Research Article

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Raloxifene hydrochloride is an adjuvant antiviral treatment of postmenopausal women with chronic hepatitis C: A randomized trial☆

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Background & Aims: Early menopause in women with chronic hepatitis C virus (HCV) infection is associated with a low likelihood of a sustained virological response (SVR) in conjunction with their antiviral treatment. This is potentially related to their reduced estrogen secretion. The study was done to determine whether selective estrogen receptor modulator administration might improve the efficacy of the current standard of care (SOC) treatment, pegylated interferon (PegIFN) α2a plus ribavirin (RBV), for postmenopausal women.

Methods: One hundred and twenty-three postmenopausal women with genotype 1b chronic hepatitis C were randomly assigned to one of two treatment groups: raloxifene hydrochloride (RLX) (60 mg/day) plus SOC (PegIFNα2a 180 μg/week and RBV 600–1000 mg/day) (n = 62) or SOC only (n = 61). Genotyping was performed of the polymorphism in the interleukin-28B (*IL28B*) gene region (rs8099917) of DNA collected from each patient.

Results: One RLX-treated patient discontinued RLX because of a systemic rash following 2 weeks of treatment. Twenty-four weeks after treatment, the SVR rate was significantly higher for RLX plus SOC patients (61.3%) than for SOC only patients (34.4%) ($p = 0.0051$). Further, the SVR rate was significantly higher for RLX plus SOC patients with *IL28B* TT (72.5%) than for SOC only patients with *IL28B* TT (39.2%) ($p = 0.0014$), but no such relationship was observed in patients carrying the minor *IL28B* allele.

Conclusions: RLX improved the efficacy of SOC in the treatment of postmenopausal women with chronic hepatitis C. RLX shows promise as an adjuvant to the standard antiviral treatment of such patients.

Keywords: Hepatitis C; Selective estrogen receptor modulator; Postmenopausal women; Interleukin-28B.

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Abbreviations: HCV, hepatitis C virus; SVR, sustained virological response; SOC, standard of care; PegIFN, pegylated interferon; RBV, ribavirin; RLX, raloxifene hydrochloride; IL28B, interleukin-28B; SERM, selective estrogen receptor modulator; WBC, white blood cell; Hb, hemoglobin; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; NR, null response; RVR, rapid virological response; cEVR, complete early virological response; cLVR, complete late virological response; ETR, end-of-treatment response; ALT, alanine aminotransferase; TNFα, tumor necrosis factor α; NS, non-structural protein; ER, estrogen receptor.

Introduction

Chronic hepatitis C virus (HCV) infection is the predominant cause of chronic liver disease. Over time, it has become apparent that men more often develop progressive liver disease than women do, with an associated, disproportionate number of men being afflicted with liver cancers [1]. However, the spontaneous clearance of HCV infection is more likely in women than in men [1]. These observations indicate sex-related differences in HCV infection status and its liver pathophysiology.

Dramatic improvements in antiviral response have been observed since the use of standard of care (SOC) treatment for chronic hepatitis C, consisting of a combination of pegylated interferon α (PegIFNα) and ribavirin (RBV), was adopted, leading to improved patient outcomes [2–5]. There are several predictors associated with the sustained virological response (SVR), including younger age, not being obese, absence of liver fibrosis, HCV non-genotype 1, and low levels of HCV RNA [2–5]. Women, particularly those who are premenopausal, respond better than men [2,5]. The response of Japanese older women is poor, with mutations in the HCV core region or iron metabolism being suggested as reasons for the comparatively poor efficacy [6–9]. These observations led to the suggestion that the low antiviral response rates of older women might be related to declining estrogen secretion [9].

Raloxifene hydrochloride (RLX) is an oral selective estrogen receptor modulator (SERM) that has estrogenic actions on bone

and anti-estrogenic actions on the uterus and breast. RLX is prescribed for postmenopausal women to prevent the progression of osteoporosis, and manifests its pharmacological activity by binding to intra-nuclear estrogen receptors and altering gene transcription [10]. Thus, SERM agents exhibit estrogen agonist and antagonist actions to produce tissue-selective estrogen-like effects. In the liver, these agents have been suggested to function as estrogen agonists that directly protect hepatocytes and control liver fibrosis [11].

To the best of our knowledge, no previous study had shown a difference in SVR between postmenopausal women and age-matched men. Recently, an Italian clinical study has reported similar SVR rates for both postmenopausal women and men [12]. These rates are significantly lower than those observed in women of childbearing age. Early menopause has been reported to be the most predictive factor for failure to achieve an SVR, and this again links SVR to estrogen secretion. In this study, we tested the hypothesis that RLX can improve the efficacy of SOC treatment for postmenopausal women with chronic hepatitis C.

Patients and methods

Patients and study design

This study was a prospective, open-label, randomized, controlled trial to investigate the SVR rate achieved when RLX administration was combined with the SOC treatment, relative to that observed with the SOC treatment alone for postmenopausal women infected with HCV genotype 1. The study design was approved by the independent ethical committee of each hospital and was carried out in accordance with the 1975 Declaration of Helsinki, as updated in 2008. Each patient gave written, informed consent after receiving comprehensive information about the study. The study was registered as a clinical trial on the University Hospital Medical Information Network (ID 000005815).

Postmenopausal Japanese women $\geqslant$45 years old with chronic hepatitis C were enrolled at seven hospitals between August 2008 and July 2010. The postmenopausal status of each patient was determined through an interview and was defined as the absence of menstrual bleeding during the preceding 12 months. Patients underwent blood tests or a liver biopsy 4 weeks before enrollment.

The inclusion criteria for the study required patients to be infected with HCV genotype 1b and serum HCV RNA positive (>5.0 log IU/ml). The exclusion criteria were history of previous interferon treatment within 3 years; Child–Pugh score >6 points; presence of hepatocellular carcinoma; ongoing treatment with systemic antiviral, antineoplastic or immune-modulating drugs; estrogen analogs; other SERMs within the previous 6 months; autoimmune liver diseases; excessive ethanol consumption ($\geqslant$60 g/day); history of venous thrombosis; malignant neoplasm; very poorly controlled diabetes, cardiopulmonary, pulmonary disorders, or thyroid diseases; severe psychiatric illness; history of attempted suicide; hepatitis B virus surface antigen positive; human immunodeficiency virus antibody positive; white blood cell (WBC) count $<3 \times 10^9$/L; neutrophil count $<1.5 \times 10^9$/L; hemoglobin (Hb) <110 g/L; platelet count $<90 \times 10^9$/L; or serum creatinine level >15 mg/L. We screened 210 female patients $\geqslant$45 years old with chronic hepatitis C; 87 patients were excluded because they did not meet the inclusion criteria. Thus, a total of 123 women aged 50–73 years (mean 59.8 years), who accepted treatment, were randomly assigned to one of the two treatment arms, as described below.

HCV genotyping and serum HCV RNA

HCV genotyping was performed by sequence determination in the 5′-non-structural region of the HCV genome [4]. The serum HCV RNA level of each patient was determined by a sensitive polymerase chain reaction (PCR) assay (COBAS TaqMan HCV Test, Roche Diagnostics, Tokyo, Japan). The COBAS TaqMan HCV test has lower and upper limits of quantitation of 15 IU/mL and 6.9×10^7 IU/mL, respectively.

Liver histology

Pretreatment liver biopsies were performed on 115 patients (93.5%). The remaining eight patients did not consent to liver biopsy due to concerns about the potential risks of the procedure. At least two well-experienced pathologists, given little clinical information, independently conducted histopathological assessments of each biopsy. Hepatic fibrosis was classified according to the METAVIR classification on a scale of F0–F4 with the following definitions: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with septa; F3, numerous septa without cirrhosis; F4, cirrhosis.

IL28B polymorphism analysis

The study also examined SVR rates relative to patient's interleukin 28B (*IL28B*) gene genotype, overall virological response during treatment, and drug regimen adherence. Single nucleotide polymorphism (SNP) testing of the *IL28B* gene (rs8099917) was done for all patients using a real-time PCR method on genomic DNA extracted from whole blood samples. Heterozygotes (TG) or homozygotes (GG) of the minor allele (G) were described as having the *IL28B* minor allele, whereas homozygotes for the major allele (TT) were described as having the *IL28B* major allele [13,14].

Treatment regimen

The 123 patients were assigned to two treatment groups: RLX plus SOC (n = 62) and SOC only (n = 61). Randomization was done at a 1:1 ratio according to a predefined, computer-generated list, using a permuted block method stratified by histological finding (F0–2 *vs.* F3–4) by each study hospital laptop computer. SOC comprised weekly subcutaneous injection of PegIFNα2a (PEGASYS®, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) and twice-daily oral RBV (COPEGUS®, Chugai Pharmaceutical Co., Ltd.) (600 mg/day for patients weighing <60 kg; 800 mg/day for those 60–79 kg; and 1000 mg/day for those >80 kg). RLX plus SOC added once-daily oral RLX (EVISTA Tablets®, Eli Lilly Japan K.K., Kobe, Japan) (60 mg/day) to SOC. Both participants and physicians were aware of which treatment was being administered. Due to the absence of an RLX placebo, neither the patients nor the investigators were blinded to the treatment group to which each patient was assigned.

The study had a planned treatment duration of 48 weeks. However, the study was designed with a futility stopping rule that would halt the trial if there was <2 $\log_{10}$ HCV RNA decline at week 12 or persistent viremia at week 24. The treatment duration was prolonged to 72 weeks only for patients who had detectable HCV RNA at week 12 but undetectable at week 24. All patients were followed for 24 weeks after cessation of treatment.

Virological response assessment

Patients with undetectable serum HCV RNA 24 weeks after cessation of treatment were designated as having an SVR. Patients who became serum HCV RNA-negative during the treatment period but became serum HCV RNA-positive within the 24 weeks following cessation of treatment were designated as having a relapse. Patients who remained HCV RNA-positive through the treatment period were designated as having a null response (NR). During the treatment, additional responses were also observed, including rapid virological response (RVR), undetectable HCV RNA at week 4; complete early virological response (cEVR), detectable HCV RNA at week 4 but undetectable HCV RNA at weeks 8–12; complete late virological response (cLVR), detectable HCV RNA during weeks 4–12 but undetectable during weeks 16–48; and end-of-treatment response (ETR), undetectable HCV-RNA at the end of treatment.

Drug adherence

The association between virological response and adherence to the planned drug regimen was assessed. This was accomplished by evaluating the medical records of each patient to determine the total dosage of PegIFNα2a and RBV actually administered during the full treatment period. Good drug adherence was defined as a total dosage $\geqslant$80% of the planned dosage of PegIFNα2a and RBV.

Statistical analysis

The study was designed to have an 80% power to detect a difference $\geqslant$25% in SVR rates between the two treatment groups. The significance level of the 2-sided test was targeted at $p = 0.05$. Based on this assumption, 61 patients were enrolled in