Preliminary Report of Anti-Hepatitis C Virus Activity of Chloroquine and Hydroxychloroquine in Huh-5-2 Cell line

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Chloroquine and hydroxychloroquine screened for antiviral activity against Hepatitis C virus in Huh-5-2 cells. Chloroquine and hydroxychloroquine reduces the HCV RNA and promote the cell growth with respect to untreated control at concentration of 10.75 µM and 6.6 µM respectively

Chloroquine is a versatile bioactive agent and reported to possess antiviral activity against human immunodeficiency virus (HIV)[1], Hepatitis A virus (HAV)[2,3] and severe acute respiratory syndrome virus (SARS)[4] infections. The documented antiviral activities of chloroquine are by the following mechanisms (a) Viral particles entry into target cells by endocytosis is blocked by chloroquine[5-8], (b) As lysosomotrophic agent chloroquine accumulates in lysosome of host cell and increases the lysosomal pH and renders lysosomal enzymes inactive and prevent the viral decoating[8-10] (c) Intercalating action; chloroquine intercalates the HBV-DNA polymerase and inhibits the enzyme activity of the virus and blocks the multiplication of HBV[11] (d) Chloroquine inhibits Duck hepatitis B virus super coiled DNA (DHBV-sc DNA) and Duck hepatitis B surface antigen also (DHBsAg)[12]. Chloroquine inhibits the replication of HIV-1 and 2 at clinically achievable concentration and antiHIV activity is by (a) inhibition of synthesis of surface antigen GP120 of HIV[13] (b) by inhibition of HIV Integrase[14], Chloroquine also inhibits the replication of both DNA and RNA human pathogenic viruses[8,9,15]. In view of antiviral activities of chloroquine against many viruses the present work is to evaluate the antiviral activity against Hepatitis C virus in Huh-5-2 cells.

Replicon assay undertaken with Huh-5-2 cells[16-19] [a cell

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line with a persistent HCV replicon 1389luc-ubi-neo/NS3-3'/5.1; replicon with firefly luciferase-lubquitin-neomycin phosphotransferase fusion protein EMCV-IRES driven NS3-5B HCV polyprotein] was cultured in RPMI medium (Gibco) supplemented with 10% fetal calf serum, 2mML-glutamine (Life Technologies); 1x non essential amino acids (Life Technologies); 100 IU/ml penicillin and 100 µg/ml streptomycin and 250 µg/ml G418 (Geneticin, Life Technologies). Cells were seeded at a density of 7000 cells per well in 96 well View Plate TM (Packard) in medium containing the same components as described above, except for G418. Cells were allowed to adhere and proliferate for 24 h. At that time, culture medium was removed and serial dilutions of the test compounds were added in culture medium lacking G418. Interferon alfa 2a (500 IU) was included as a positive control. Plates were further incubated at 37° and 5% CO_2 for 72 h. Replication of the HCV replicon in Huh-5 cells results in luciferase activity in the cells. Luciferase activity is measured by adding 50 µl of Gloysis buffer (Promega) for 15 min followed by 50 µl of the Steady-Glo Luciferase assay reagent (Promega). Luciferase activity is measured with luminometer and signal in each individual well is expressed as a percentage of the untreated cultures. Parallel cultures of Huh-5-2 cells, seeded at a density of 7000 cells/well of classical 96-well cell culture plates (Becton-Dickinson) are treated in a similar fashion except that no Glo-lysis buffer or Stady-Glo Luciferase reagent is added. Instead the density of the culture is measured by means of the MTS method (Promega). AntiHCV data of Chloroquine and hydroxychloroquine present in Table 1.

TABLE 1: ANTIHCV ACTIVITY OF CHLOROQUINE AND HYDROXYCHLOROQUINE IN HUH-5-2 CELLS

Compounds	Conc. (µM)	Cell growth*	Viral RNA*
Chloroquine	96.9	15	2
	32.17	54	5
	10.75	**91**	**7**
	3.585	102	70
	1.201	104	82
	0.405	101	74
	0.13	109	85
	0.044	108	87
	0.013	116	86
	0.0038	109	39
Hydroxychloroquine	33	12	29
	6.6	**81**	**7**
	1.32	97	93
	0.26	97	100
	0.05	97	76
	0.01	120	99
	0.002	94	74
	0.0004	102	100
	0.00008	94	100
	0.000016	102	100

*(% of untreated control), Interferon alfa -2b at 10.000 units/well reduced the signal in the viral RNA (luciferase) assay to background levels; without any cytostatic activity

Chloroquine and hydroxychloroquine were screened for *in vitro* antiviral activity against HCV in Huh 5-2 cells using interferon alpha 2b as positive control. Parameters such as cell growth and viral RNA were measured to evaluate antiviral activity. When the compound reduced the viral RNA below 25% and promoted cell growth more than 80% with respect to the untreated control is considered as positive antiviral activity. We have found in the study that chloroquine reduced the viral RNA to below 7% and promoted cell growth to more than 91% with respect to the untreated control at the concentration of 10.75 µM. hydroxychloroquine reduced the viral RNA to below 7% and promoted cell growth to more than 81% with respect to the untreated control at the concentration of 6.6 µM. Chloroquine and hydroxylchloroquine can be useful additive agents which are long acting, cheap and least toxic, to be utilized in combination with Interferon alfa, ribavirin and amantadine and achieve radical cure of viral hepatitis C infection. So the docile antimalarial chloroquine can be designated as a effective small arm in the armamentarium against all viral infections. We would like to state that it is the first report of antiHCV activity of chloroquine and hydroxychloroquine in the world literature.

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