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Inhibitory effect of fasiglifam on hepatitis B virus infections through suppression of the sodium taurocholate cotransporting polypeptide

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ABSTRACT

Fasiglifam is a selective partial agonist of G-protein–coupled receptor 40 (GPR40), which was developed for the treatment of type 2 diabetes mellitus. However, the clinical development of fasiglifam was voluntarily terminated during phase III clinical trials due to adverse liver effects. Fasiglifam showed an inhibitory effect on sodium taurocholate cotransporting polypeptide (NTCP) in human and rat hepatocytes. Recently, NTCP was reported to be a functional receptor for human hepatitis B virus (HBV) infections. Therefore, in this study, we hypothesised that fasiglifam would be a good candidate for a novel HBV entry inhibitor, and its effects were evaluated by using NTCP-overexpressing HepG2 cells, human hepatocyte cell lines and human hepatocytes (PXB cells) obtained from PXB mice. Pre-treatment with fasiglifam at a concentration of 30 μM prior to HBV infection significantly suppressed supernatant HBV DNA levels after HBV infection in NTCP-overexpressing HepG2 cells, human hepatocyte cell lines and PXB cells. Fasiglifam did not suppress supernatant HBV DNA levels up to 50 μM in HepG2.2.15.7 cells, which are stably transfected with a complete HBV genome without HBV infection. These results indicated that fasiglifam only affect on HBV infection via NTCP inhibition. For HBV treatment of fasiglifam, further investigation including additional non clinical research in addition to the evaluation of safety and efficacy in humans would be needed in the future study.

1. Introduction

Fasiglifam is an orally available, potent, and selective partial agonist of G-protein–coupled receptor 40 (GPR40) that was developed for the treatment of type 2 diabetes mellitus. In a previous study, fasiglifam significantly improved glycaemic control via its glucose-dependent mechanism of action, which made it a promising investigational candidate for the treatment of type 2 diabetes mellitus, combined with its low risk of hypoglycaemia, compared with classic insulin secretagogues [1]. However, the clinical development of fasiglifam was terminated at the phase III clinical trials because of liver safety concerns [2,3]. Bile acid (BA) uptake into hepatocytes is mediated by sodium taurocholate cotransporting polypeptide (NTCP) and organic anion transporting polypeptides (OATPs), while export is mediated by the bile salt export pump (BSEP) and multidrug resistance-associated proteins (MRPs) [4]. Wolenski et al. [5] and Li et al. [6] reported that fasiglifam showed an inhibitory effect on multiple influx (NTCP and OATPs) and efflux (BSEP and MRPs) hepatobiliary BA transporters in human and rat hepatocytes. The ability of fasiglifam to inhibit the human version of these transporters was evaluated and the corresponding half maximal inhibitory concentration (IC_{50}) values

Abbreviations: GPR40, G-protein–coupled receptor 40; BA, bile acid; NTCP, sodium taurocholate cotransporting polypeptide; OATPs, organic anion transporting polypeptides; MRPs, multidrug resistance-associated proteins; DMSO, Dimethyl sulfoxide; HBIG, Hepatitis B immunoglobulin.

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were determined. In rat hepatocytes, fasiglifam inhibited NTCP with an IC_{50} value of 10.9 μM. In human hepatocytes, fasiglifam also inhibited NTCP with an IC_{50} value of 2.0 μM [5,6]. Recently, NTCP was reported to be a functional receptor for human hepatitis B and D virus infections [7], and this discovery was validated by three further studies [8–10]. Currently approved therapies for HBV infection include interferon and a growing set of nucleos(t)ide analogues, but are rarely associated with complete recovery [11] due to the remaining HBV covalently closed circular DNA (cccDNA) in the nucleus [12]. If nucleotide analogue therapy were stopped, HBV replication would still occur from HBV cccDNA. Therefore, HBV infection remains a serious public health problem worldwide [13]. In addition, HBV remains prevalent among needle sharing drug users within developing countries. Furthermore, vaccine escape and nucleos(t)ide-resistant HBV strains have evolved and present a serious problem that must be addressed for future HBV prevention and treatment [14,15]. Therefore, the design and development of new *anti*-HBV strategies, and drugs that interfere with different stages in the HBV life cycle, are urgently required. The discovery of NTCP as a functional receptor for HBV opened up new possibilities for the study of HBV infection and the development of new antiviral agents. It may be very valuable to explore NTCP itself or other regulatory factors [16] as potential *anti*-HBV targets. Using the HepG2.N9 cell line, two small molecules, cyclosporine A [17,18] and irbesartan [19] were used as new inhibitors of HBV entry through NTCP inhibition. Irbesartan is used as an antihypertensive and to prevent kidney damage in patients with type 2 diabetes mellitus [20]. Blanchet et al. [21] showed that irbesartan could also exert *anti*-HDV effects and this might be helpful for HBV/HDV co-infection therapy, however, efficacy of irbesartan on HBV was not so stronger than that of cyclosporine. Regarding cyclosporine, as there are some side effects such as infection, headache, dizziness, unusual growth of body hair, nausea/vomiting, diarrhea, stomach upset, or flushing, cyclosporine seemed not to be suitable for long time use for HBV treatment. Similar to cyclosporine A and irbesartan, fasiglifam also inhibited NTCP in human and rat hepatocytes. As a result of the findings from previous work, we hypothesised that fasiglifam would be a good candidate for a new entry inhibitor of HBV.

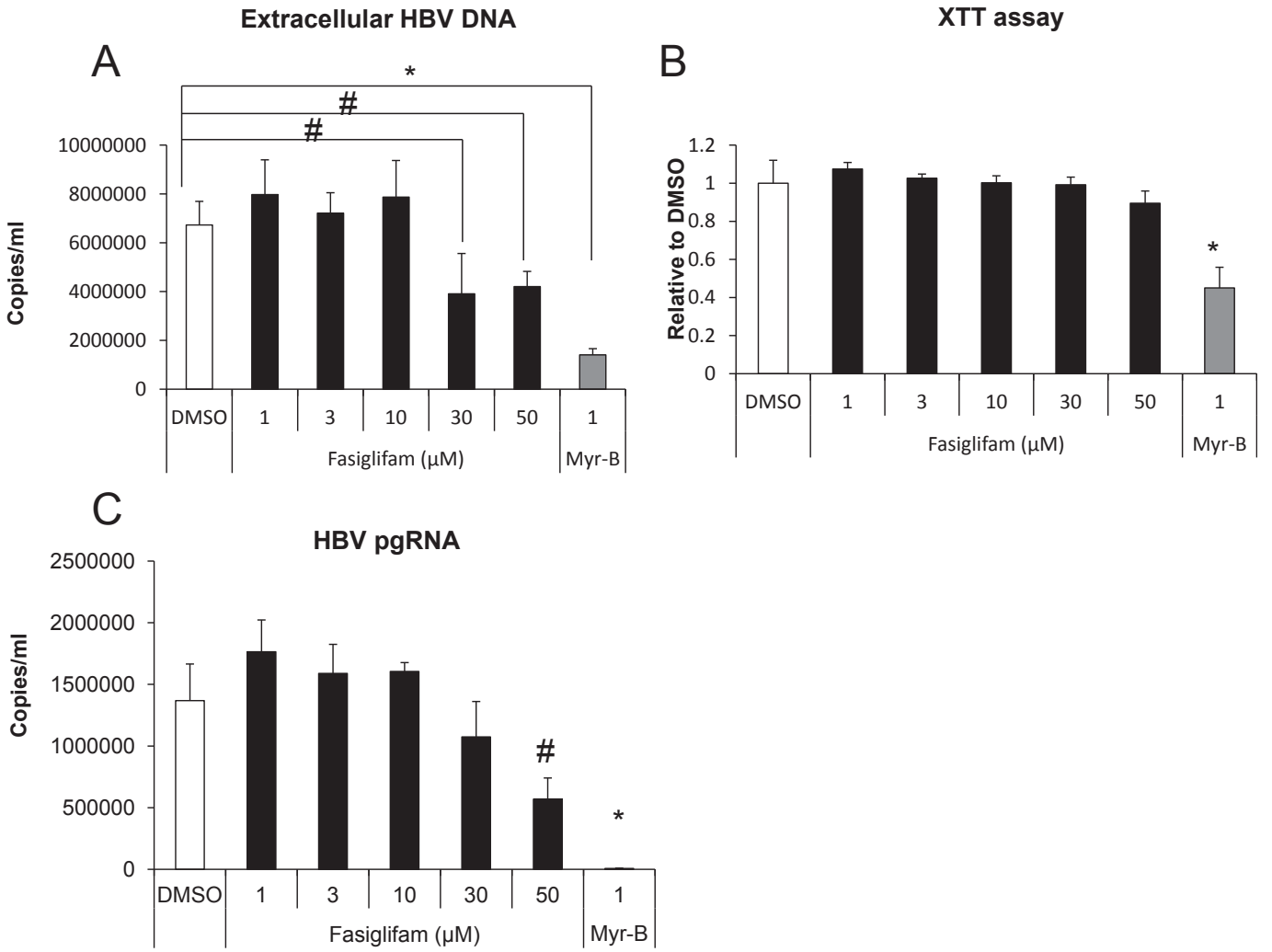

Fig. 1. Effect of fasiglifam on HBV infection using HepG2-hNTCP-C4 cells. HepG2-hNTCP-C4 cells were seeded 2 days before HBV infection and DMSO (white bar), fasiglifam at 1, 3, 10, 30, 50, and 100 μM (black bars), and Myrcludex B (grey bar) at 1 nM were administered from 3 h before infection. Fasiglifam and Myrcludex B were treated for 15 days after infection. After 15 days treatment, the extracellular HBV DNA (A) and cell viability (B) were quantified by qPCR and XTT assay, respectively. HBV pgRNA levels in the culture media of fasiglifam (black bars) and Myrcludex B (grey bar) were also measured by qPCR (C). Results are expressed as the mean ± S.D ($n = 3$ per group). #, $P < 0.025$ vs. DMSO by the one-tailed Williams test. *, $P < 0.05$ vs. DMSO by the Student's t-test.

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