# Antibiotic Pretreatment Alleviates Liver Transplant Damage in Mouse and Man

Kojiro Nakamura[1*], Shoichi Kageyama[1*], Takahiro Ito[1*], Hirofumi Hirao[1*], Kentaro Kadono[1], Antony Aziz[1], Kenneth J. Dery[1], Matthew J. Everly[2], Kojiro Taura[3], Shinji Uemoto[3], Douglas G. Farmer[1], Fady M. Kaldas[1], Ronald W. Busuttil[1] and Jerzy W. Kupiec-Weglinski[1]

[1] The Dumont-UCLA Transplantation Center, Department of Surgery, Division of Liver and Pancreas Transplantation, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

[2] The Terasaki Research Institute, Los Angeles, CA 90024, USA

[3] Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

*These authors contributed equally

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**Recipients' peri-operative variables (biopsied 52 cases)**

| Variables | Control (n=24) | Abx (n=28) | p value |
|---|---|---|---|
| Age (years) | 60 (29-73) | 52 (23-71) | **0.008** |
| Gender (M/F) | 18 (75.0%) / 6 (25.0%) | 18 (64.3%) / 10 (35.7%) | 0.549 |
| Race | | | 0.102 |
| White | 8 (33.3%) | 13 (46.4%) | |
| Hispanic | 8 (33.3%) | 11 (39.3%) | |
| Black | 1 (4.2%) | 2 (7.1%) | |
| Asian | 5 (20.8%) | 0 | |
| Others | 2 (8.3%) | 1 (3.6%) | |
| Height (cm) | 170.2 (149.9-182.9) | 176.3 (152.0-195.6) | 0.120 |
| BMI (kg/m$^2$) | 26.2 (16.6-38.3) | 28.7 (14.5-47.5) | 0.086 |
| Past Histroy | | | |
| Smoking | 14 (58.3%) | 11 (39.2%) | 0.266 |
| Diabetes | 9 (37.5%) | 9 (32.1%) | 0.774 |
| Hypertention | 11 (45.8%) | 8 (28.6%) | 0.253 |
| Heart disease | 4 (16.7%) | 3 (10.75) | 0.690 |
| Abdominal surgery | 9 (37.5%) | 8 (28.6%) | 0.562 |
| Disease ethiology | | | 0.125 |
| HBV | 2 (8.3%) | 0 | |
| HCV | 14 (58.3%) | 11 (39.3%) | |
| EtOH | 1 (4.2%) | 7 (25.0%) | |
| Cryptogenic cirrhosis / NASH | 4 (16.7%) | 7 (25.0%) | |
| ALF | 0 | 1 (3.6%) | |
| Others | 3 (12.5%) | 2 (7.1%) | |
| HCC (with/without) | 16 (66.7%) / 8 (33.3%) | 4 (14.3%) / 24 (85.7%) | **<0.001** |
| ABO | | | 0.590 |
| identical | 22 (91.7%) | 27 (96.4%) | |
| compatible | 2 (8.3%) | 1 (3.6%) | |
| MELD score | 15 (6-42) | 37 (16-47) | **<0.001** |
| Pre-transplant AST (IU/L) | 56.0 (23-268) | 58.5 (23-283) | 0.601 |
| Pre-transplant ALT (IU/L) | 50 (15-299) | 29.5 (11-217) | 0.189 |
| Abx use during OLT | | | 0.833 |
| ampicillin-sulbactam | 18 (75.0%) | 19 (67.9%) | |
| piperacillin/tazobactam | 3 (12.5%) | 4 (14.3%) | |
| Others | 3 (12.5%) | 5 (17.9%) | |
| Post-OLT Abx use within 7 days | 14 (58.3%) | 20 (71.4%) | 0.388 |
| Post-OLT Basiliximab use | 10 (41.7%) | 19 (67.9%) | 0.093 |

**Table S1: Recipients' peri-operative variables (biopsied 52 cases)**

**Donors' and grafts' parameters (biopsied 52 cases)**

| Variables | Control (n=24) | Abx (n=28) | p value |
|---|---|---|---|
| Age (years) | 44.5 (7-65) | 34 (13-67) | 0.142 |
| Gender (M/F) | 10 (41.7%) / 14 (58.3%) | 16 (57.1%) / 12 (42.9%) | 0.404 |
| Race | | | 0.225 |
| White | 14 (58.3%) | 16 (57.1%) | |
| Hispanic | 8 (33.3%) | 9 (32.1%) | |
| Black | 2 (8.3%) | 0 | |
| Asian | 0 | 3 (10.7%) | |
| Others | 0 | 0 | |
| Height (cm) | 167.5 (131.0-187.9) | 170.1 (152.4-190.0) | 0.357 |
| BMI (kg/m$^2$) | 25.6 (13.4-42.6) | 27.0 (19.6-34.9) | 0.709 |
| Pre-transplant AST (IU/L) | 43.5 (12-314) | 43 (13-189) | 0.912 |
| Pre-transplant ALT (IU/L) | 24 (11-403) | 28 (8-730) | 0.978 |
| T-Bil (g/dl) | 0.7 (0.2-4.9) | 0.7 (0.3-1.9) | 0.599 |
| PT-INR | 1.25 (1-2.0) | 1.2 (1-1.8) | 0.688 |
| Warm ischemia time (min) | 52.5 (23-79) | 53.0 (29-78) | 0.818 |
| Cold ischemic time (min) | 485.5 (328-762) | 434 (150-747) | 0.177 |
| Donor Abx use (yes/ no or unknown) | 16 (66.7%) / 8 (33.3%) | 16 (57.1%) / 12 (42.9%) | 0.573 |
| Donation status (DCD / DBD) | 1 (4.2%) | 2 (7.1%) | 0.566 |

**Table S2: Donors' and grafts' parameters (biopsied 52 cases)**

**Recipients' preoperative status and surgical parameters (264 cases, October/2013-August/2015)**

| Variables | Control (n=**108**) | Abx (n=**156**) | p value |
|---|---|---|---|
| Pre-OLT blood culture postive | 3 (2.8%) | 31 (19.9%) | **<0.001** |
| Pre-operative hospital stay (days) | 1 (0-136) | 15 (0-152) | **<0.001** |
| Pre-operative ICU stay (days) | 0 (0-72) | 5 (0-145) | **<0.001** |
| RBC transfused during surgery(ml) | 3375 (500-26000) | 6000 (1250-30750) | **<0.001** |
| FFP transfused during surgery(ml) | 3875 (0-28750) | 6000 (110-25000) | **<0.001** |
| PLT transfused during surgery(ml) | 2341 (0-1195) | 462 (0-1877) | **<0.001** |

**Table S3: Recipients' preoperative status and surgical parameters (264 cases, October/2013-August/2015)**

**Donors' and grafts' parameters (264 cases, October/2013-August/2015)**

| Variables | Control (n=**108**) | Abx (n=**156**) | p value |
|---|---|---|---|
| Age (years) | 39 (7-76) | 36.5 (13-72) | 0.306 |
| Gender (M/F) | 70 (64.8%) / 38 (35.2%) | 94 (60.3%) / 62 (39.7%) | 0.519 |
| Race | | | 0.482 |
| White | 63 (58.3%) | 88 (56.4%) | |
| Hispanic | 31 (28.7%) | 48 (30.8%) | |
| Black | 4 (3.7%) | 12 (7.7%) | |
| Asian | 8 (7.4%) | 6 (3.8%) | |
| Others | 2 (1.9%) | 2 (1.3%) | |
| Height (cm) | 172.7 (131.0-196.0) | 170.2 (134.6-198.0) | 0.260 |
| BMI (kg/m$^2$) | 25.6 (13.4-44.4) | 26.5 (17.0-49.6) | 0.459 |
| Pre-transplant AST (IU/L) | 37.0 (10-314) | 39.5 (8-669) | 0.584 |
| Pre-transplant ALT (IU/L) | 29 (8-403) | 33 (6-730) | 0.357 |
| T-Bil (g/dl) | 0.7 (0.1-11.5) | 0.7 (0.2-3.5) | 0.111 |
| PT-INR | 1.2 (0.9-4.7) | 1.2 (0.9-2.8) | 0.420 |
| Warm ischemia time (min) | 48 (23-79) | 49 (20-81) | 0.546 |
| Cold ischemic time (min) | 445 (147-878) | 443.5 (138-817) | 0.876 |
| Donor Abx use (yes/ no or unknown) | 54 (50.0%) / 54 (50.0%) | 86 (55.1%) / 70 (44.9%) | 0.453 |
| Donation status (DCD / DBD) | 4 (3.7%) / 104 (96.3%) | 8 (5.1%) / 148 (94.9%) | 0.766 |

**Table S4: Donors' and grafts' parameters (264 cases, October/2013-August/2015)**

**Incidence of post-OLT infection (264 cases, October/2013-August/2015)**

| Variables | Control (n=**108**) | Abx (n=**156**) | p value |
|---|---|---|---|
| SSI (grade ≥IIIb) | 7 (6.5%) | 2 (1.3%) | 0.317 |
| Post-OLT blood culture postive | 4 (3.7%) | 11 (7.1%) | 0.291 |

**Table S5: Incidence of post-OLT infection (264 cases, October/2013-August/2015)**

**Recipients' preoperative status and surgical parameters (180 cases in rifaximin study, May /2013-April /2016)**

| Variables | Control (n=123) | RFX (n=57) | p value |
|---|---|---|---|
| Pre-OLT blood culture postive | 0 | 0 | NA |
| Pre-operative hospital stay (days) | 1 (0-136) | 1 (0-72) | **0.012** |
| Pre-operative ICU stay (days) | 0 (0-11) | 0 (0-27) | 0.912 |
| RBC transfused during surgery(ml) | 2750 (250-14500) | 5250 (750-30750) | **<0.001** |
| FFP transfused during surgery(ml) | 3500 (0-13750) | 5500 (750-25000) | **<0.001** |
| PLT transfused during surgery(ml) | 230 (0-1195) | 400 (0-1877) | **0.001** |

**Table S6: Recipients' preoperative status and surgical parameters (180 cases in rifaximin study, May /2013-April /2016)**

**Donor and graft parameters (180 cases in rifaximin study, May /2013-April /2016)**

| Variables | Control (n=**123**) | Rifaximin (n=**57**) | p value |
|---|---|---|---|
| Age (years) | 39 (7-76) | 35 (12-70) | 0.782 |
| Gender (M/F) | 75 (61.0%) / 48 (39.0%) | 36 (63.2%) / 21 (36.8%) | 0.869 |
| Race | | | **0.008** |
| White | 65 (52.8%) | 29 (50.9%) | |
| Hispanic | 37 (30.1%) | 13 (22.8%) | |
| Black | 10 (8.1%) | 13 (22.8%) | |
| Asian | 10 (8.1%) | 0 | |
| Others | 1 (0.8%) | 2 (3.5%) | |
| Height (cm) | 170.2 (124.5-196.0) | 173.0 (152.4-198.0) | 0.277 |
| BMI (kg/m$^2$) | 25.7 (13.4-47.58) | 25.4 (18.5-49.6) | 0.936 |
| Pre-transplant AST (IU/L) | 40 (9-267) | 47 (13-747) | 0.244 |
| Pre-transplant ALT (IU/L) | 32 (10-245) | 37 (9-280) | 0.661 |
| T-Bil (g/dl) | 0.8 (0.2-11.5) | 0.7 (0.2-2.7) | 0.890 |
| PT-INR | 1.2 (0.9-2.2) | 1.2 (0.9-2.8) | 0.627 |
| Warm ischemia time (min) | 48 (23-138) | 49 (27-81) | 0.342 |
| Cold ischemic time (min) | 440 (163-878) | 418 (210-760) | 0.374 |
| Donor Abx use (yes/ no or unknown) | 67 (54.5%) | 36 (63.2%) | 0.332 |
| Donation status (DCD / DBD) | 6 (3.8%) | 3 (6.1%) | 0.492 |

**Table S7: Donor and graft parameters (180 cases in rifaximin study, May /2013-April /2016)**

**Incidence of post-OLT infection (180 cases in rifaximin study, May /2013-April /2016)**

| Variables | Control (n=123) | RFX (n=57) | p value |
|---|---|---|---|
| SSI (grade ≥IIIb) | 5 (4.1%) | 2 (3.5%) | >0.999 |
| Post-OLT blood culture postive | 6 (4.9%) | 1 (1.8%) | 0.434 |

**Table S8: Incidence of post-OLT infection (180 cases in rifaximin study, May /2013-April /2016)**

Table S9: Primer Sequences Used for Real-Time Quantitative PCR

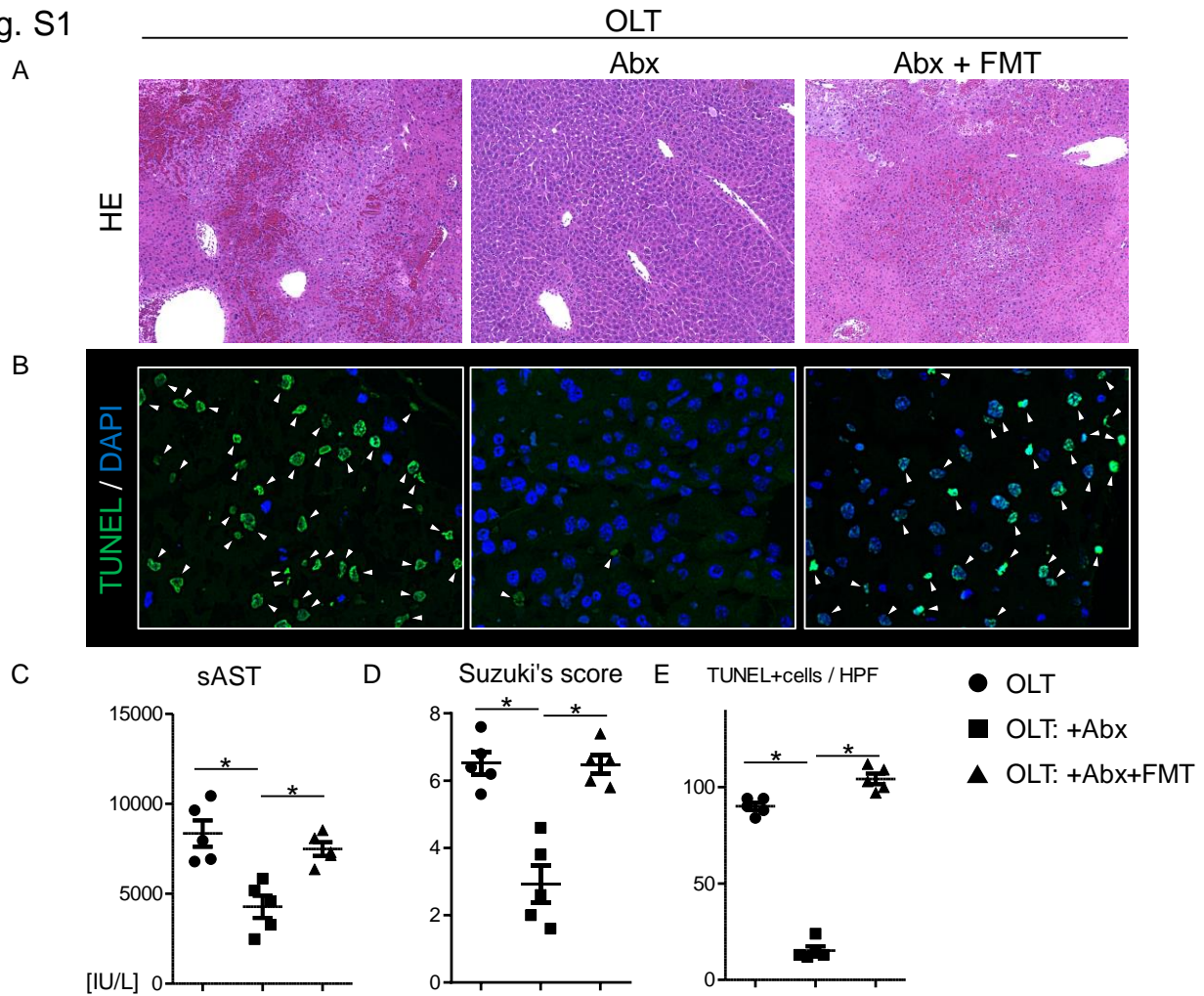| Gene | Forward | Reverse |
| --- | --- | --- |
| Mouse CD68 | 5'-CCATCCTTCACGATGACACCT-3' | 5'-GGCAGGGTTATGAGTGACAGTT-3' |
| Mouse Ly6G | 5'-TGGACTCTCACAGAAGCAAAG-3' | 5'-GCAGAGGTCTTCCTTCCAACA-3' |
| Mouse MCP1 | 5'-CATCCACGTGTTGGCTCA-3' | 5'-GATCATCTTGCTGGTGAATGAGT-3' |
| Mouse CXCL2 | 5'-ACTTCAAGAACATCCAGAG-3' | 5'-CTTTCCAGGTCAGTTAGC-3' |
| Mouse CXCL10 | 5'-GCTGCCGTCATTTTCTGC-3' | 5'-TCTCACTGGCCCGTCATC-3' |
| Mouse EP4 | 5'-ACCATTCCTAGATCGAACCGT-3' | 5'-CACCACCCCGAAGATGAACAT-3' |
| Mouse HPRT | 5'-TCAACGGGGGACATAAAAGT-3' | 5'-TGCATTGTTTTACCAGTGTCAA-3' |
| Mouse TBP | 5'-GGGGAGCTGTGATGTGAAGT-3' | 5'-CCAGGAAATAATTCTGGCTCA-3' |
| Human CD68 | 5'-GGAAATGCCACGGTTCATCCA-3' | 5'-TGGGGTTCAGTACAGAGATGC-3' |
| Human Cathepsin G | 5'-GAGTCAGACGGAATCGAAACG-3' | 5'-CGGAGTGTATCTGTTCCCCTC-3' |
| Human GAPDH | 5'-GGAGCGAGATCCCTCCAAAAT-3' | 5'-GGCTGTTGTCATACTTCTCATGG-3' |

**Figure S1: Fecal microbiota transfer (FMT) recreates liver IRI in Abx pretreated OLT recipients.**

BALB/c livers subjected to 18h of cold storage were transplanted orthotopically to C57BL/6 mice, followed by serum/liver sampling at 6h post-OLT. Some OLT recipient were treated with Abx on days –11 to –1, and then administered with (Abx+FMT) or without (Abx) fecal samples on day -1 and 0 by gavage. **(A)** Representative H&E staining (original magnification, x100). **(B)** Representative TUNEL staining (original magnification, x200). Arrowheads indicate TUNEL-positive dead cells. **(C)** Serum AST levels (IU/L, n=5/group). **(D)** Suzuki's histological grading of liver IRI (n=5/group). **(E)** Quantification of TUNEL-positive cells/HPF (n=5/group). Data shown in dot plots and bars indicate mean±SEM ($^{*}$p<0.01, 1-way ANOVA followed by Tukey's HSD test).
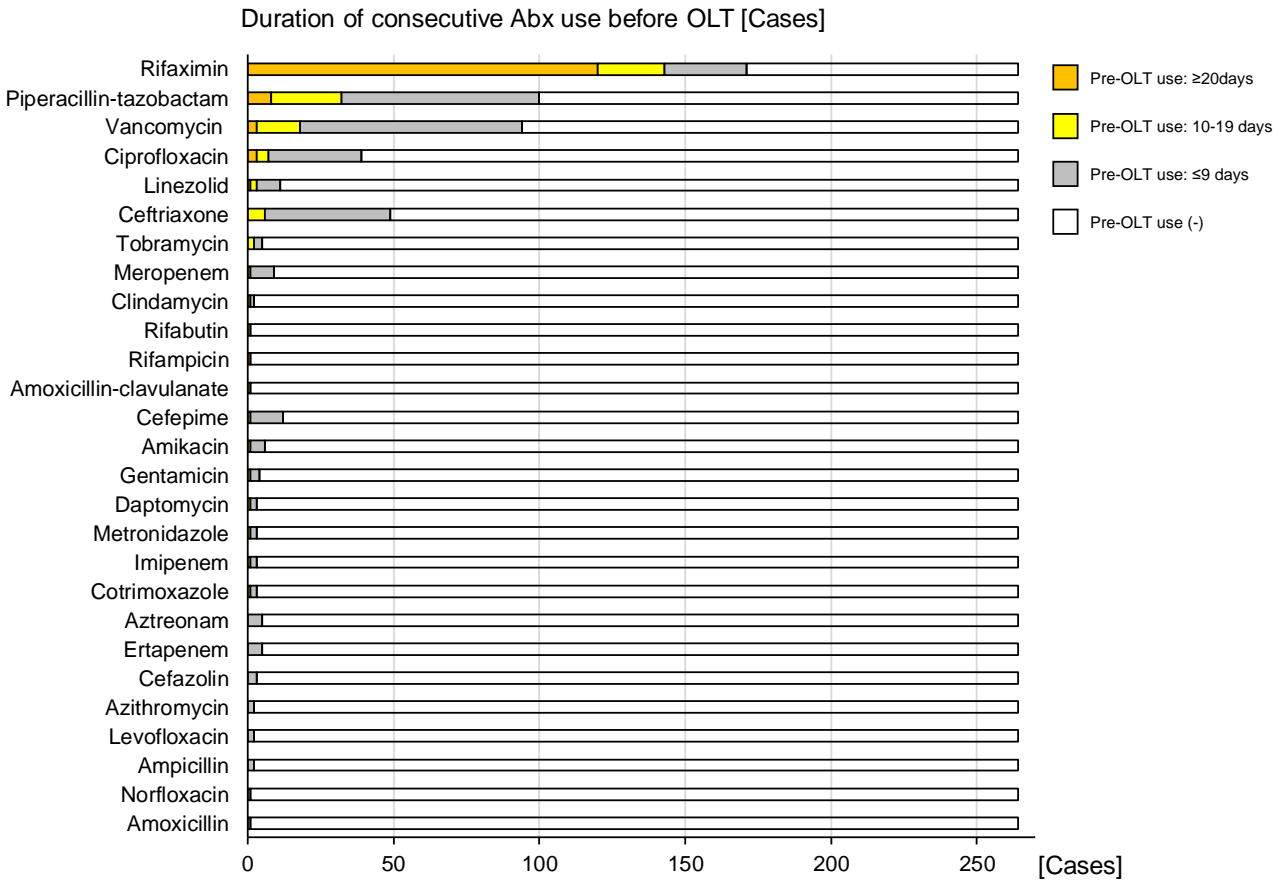
Fig. S2



Figure S2: Duration of consecutive Abx use prior to OLT in 264 recipients.

Twenty-seven different Abx were used in primary OLT patients (n=264; October 2013-August 2015). Number of treated cases and consecutive treatment periods are shown with respect to each Abx modality.