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Prevention of HCV infection using a broad cross-neutralizing monoclonal antibody (AR4A) and Epigallocatechin-Gallate

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Abstract

The anti-HCV activity of a novel monoclonal antibody (mAb; AR4A) and Epigallocatechin-gallate (EGCG) were studied *in-vitro* using an HCV cell culture system and *in-vivo* using a humanized liver mouse model capable of supporting HCV replication. Alone, both exhibit reliable cross-genotype HCV inhibition *in-vitro*, and combination therapy completely prevented HCV infection. *In-vivo* AR4A mAb (alone and combined with EGCG) robustly protects against the establishment of HCV genotype 1a infection. EGCG alone fails to reliably protect against HCV challenge.

Conclusion—AR4A mAb represents a safe and efficacious broadly neutralising antibody against HCV applicable to strategies to safely prevent HCV re-infection following liver transplantation, and lends further support to the concept of HCV vaccine development. The poor bioavailability of EGCG limits HCV anti-viral activity *in-vivo*.

Keywords

Liver transplantation; HCV prevention; anti-HCV monoclonal antibody; Epigallocatechin-gallate

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Conflict of Interest

The authors declare no conflict of interest.

The management of chronic Hepatitis C virus (HCV) infection continues to rapidly evolve. Novel direct acting anti-virals (DAAs) capable of achieving universally high cure rates carry much promise (1). Equitable access to these agents represents a global challenge with a large proportion of those chronically infected worldwide likely to have very limited access for the considerable future (2, 3).

DAAs will also feature prominently in the global resolve to eradicate HCV infection. HCV prevention, however, will remain fundamental to realising this aspiration (3, 4). Primary prevention via vaccination has been undermined by the lack of a convenient animal model and the challenge presented by the diversity amongst HCV genotypes and quasispecies (4-6). Likewise capitalising on the unique opportunity afforded by liver transplant to prevent HCV re-infection has been unsuccessful to date (7–9).

HCV associated liver disease (cirrhosis or hepatocellular carcinoma) is long established as the leading indication for liver transplantation worldwide (10). In those undergoing this life saving intervention, HCV re-infection is presently universal and drives inferior post-transplant outcomes (10–12). A window exists peri-transplantation to optimise post transplant outcomes by preventing allograft HCV infection. A precedent exists within liver transplantation whereby Hepatitis B virus re-infection can be prevented post-transplant (13, 14). To date replicating this in relation to HCV has proven elusive (15).

Strategies to employ existing therapies (Pegylated-Interferon/Ribavirin with or without first generation protease inhibitors) in a pre-emptive role pre-transplant or early post transplant have been unsuccessful. The intolerability and contraindications of these agents in highly complex and medically unstable patients severely limits the applicability of such approaches (7, 8). A randomised trial using hepatitis C immune globulin concluded that this was a safe and tolerable agent in liver transplant recipients but no beneficial effect on the rate of HCV recurrence was observed (9).

Very recently, limited data in select populations employing sofosbuvir and ribavirin pre-transplantation have proven the feasibility of this approach (16). Generalising this approach to complex, and often compromised patients pre and post-liver transplant is however the subject of ongoing studies.

Many monoclonal (mAb) and polyclonal antibodies targeting linear or conformational epitopes within the HCV surface glycoprotein (E1-E2) have been described that effect HCV neutralization *in-vitro* (17–19). E1/E2 interacts with a number of cell surface molecules to mediate HCV entry, e.g. C-type lectins, CD81, Scavenger receptor-B1 (20). The *in-vivo* performance of HCV neutralizing antibody has been more variable (21). Law et al characterised a number of antigenic regions on E2 and identified numerous human mAbs with cross neutralizing activity (22). This group has identified further antigenic regions of E1/E2, and generated a human mAb (AR4A) targeting a discontinuous epitope, outside the CD81 binding site on HCV E1/E2. This epitope is highly conserved across genotypes, and AR4A demonstrates potent, cross neutralizing activity *in-vitro* (23, 24).

EGCG is the most abundant catechin present in green tea extract. Green tea extracts have a long history of safe human consumption and have many purported benefits (25, 26). Green

tea extracts are safe, widely available, and inexpensive (27). Two groups independently published data reporting an *in-vitro* anti-viral effect of EGCG against HCV (28, 29). This was primarily attributed to prevention of initial attachment of the viral particle to hepatocytes. EGCG, however, also inhibited the direct cell-cell mode of HCV transmission (28). *In-vivo* evidence of the anti-HCV effect of EGCG is lacking.

Using the HCV cell culture system and the SCID/uPA humanised liver mouse model we examined the anti-HCV effect of AR4A and EGCG alone and in combination. We hypothesised that combining safe, tolerable and effective HCV therapies acting at different sites of HCV cell entry could reliably protect against HCV challenge *in-vivo*.

Materials and Methods

Cells and viruses

Huh7.5 cells (kindly provided by Dr. Charles Rice) were maintained in complete Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (vol/vol) fetal bovine serum (FBS), 0.1mM non-essential amino acids (NEAA), 100U/mL penicillin and streptomycin 100 mg/mL. Cells were incubated at 37°C in conditions of 5% CO_2 (30).

Plasmids encoding chimeric HCV genomes (provided by Dr. Charles Rice) representing genotypes 1–6 were used to generate cell culture derived HCV (HCVcc) as previously described (31).

Compounds

EGCG (Cat. number: E4143, 95% purity, Sigma-Aldrich, St Louis, MO) was dissolved in double distilled H_2O and stored at −20°C. Recombinant human interferon alpha 2a (IFN-α2a, positive control) was obtained from PBL interferon source, (Piscataway, NJ, cat. number: 11100-1) and stored as instructed. Human anti-E1/E2 mAb AR4A has previously been characterised in detail (23). Murine IgG obtained from BD biosciences (Franklin Lakes, NJ) was used as an isotype control antibody for *in-vitro* assays. Mouse monoclonal anti-human CD81 antibody from BD biosciences (Franklin Lakes, NJ, Cat number 555675) represented a positive antibody control. An isotype human anti-HIV-1 IgG was administered to the control group of mice during *in-vivo* studies. All antibodies were stored at 4°C.

HCV inhibition assays

96 well plates were coated with poly-lysine (Trevigen, Gaithersberg, MD, Cat number: 3438-100-01) prior to plating with 10^4 Huh 7.5 cells in 100 μL of growth media and incubated overnight. Serially diluted concentrations of EGCG were added as appropriate pre, simultaneous with, or after inoculation with HCVcc at a multiplicity of infection (MOI) of 0.01. For antibody neutralization assays the relevant antibody was serially diluted to the required concentrations and pre-incubated with HCVcc for one hour prior to addition to Huh 7.5 cells. IFN-α2a 100 IU/mL and anti-human CD81 antibody (2.5μg/mL) served as positive controls. Murine IgG (10μg/mL) served as an isotype antibody control. After ten hours of incubation with HCVcc the cells were washed to remove unbound virus and fresh media or the relevant concentration of the investigational agents was added. HCV infectivity was

determined at 48 hours using NS5a immune-histostaining (mouse monoclonal NS5a antibody (9E10), Dr. Charles Rice).

Cell viability assay

Cell Proliferation Kit I assay (Roche, Basel, Switzerland Cat Number: 11465007001) was performed as recommended by the manufacturer.

Animal Care

Homozygous albumin/urokinase plasminogen activator severe combined immunodeficient mice (SCID/uPA) mice were kept virus- and antigen-free and housed in the provincial laboratory vivarium at the University of Alberta (32, 33). The University of Alberta Health Sciences Animal Welfare Committee approved experimental protocols, and animals were cared for in accordance with the 1993 guidelines of the Canadian Council on Animal Care. Mice were anaesthetized for transfer of human hepatocytes via intrasplenic injection. Full ethical approval for the use of human tissue was obtained from the Human Research Ethics Board of the University of Alberta Faculty of Medicine. Informed consent was obtained from all donors.

Human alpha-1-Antitrypsin levels (hAAT)

The extent and stability of human liver chimerism can be assessed by serial measurements of hAAT. Serum hAAT levels were analysed as previously described (34). Animals with low serum hAAT levels were used in tolerability/toxicology studies; high hAAT mice were used in the HCV challenge studies.

Dosing and administration of agents

Anti E1/E2 monoclonal antibody (AR4A)—An initial dose of 200mg/kg was administered via intraperitoneal (IP) injection 24 hours prior to HCV inoculation. Prior studies have shown that this dose yields mAb serum values in excess of *in-vitro* 90% neutralization titers (22). A further four mAb doses of 50mg/kg were administered IP at intervals of 5 days throughout the experiment. Mice in the control group received equivalent doses of an isotype antibody; human anti-HIV-1 IgG.

EGCG—Considering the available information on *in-vivo* EGCG pharmacokinetics, toxicity, and efficacy, a dosing schedule of 100mg/kg twice daily by gavage(IG) was employed (35). This dose was higher than that which had demonstrated efficacy in previous mouse studies but lower than that with which toxicity had been observed with repeated dosing (36). Further this dosing schedule was within that tolerated by SCID/uPA mice with reference to volume and frequency of gavage. EGCG dosing began 48 hours prior to HCV inoculation and continued for 14 days.

Experimental Protocol

Screening of serum hAAT levels was undertaken six weeks following transplantation of human hepatocytes. Mice with serum hAAT levels (>500 μg/mL) reflecting high

engraftment of human hepatocytes were selected to go forward into the HCV challenge studies. In total 11 mice were assigned to each of 4 study groups:

Group 1: Control mice receiving isotype antibody IP and water IG

Group 2: Mice receiving EGCG IG only

Group 3: Mice receiving AR4A mAb IP only

Group 4: Mice receiving both AR4A IP and EGCG IG

The study outline and protocol employed is illustrated in figure 1.

HCV inoculation was administered by intrajugular injection. The inoculum used (50 μL, 1.5×10^7 IU/mL) was patient derived HCV genotype 1a serum. Blood sampling was conducted weekly by drawing 100 μL via tail bleeds for measurement of HCV titers and serum hAAT levels.

EGCG Tolerability/Toxicology: EGCG 200mg/kg/day was administered to uninfected low hAAT level animals. Control animals received an equivalent volume of water. The health status of the animals was monitored by their general condition and weight change. At the end of the 14 day dosing period the animals were euthanized. Cardiac puncture was performed to obtain serum for measurement of EGCG levels and liver tissue was snap frozen and stored at −80°C for later measurement of EGCG.

HCV RNA quantification

Viral RNA was extracted from aliquots of mouse serum using a guanidine extraction method (Buffer AVL. Qiagen, Valencia, CA, Cat number: 19073) as per the manufacturer's instructions and quantitated as described previously (34). The lower limit of quantification of this assay is 300 IU/mL.

EGCG quantification

Plasma and liver tissue levels of EGCG were analyzed using high performance liquid chromatography (HPLC) methodology as previously published (35).

Statistical analysis

Statistical analyses were performed using Stata (Version 12.0, StataCorp LP, College Station, TX) and GraphPad prism (version 6.0, La Jolla, CA) software. Continuous variables were compared between groups using a Mann Whitney U test. A P value less than 0.05 was considered significant. The drug concentration at which HCV infection was inhibited by 50% (IC_{50}) was calculated using the following equations:

$$\text{Best fit curve} \qquad y = a^{*}e^{-\text{slope}^{*}x}$$

$$IC_{50} \qquad x = \text{Log}_{10}(50/a)/\text{slope}^{*}\text{Log}(\text{EXP}(1))$$

In the HCV challenge experiments, animals with HCV RNA detectable above the threshold (1000 IU/mL) by PCR at day 7 or thereafter were considered 'infected'. Animals not reaching this threshold were 'censored' and considered free from HCV infection. A Kaplan-

Meier survival curve ('Survival free from infection') was thus generated. Statistical significance between the groups was calculated using a two-tailed log rank test.

Results

AR4A demonstrates effective HCV neutralizing activity *in-vitro*

The *in-vitro* neutralizing capability of AR4A against HCVcc expressing surface glycoproteins of genotypes (gt) 1–6 was examined. Focusing on gt1a (genotype utilized for mouse challenge experiments) and 2a (parent JFH genotype), AR4A exhibited superior neutralizing activity against HCV genotype 1a when compared with genotype 2a; consistent with prior reports (23, 37). IC_{50} estimates of 1.28 μg/mL and 4.37 μg/mL were obtained for genotypes 1a and 2a, respectively (Figure 2 a,b).

Epigallocatechin-gallate (EGCG) inhibits HCV infection *in-vitro*

A dose dependent decline in HCV infection was observed when Huh 7.5 cells had been simultaneously treated with various concentrations of EGCG (0–100 μg/mL) (Figure 2 c,d). The EGCG IC_{50} was similar for HCVcc gt 1a and 2a infections (6.6 ug/ml vs. 5.6 ug/ml respectively). At concentrations greater than 25 μg/ml, EGCG consistently achieved almost complete inhibition of HCVcc infection. This inhibitory effect of EGCG was independently confirmed using a firefly luciferase reporter virus, and EGCG did not negatively impact cellular viability. Time of addition assays confirmed that optimal HCV inhibition was critically dependent upon the presence of EGCG at the time of infection (not shown).

Additive reduction in HCV infection combining AR4A and EGCG

AR4A and EGCG primarily act early in the HCV life cycle to inhibit HCV cellular attachment and entry. Using the HCVcc system, we studied the anti-HCV activity of both agents in combination.

Using gt1a HCVcc, AR4A (2 μg/mL) combined with EGCG (10 μg/mL) demonstrated significantly increased inhibition compared to either agent alone; 88.7% inhibition compared to 61% for AR4A alone (P=0.01), 56% for EGCG alone (Figure 3a). Additive efficacy was again demonstrated with HCV gt 2a(Figure 3b).

High dose AR4A with low dose EGCG completely inhibits HCV infection *in-vitro*

To replicate the clinical scenario whereby therapeutic antibody products are administered at high concentrations, and knowing that the *in-vivo* bioavailability of EGCG may prove a limiting factor we next titrated AR4a and EGCG to identify conditions capable of completely inhibiting HCV infection. The mAb AR4A, at a concentration of 10 μg/mL, consistently achieved approximately 95% neutralization of genotype 1a HCV. Even at the highest dose of AR4A used (50 μg/mL), residual infection could be detected. Despite 95% neutralization with AR4A (10 μg/mL) alone, the addition of EGCG at 10 μg/mL resulted in a significant further inhibition of HCV (P<0.001 for AR4a alone vs. combination), with complete inhibition of HCV infection attained in a number of experimental repeats (Figure 4). The activity of AR4A (10 μg/mL) and EGCG (10 μg/mL) in combination was further assessed against JFH-1 chimeric constructs expressing the structural proteins of genotypes

2–6. High titer AR4A strongly neutralized genotypes 4a, 5a and 6a; a finding in keeping with prior reports (23).

Animal HCV Challenge Experiments

All therapeutic regimens were well tolerated by the animals. Serum hAAT levels in mice were evenly distributed through the four groups, and remained broadly stable throughout the experiment. Two mice did not recover following the intrajugular HCV inoculation procedure, one animal in each of the control and combination groups. Additionally two mice from the EGCG alone, and the combination groups became morbid following the day seven blood draw, and one animal in the AR4A alone arm was lost after day 14.

AR4A containing treatment arms robustly protected against HCV infection in SCID/uPA mice; EGCG alone has no apparent protective effect—HCV infection was established in eight of ten control animals, with five progressing to high level replication over 42 days of follow-up.

AR4A demonstrated clear efficacy. Only 2/11 mice receiving AR4A alone and 1/10 mice in the combination arm had HCV RNA detected above the threshold of 1000IU/mL throughout study follow-up. 'Survival free from HCV infection' was significantly increased in AR4A treated groups. Given the small number of events in these study groups no difference was demonstrable between the groups receiving AR4A alone or both AR4A and EGCG.

EGCG monotherapy failed to reliably protect from HCV infection and observed outcomes were no different from the control group. The viral kinetics and a Kaplan-Meier curve of 'Survival Free from HCV Infection' are illustrated in figure 5 and 6 respectively.

EGCG levels in plasma and liver tissue of mice following 14 consecutive days of administration—In order to assess if repeated high doses of EGCG 200mg/kg/day were capable of achieving sufficient levels of EGCG (and its metabolites) in plasma and liver we collected samples after 14 consecutive days of dosing. For this analysis samples were obtained four hours following the final dose. Figure 7 illustrates the levels of detection of EGCG and its metabolites. The parent compound itself or its metabolites were detectable in all treated animals, however importantly the levels in both plasma and liver tissue were low (in the nanomolar range) when measured four hours post administration.

Discussion

HCV related liver disease is the leading indication for liver transplantation. Re-infection continues to drive inferior outcomes (12). Numerous unsuccessful attempts to address this discrepancy have been made. Recently, novel approaches provide some cause for optimism (16, 38), potentially shifting the paradigm whereby the treatment of HCV after liver transplantation is restricted to a delayed phase post transplantation when reinfection and histological disease has already been established (15, 39, 40).

There is a need for safe, effective and tolerable therapies that can avail of the window of opportunity provided by liver transplantation to prevent HCV re-infection. Complex drug-

drug interactions require due consideration as does treatment durability in the face of a dynamic virus with high replicative capacity in the setting of impaired host humoral and cell mediated immunity. Effective inhibition of HCV entry into 'naïve' allograft hepatocytes represents a primary objective of preventative strategies.

In this work we investigated agents acting primarily to inhibit HCV cell entry. Consistent with prior reports AR4A and EGCG both reliably demonstrate cross genotype inhibition of HCV *in-vitro*. Using these agents in combination was additive and the value of EGCG maintained significance even at high AR4A titers.

SCID/uPA mice receiving AR4A mAb by intraperitoneal injection were significantly less likely to develop established infection following HCV challenge with a genotype 1a inoculum. This is the first data reporting the anti-HCV efficacy of AR4A monotherapy in an animal model capable of sustaining HCV replication. AR4A clearly provides robust protection against the initial establishment of infection, demonstrating durable activity, which compares favourably with prior studies employing immunotherapy (21, 22).

Despite clear *in-vitro* inhibition, administration of EGCG alone, whilst well tolerated by SCID/uPA mice, did not offer protection against HCV challenge *in-vivo*. This is the first report concerning the *in-vivo* anti-HCV activity of EGCG. This lack of efficacy was likely driven by the low bioavailability of orally-administered EGCG (41, 42). Previous studies have shown that peak plasma and liver concentrations of unconjugated EGCG in mice were 40 nmol/L and 3.5 nmol/g, respectively following treatment with intragastric EGCG (75 mg/kg). EGCG undergoes extensive Phase II metabolism resulting in the formation of glucuronide conjugates and methylated metabolites. The levels of these metabolites have been shown in animal models to exceed the levels of the parent compound (41, 43). Studies have shown that EGCG is rapidly metabolized by catechol-*O*-methyltransferase (COMT) resulting in the formation of MeEGCG and diMeEGCG (44). These methylated metabolites have been shown to have reduced biological activity in a number of systems compared to the unmetabolized parent compound (45, 46).

To date, no studies have examined the effects of methylation on the antiviral activity of EGCG. A recent study of influenza virus reported that methylated (−)-epigallocatechin has reduced inhibitory potency (IC_{50} = 33.4 μg/mL) compared to unmetabolized (−)-epigallocatechin (IC_{50} = 13.5 μg/mL) *in vitro* (47). Given the extensive Phase II biotransformation of EGCG *in vivo,* further studies on the anti-viral activity of the major metabolites are warranted.

Calland et al have recently proposed a novel mechanism whereby EGCG and related natural compounds disrupt initial HCV attachment *in vitro*. Through an interaction with surface glycoproteins such compounds alter the shape of HCV viral particles inhibiting efficient cellular attachment (48). With a proposed mechanism of action reliant on direct disruption of the initial stages of HCV cellular attachment and direct cell-cell transmission, failing to maintain adequate local concentrations will clearly limit the efficacy of EGCG *in-vivo*.

Prior clinical studies administering passive immunotherapy to HCV patients undergoing liver transplantation have been disappointing. Polyclonal immunoglobulin failed to prevent

HCV re-infection (9). Similarly an anti-E2 mAb (HCV-AbxTL68), whilst effecting some decline in titers, did not prevent HCV recurrence in patients undergoing liver transplantation (49). Another group administered an anti-E2 mAb (MBL-HCV1) to six HCV transplant recipients. All eventually experienced re-infection with viral species harboring mutations in the target epitope (50).

AR4A consistently demonstrated a robust ability to protect against HCV challenge with a patient derived genotype 1a inoculum, and its *in-vitro* characteristics compare very favorably with those of other anti-HCV mAbs (23, 37, 51). The increased efficacy of AR4A mAb can be proposed by it uniquely targeting an epitope region abridging both E1 and E2, and containing a residue which is highly conserved across HCV species.

The SCID/uPA humanized liver mouse model is an extremely useful tool for conducting *in-vivo* HCV studies (32, 33, 37). These mice lack an adaptive immune response, thus caution is required when translating findings to the clinical situation, considering that pre-existing host antibody bound to HCV viral particles can competitively inhibit effective neutralization by anti-HCV mAbs (52, 53). Likewise it is possible that the native viral particle in the experimental systems used is physically different to that which circulates in association with human lipoproteins. An overestimation of efficacy could contribute to the comparatively poor activity observed in patient studies to date (9, 49, 50).

Another limitation is the use of a patient derived genotype 1a inoculum only. Genotype 1 is the predominant HCV genotype in Europe and North America, however the immediate generalizability to individuals with HCV of diverse genotypes awaiting liver transplant is somewhat limited. However clear *in-vitro* cross genotype activity of AR4A was demonstrated. Further, the patient inoculum provided a heterologous HCV species challenge against which AR4A demonstrated impressive preventative capacity.

Despite these limitations this study has yielded a number of important findings. We have demonstrated the proficiency of the anti-E1/E2 mAb AR4A to prevent the establishment of replicating HCV infection *in-vivo*. On the other hand, EGCG, though an effective *in-vitro* inhibitor, demonstrated a lack of definitive efficacy to protect SCID/uPA mice against HCV infection.

The results identify next generation anti-E1/E2 monoclonal antibodies as a potential therapeutic advance, which can constitute a primary component of future preventative approaches. The cross neutralizing ability of AR4A targeting a highly conserved epitope also provides further proof of principle that effective pre-formed antibody can contribute to protection against HCV challenge. A candidate vaccine has been shown to elicit such antibodies in vaccinated human volunteers, providing encouragement for demonstration of future preventive efficacy (54).

To optimize the efficacy of this mAb in liver transplantation, additional agents to protect against virologic breakthrough and resistant mutants will be a prerequisite. Future clinical studies of anti-HCV mAbs incorporating novel pan-genotypic DAAs in complex transplant candidates with advanced HCV-related liver disease are required.

The application of EGCG, though eminently more available worldwide than DAAs, is limited by poor bioavailability. Further characterization of the interaction between the HCV viral particle and EGCG carries the potential to identify new agents targeting HCV cell entry (48). Moreover, capitalizing upon the unique circumstances of liver transplantation, novel applications of non-toxic compounds such as EGCG or derivatives could provide an opportunity to prime liver allografts *ex-vivo* against HCV reinfection following implantation.

Employing therapies pre or immediately at transplantation stands to deliver timely, effective, and tolerable HCV prophylactic combinations. This provides considerable optimism that in the future routine prevention of HCV re-infection after liver transplantation is in fact attainable. In achieving this goal, outcomes for patients with HCV undergoing liver transplant will be brought back in line with that of their non-HCV counterparts, whilst ensuring optimal use of a highly valuable resource – human livers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DAA	direct acting antivirals
Di-Me EGCG	Dimethylated Epigallocatechin gallate
DMEM	Dulbecco's Modified Eagle's Medium
EGCG	Epigallocatechin gallate
FBS	Fetal bovine serum
h-AAT	human alpha-1-antitrypsin
HCV	Hepatitis C virus
HCVcc	cell culture derived Hepatitis C Virus
HPLC	High performance liquid chromatography
IC_{50}	50% inhibitory concentration
IFN	Interferon

IG	Intragastric
IP	Intraperitoneal
mAb	Monoclonal antibody
Me-EGCG	Methylated epigallocatechin gallate
MOI	multiplicity of infection
NEAA	Non-essential amino acids
PCR	Polymerase Chain reaction
SCID/uPA	Severe combined immunodeficiency/Albumin/urokinase plasminogen activator

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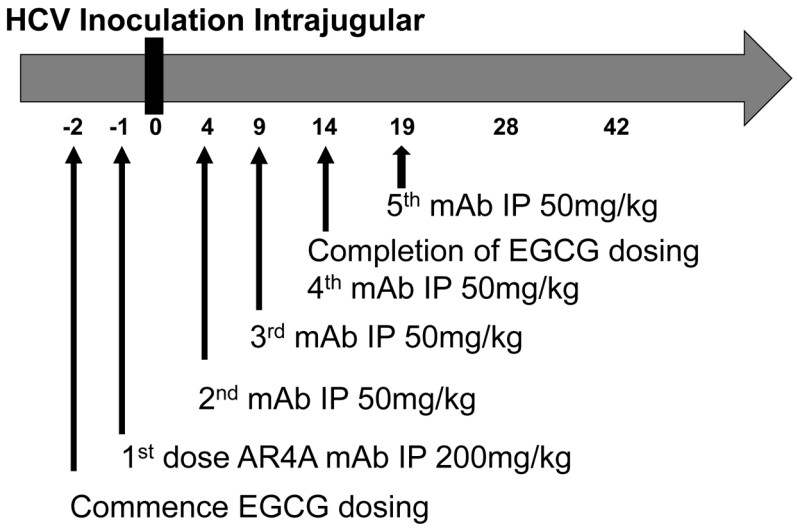

Figure 1.

Schematic outline of SCID/uPA mouse HCV challenge experiments. Animals were pretreated with EGCG and/or AR4A prior to challenge with HCV. Treatment continued as indicated. mAb: monoclonal antibody, IP: intraperitoneal, * four doses of mAb at 50mg/kg.

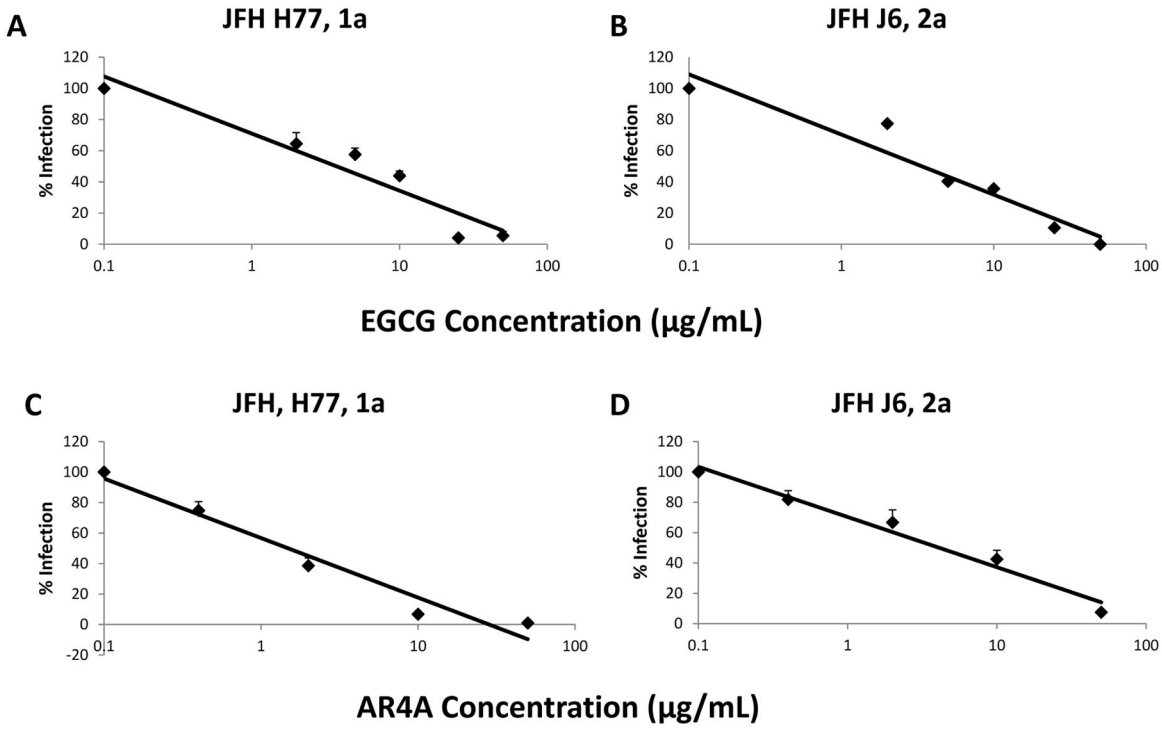

Figure 2.

Anti E1/E2 mAb AR4A (A,B) and EGCG (C,D) dose dependently inhibits infection with JFH-1 1a(A,C) and 2a(B,D) constructs *in-vitro*. Huh 7.5 cells were treated with EGCG simultaneous with addition of JFH-1 (HCVcc MOI 0.01). AR4A was pre-incubated with JFH-1 for 1 hour prior to addition to Huh 7.5 cells. Ten hours after JFH-1 addition, cells were washed and residual infection in Huh 7.5 cells was detected by NS5A staining at 48 hours. Mean % residual HCV infection is shown. Error bars indicate sem.

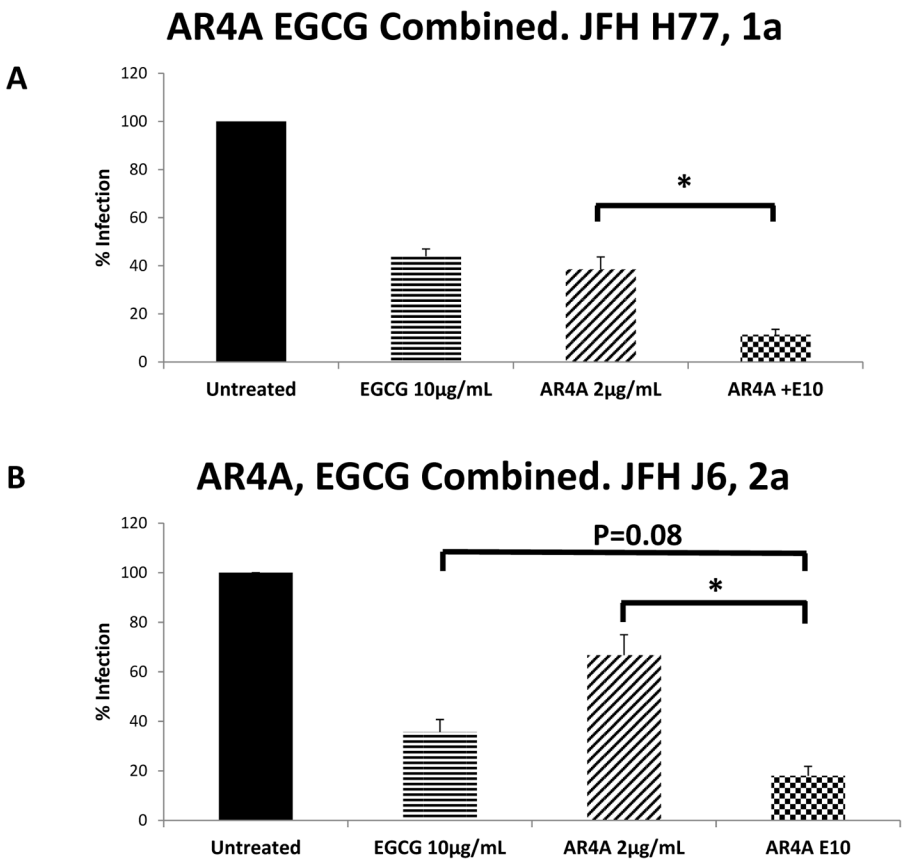

Figure 3.

AR4A mAb and EGCG additively inhibit infection with JFH-1 1a(A) and 2a(B) constructs *in-vitro*. Huh 7.5 cells were treated with EGCG simultaneous with addition of JFH-1 (HCVcc MOI 0.01). AR4A was pre-incubated with JFH-1 for 1 hour prior to addition to Huh 7.5 cells. Ten hours after JFH-1 addition, cells were washed and residual infection in Huh 7.5 cells was detected by NS5A staining at 48 hours. Mean % residual HCV infection is shown. Error bars indicate sem. * P=0.01.

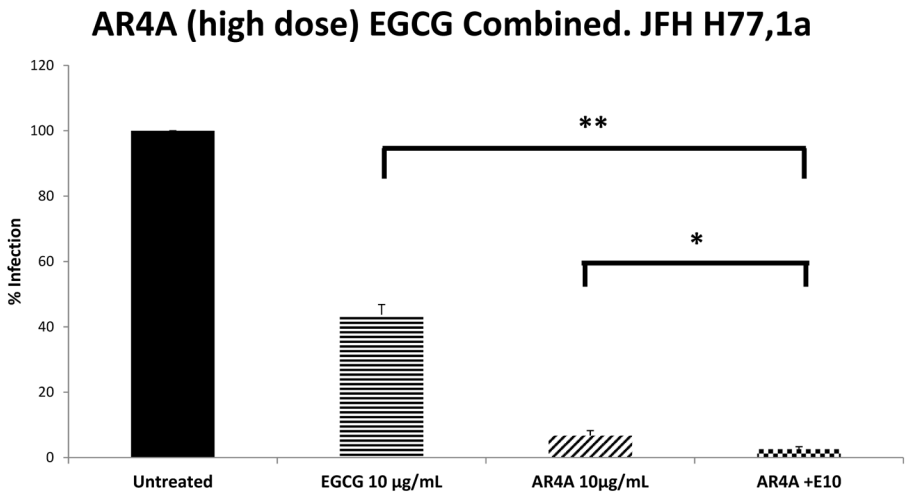

Figure 4.

High dose AR4A mAb and low dose EGCG combined robustly inhibit infection with JFH-1 genotype 1a *in-vitro*. Huh 7.5 cells were treated with EGCG simultaneous with addition of JFH-1 (HCVcc MOI 0.01). AR4A 10µg/mL was preincubated with JFH-1 (HCVcc MOI 0.01) and added to Huh 7.5 cells simultaneous with or without EGCG 10µg/mL. Ten hours after JFH-1 addition, cells were washed and residual infection in Huh 7.5 cells was detected by NS5A staining at 48 hours. Mean % residual HCV infection is shown. Error bars indicate sem. Data from 5 independent experiments conducted in triplicate. * P=0.02; ** P<0.001.

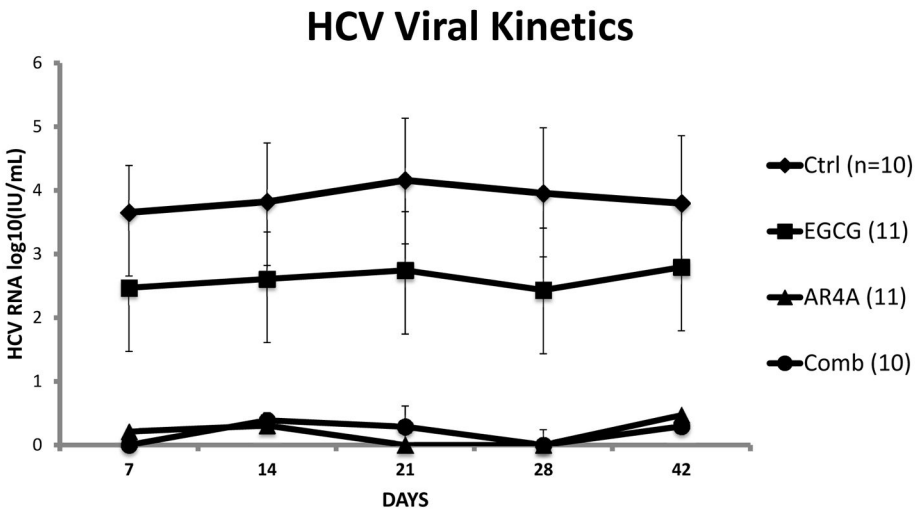

Figure 5.

HCV viral kinetics in mice according to intervention group. Mean $\log_{10}$ HCV RNA values are shown. Error bars indicate sem. Animal treatment groups: Control mice were treated with isotype human IgG; Animals treated with EGCG alone (200mg/kg/day for 14 days); Animals treated with AR4A alone (5 intraperitoneal injections); Animals treated with both AR4A and EGCG.

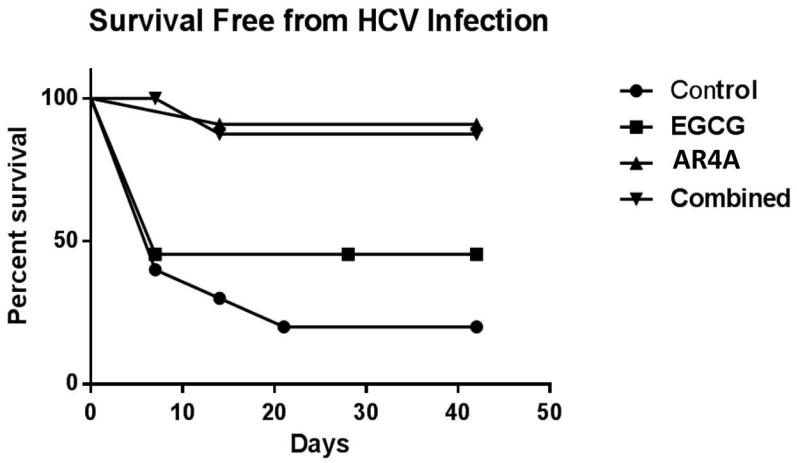

Figure 6.

Kaplan-meier 'Survival Free From HCV Infection'. Mice failing to achieve the pre-determined threshold for active HCV replication (HCV RNA > 1000IU/mL at or after day 7) were censored. Animal treatment groups: Control mice were treated with isotype human IgG; Animals treated with EGCG alone (200mg/kg/day for 14 days); Animals treated with AR4A alone (5 intraperitoneal injections); Animals treated with both AR4A and EGCG.

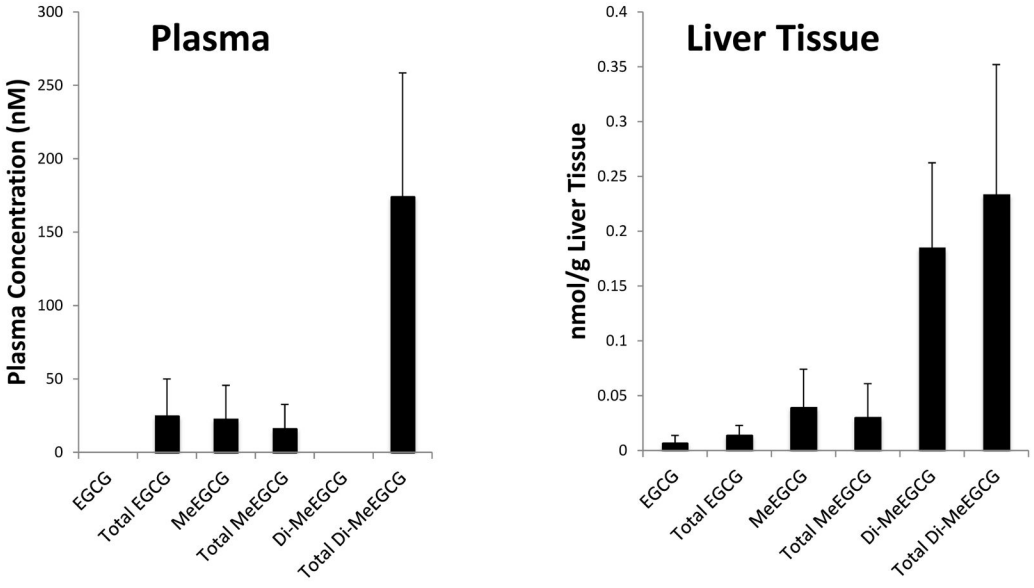

Figure 7.

Detection of EGCG, Monomethylated EGCG (MeEGCG) and Dimethylated EGCG (Di-MeEGCG) in plasma and liver tissue of treated mice. SCID/uPA Mice underwent daily intragastric instillation of EGCG 200mg/kg/day for 14 days. 4 hours following the final dose the animals were euthanized. Serum was stored, and liver tissue snap frozen for later assays of levels of EGCG and its metabolites. The mean value of 3 treated mice are presented, bars indicate sem. Total: includes estimates of parent compound, in addition to the glucuronide and sulfated metabolites.