induces a dose-dependent and striking increase in plasma HGF levels in healthy humans. This may constitute a novel pharmacological effect of the drug, providing a biologically plausible support to its emerging pleiotropic properties.

The rapid rise in plasma HGF following i.v. sulodexide injection (evident after 10 min) is most likely due to the release of the growth factor from its easily accessible endothelial and extracellular matrix stores, as it was previously shown for the structurally similar heparin (Seidel et al., 1999; Salbach et al., 2000; Borawski et al., 2003). In support, we found remarkable inverse correlations between baseline HGF levels and the magnitude of its increase after both 10 min and 120 min; the associations were confirmed on repeated i.v. injection of the drug. From a pharmacokinetic point of view, such regression patterns may indicate the specific and, notably, reciprocal equilibrium between the circulating and sulodexide-releasable HGF pools. Moreover, we showed that the extent of HGF release depends on the dose of i.v. sulodexide. This effect was much more evident while the medication was injected following its 12-day oral administration compared with the initial i.v. administration. Thus, it seems not unreasonable to speculate on the existence of a vague process of ongoing "adaptation" to sulodexide administration, which may result in increased sensitiveness to the drug; the issue deserves further studies.

Regarding the effects of oral sulodexide, we found no change in plasma HGF levels at 2 h after p.o. drug administration. This

single point measurement, which was chosen according to previous studies on its oral absorbance (Harenberg, 1998; Ofosu, 1998), does not, however, allow definite denial of the possibility that enteral sulodexide also may affect HGF release. For this purpose, more frequent and likely — more delayed HGF measurement and/or higher oral sulodexide doses are needed. The limitation of our study is also a lack of a placebo group. The experiments regarding the effects of i.v. sulodexide administration on the extent of HGF release were, however, performed twice, and yielded similar results. Altogether, they prove a novel action of i.v. sulodexide on multipotential HGF, and suggest that some of the pleiotropic effects of this heparinoid compound may be mediated by HGF.

To verify the rationale of the hypothesis, we reviewed to-date literature regarding clinical effects of sulodexide vs those of HGF. The Pubmed search has revealed that the drug is protective against common pathological states and/or diseases such as endothelial dysfunction (Kristová et al., 2000), cardiovascular complications and postischemic heart injury (Condorelli et al., 1994; Park et al., 1997; Lauver et al., 2005), atherosclerotic peripheral artery disease (Coccheri et al., 2002b), diabetic nephropathy (Gambaro et al., 2002; Achour et al., 2005; Abaterusso and Gambaro, 2006), and vascular dementia (Parnetti et al., 1997). Oral sulodexide also was reported to improve peritoneal membrane function and diminish systemic inflammation in end-stage renal failure patients treated with peritoneal dialysis (Fracasso et al., 2003). Due to the HGF release sulodexide may: protect vascular wall (Date et al., 2004; Zhou et al., 2006), prevent progression of heart failure (Jayasankar et al., 2005, Sakaguchi et al., 2005), improve arterial function and performance (Morishita et al., 2004; McKinnon et al., 2006), have a strong therapeutic potential in kidney diseases including diabetic nephropathy (Funakoshi and Nakamura, 2003; Liu, 2004), enhance cerebral functions (Date et al., 2004; Shimamura et al., 2006), and prevent peritoneal fibrosis (Matsuo et al., 2005). This literature comparison indicates that sulodexide and HGF share a number of therapeutic properties. Based on the above facts and the results of our study, it is attractive to speculate that some of the extra-anticoagulant effects of sulodexide may be mediated by HGF. This pleiotropic growth factor has an emerging therapeutic value in liver diseases (Kim et al., 2005; Xia et al., 2006), lung fibrosis (Watanabe et al., 2005), inflammatory bowel disease (Ido et al., 2005), wound healing (Conway et al., 2006) and regeneration of pancreatic β-cells (Izumida et al., 2005). Interestingly, HGF gene transfer was found to be effective in the treatment of some of these diseases (Morishita et al., 2004; Shimamura et al., 2006; Watanabe et al., 2005). However, the present finding that sulodexide depletes vascular stores of HGF — comparably with the anti-angiogenic heparin which is currently commonly used in oncology (Norrby, 2006), suggests that sulodexide also may be regarded as a putative anti-neoplastic drug.

The recently emerging and gaining importance issue is that heparin also affects other multipotential growth factors, cytokines and enzymes — often by their release from the endothelial stores and extracellular matrix (Norrby, 2006). The growing list of these heparin-binding proteins includes vascular endothelial growth factor, transforming growth factor-β, basic fibroblast growth factor, epidermal-like growth factor, connective tissue growth factor, bone morphogenetic proteins, activin A, endostatin, tumstatin, midkine, survivin, macrophage stimulating protein-1/CCL-2, myeloperoxidase, and others. Therefore, it is plausible and intriguing to suppose that also the heparin-like sulodexide may influence the metabolism and biological effects of those vital endogenous substances. However, at the current state of knowledge, it is much premature to discuss the clinical relevance of the i.v. sulodexide-mediated HGF release.

In conclusion, sulodexide — so far regarded as the useful but mostly supportive anticoagulant agent also has a remarkable and stimulating effect on the pleiotropic hepatocyte growth factor in humans. This novel action of sulodexide may, hopefully, encourage pharmacological research and clinical medicine.

Acknowledgements

This work was funded by the Medical University of Bialystok, Poland (grant No 3-54860). The authors were not supported by the manufacturer of sulodexide.

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