European Journal of Cell Biology 92 (2013) 12–20

Contents lists available at SciVerse ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

The kinase inhibitor Sorafenib impairs the antiviral effect of interferon α on hepatitis C virus replication

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ARTICLE INFO

Article history:
Received 26 June 2012
Received in revised form 8 September 2012
Accepted 14 September 2012

Keywords:
Hepatitis C virus
Sorafenib
Signal transduction
Cell biology
Interferon α

ABSTRACT

Recently, it was shown that the kinase inhibitor Sorafenib efficiently blocks HCV replication by inhibition of c-Raf. However, a longer treatment with higher doses of Sorafenib might be associated with adverse effects. Therefore, it was analysed whether a decreased dose of Sorafenib can be applied in combination with interferon α to obtain additive antiviral, but at the same time decreased adverse effects. However, Sorafenib abolishes the inhibitory effect of interferon α on HCV replication and vice versa. In order to reveal the underlying mechanisms, we observed that on the one hand IFNα activates c-Raf and thereby counteracts the inhibitory effect of Sorafenib on HCV replication that is based on the Sorafenib-dependent inhibition of c-Raf. On the other hand we found that the IFNα-induced PKR-phosphorylation depends on c-Raf. So, Sorafenib as a potent inhibitor of c-Raf prevents the IFNα-dependent PKR phosphorylation. Moreover, Sorafenib inhibits c-Raf-independent the phosphorylation of STAT1 resulting in an impaired induction of IFNα-dependent genes. Taken together, these data indicate that a combined application of Sorafenib and interferon α in order to obtain an antiviral effect is not useful since Sorafenib exerts an inhibitory effect on targets that are crucial for the transduction of interferon α-dependent antiviral response.

Introduction

Hepatitis C virus (HCV) infection results in chronic hepatitis in more than 70% of infected individuals. At present more than 170 million people are persistently infected with HCV worldwide (Koziel and Peters, 2007). Persistent HCV infection is associated with chronic inflammation of the liver (hepatitis), which can progress to liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). The standard treatment of chronic hepatitis C (CHC) is peginterferon alpha (PEG-IFNα) plus ribavirin (RBV) for 48 weeks in patients infected with genotype 1, and 24 weeks for those infected with genotype 2 or 3 (Torriani et al., 2004; Zeuzem et al., 2008). However, the frequency of adverse effects associated with the use of interferon is significant. Adverse effects include the flu-like syndrome, haematological side effects, psychiatric side effects, etc. (Askarieh et al., 2010).

Recent approaches to affect HCV replication are based on the development of HCV-specific protease inhibitors and on kinase inhibitors (Soriano et al., 2009). It has been shown by us recently that the kinase inhibitor Sorafenib efficiently impairs HCV replication of infectious HCV particles by inhibition of c-Raf as observed in HCV infected Huh7.5 cells or primary human hepatocytes (Himmelsbach et al., 2009). This promising approach might be limited by the fact that the main target of Sorafenib c-Raf as a central kinase is involved in the control of a variety of intracellular signal transduction cascades: Therefore, in case of Sorafenib treatment over a longer time period, significant adverse effects can be expected as described for RCC or HCC patients treated with this substance (Welker et al., 2010). In light of this it was tempting to speculate whether a combination of lower doses of IFNα and of Sorafenib could help to decrease adverse effects, but results in additive antiviral effects.

Materials and methods

Plasmids

Plasmids pJFH1, pJFH1/GND, pFK-lucJFH1/wt and pRafC4 have been described previously (Himmelsbach et al., 2009; Bruder et al., 1992; Carvajal-Yepes et al., 2011; Wakita et al., 2005). The overexpression of the RafC4 mutant was demonstrated by Western blot analysis (data not shown). For analysis of ISRE-dependent gene expression pISREluc (Stratagene, La Jolla, CA, USA) was used as reporter construct.

Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; IFN, interferon; tdn, trans dominant negative; RBV, ribavirin; OAS, oligoadenylate synthase; PKR, protein kinase R.

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http://dx.doi.org/10.1016/j.ejcb.2012.09.001

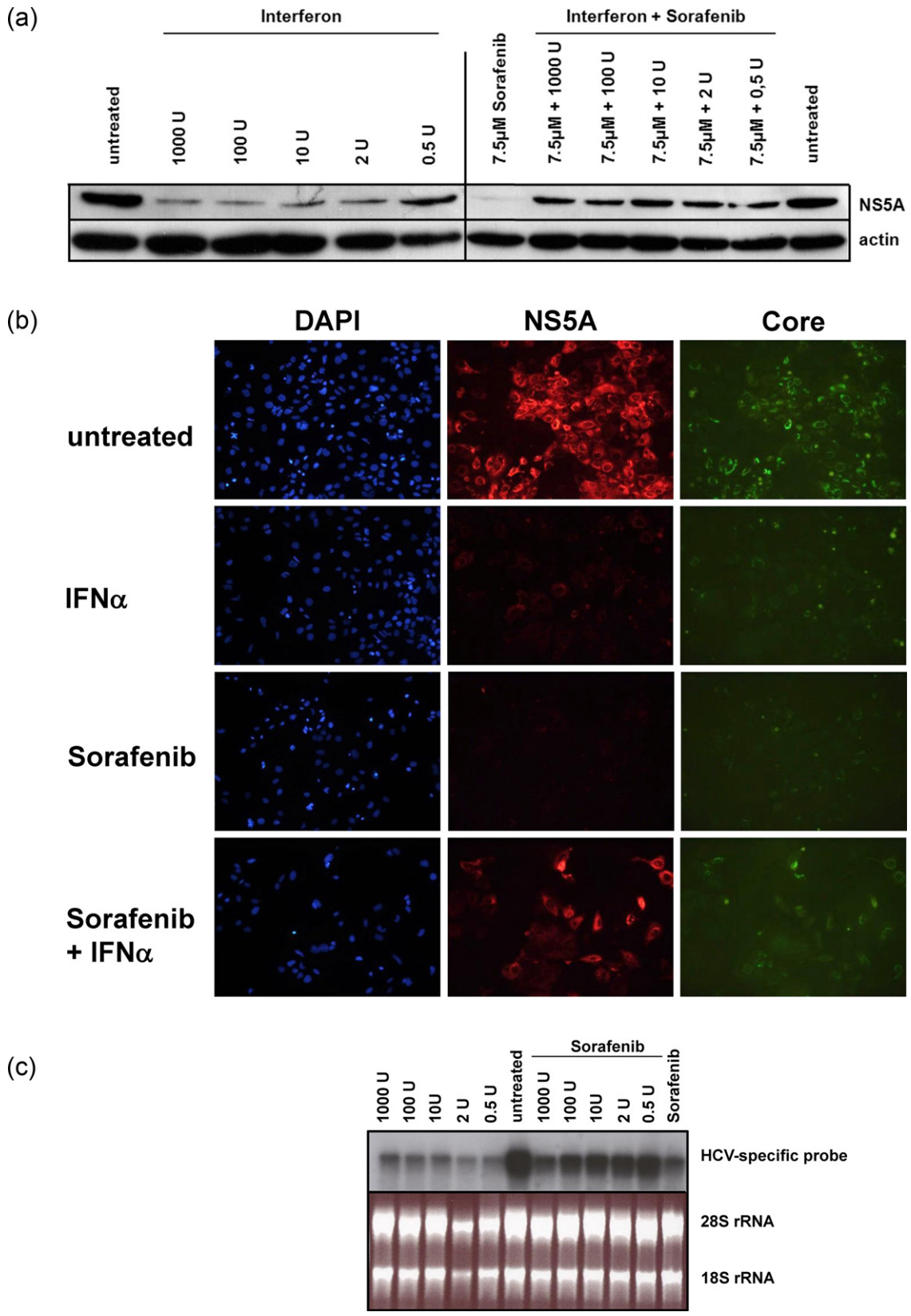

Fig. 1. Sorafenib impairs the inhibitory effect of IFNα on HCV replication. (a) Western blot analysis of cellular lysates derived from HCV replicating Huh7.5 cells stimulated with the indicated amounts of IFNα in the absence or presence of 7.5 μM Sorafenib. NS5A was detected using a polyclonal rabbit derived serum (Bruder et al., 1992), actin was detected to control equal loading. (b) Immunofluorescence microscopy of HCV replicating cells using core- (green) or NS5A- (red) specific antisera. Nuclei were stained with DAPI. Untreated HCV-positive cells served as control. Cells were grown in the presence of 100 U/ml IFN for 6 h or 5 μM Sorafenib or both reagents were administered simultaneously. (c) Northern blot analysis of total RNA isolated from HCV replicating cells that were incubated with the indicated amounts of IFNα for 6 h in the presence or absence of 5 μM Sorafenib. Untreated HCV positive cells served as control. For detection of HCV a NS5A-specific probe was used. Detection of 28S and 18S rRNA served as loading control.