Ampicillin inhibition effect on HCT116 cell line

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Abstract

Ampicillin is a common antibiotic used treat infections derived by gram negative and positive bacteria. The hypothesis of this work is to uncover the therapeutic action of Ampicillin in case of human colorectal carcinoma as a model of gastric malignancy in the presence or absence of bacterial toxin. The results indicated that HCT116 viability was significantly attenuated by relatively high doses of Amp in presence or absence of LPS. However LPS treatment alone of high impact on HCT116 cells growth. We conclude that Ampicillin may have therapeutic effect on colorectal cancer upon and lower intestine bacterial infection. Further studies required revealing Ampicillin effect on normal intestinal cells and underneath molecular mechanisms

Keywords: Colorectal cancer, Ampicillin, anti-cancer effect.

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Introduction

Ampicillin, whose chemical formula C16H19N3O4Sand 3D structure given in Fig.1 (NCBI, 2016), is a wide spectrum antibiotic used either topically, intravenous or intramuscular injection.

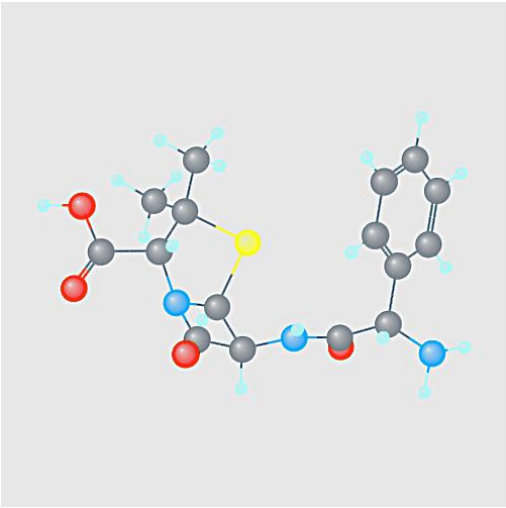


Figure 1. Three dimension conformer of Ampicillin. The imageadapted from PUBCHEM NCBI, (2016)

Around 50 years ago (Nash and Hugh, 1967)concluded the importance of locally administration of Ampicillin in site of large intestine operation incision before closure to prevent post sequels such as contamination and sepsis Amp. Later in other clinical trials it have been used successfully in during colon surgical operations to prevent infection (Gui et al., 1990) and (Menzel et al., 1996), however another researchers found that previous intake of systemic antibiotic can work the same as a prophylaxis(Raahave et al., 1989) Amp. Also used with surgery of gastric cancer removal(Ise et al., 2004). Lipopolysaccharides (LPS)from *Escherichia coli* are suggested in order to create inflammatory condition similar to bacterial infection (Oh et al., 2017).

Between various types of cancer, colon or colorectal cancer, which is the cancer of lower part of intestine, is the widest spread gastric cancer in adults. Characterized by up regulationof specific biomarkers mainly Fukutin FKTN (Oo et al., 2016), TM9SF3(Oo et al., 2014), and (ZDHHC14, BST2, DRAM2, DSC2) as demonstrated by (Anami et al., 2010). Same researcher identified tetraspanin 8 (TSPAN8), that encodes above-mentioned proteins and promote this type of cancer(Anami et al., 2016). Alongside with Cancer biomarkers, metabolic parameters are employed to predict the development of the colon cancer(Ferroni et al., 2016) also colonoscopy or screening (Telford et al., 2016). The aim of this study was to investigate whether Ampicillin treatment can effect on cells activity rather than preventing infection.

Materials and methods

Viability assay

Proliferation rate of the cells was determined by using Cell Titer 96 -Non-Radioactive Cell Proliferation Assay (MTT) Catalog no G4000. HCT116 cancer cells fromATCC at a passage number 18, and maintained in RPMI medium supplemented with the following (10% Fetal calf serum, 1% L-Glutamine, 1% Penicillin-streptomycin) and cells maintained on density 1-2 $x10^6$ cell per ml. Ampicillin from sigma catalog number A1593.

.Cells were normally cultured and counted, afterwards 5000 cells per 100 µl were loaded per each well of F-shape 96 well plate, the total number of wells / samples was 24 (8 groups, three samples per each group) and incubated for 24h, then certain doses (1, 25, 50 mg/ml) in presence and absence of LPS 1µg/ml are added in 100 µl for each well except the control were only added media to the cells Fig.1 first black column.Lipopolysaccharidesfrom *Escherichia coli* 055:B5, purchased from Sigma catalog number L6529-1MG

Plates are incubated in 37ºC and 5% CO_2, humidified incubator for 96 hours. At the end of incubation time 50µl of MTT dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) added to the wells and incubated for 3 hours inside the incubator.

Following formation of Formazan crystals at the bottom of the wells, gently aspirated the fluid and 200 µl of DMSO solvent added to the well and mixed thoroughly. The absorbance of the plate was read on spectrophotometer at 540-690 nm.

Stimulatory molecular assay

In order to investigate the subcellular interactions of the studied gent , in silico prestige are carried out . An online tool named Similarity ensemble approach (SEA)are employed for this purpose(as seen in fig.3),the compound SMILES and name are used in the search tool to investigate the affected biological receptors according to (Keiser et al., 2007). Statistical analysis carried out using TTEST and least significant difference ($p<0.05$)

Results and discussion

Overall, high and low concentrations of Ampicillin reduced the growth of the cells with or without presence of LPS.

LPS treatment alone inhibited 60%of the growth, Extremely high dose 50 mg /ml Ampicillin. Proliferation reduced less than 40%, at same dose with LPS the viability reduced to less than 20%.

The IC50 recorded with second high dose 25mg/ml reduced the growth to 50% while presence of LPS inhibited the growth 5% more at same dose. Interestingly low dose 1% exerted similar inhibition profile to 25 mg/ml of Amp with/out LPS. The results demonstrated the ability of Amp. To interfere with the viability of HCT116 cells this may be via interacting with specific colorectal cancer cell surface receptors, which are usually expressed in cancer cells rather than normal. Those cancer biomarker are play important role in therapy as most anticancer drugs are ligands of most these receptors and could modulate their mode of action to inhibit or stop cancer progression(Hayashi et al., 2013) cells, likewise browsing the possibilities of Ampicillin act as a Ligand (see fig.3.) we have found 14 differenthuman and animal molecular receptor capable of binding with this drug at various potential degrees which support the objectives of this work.

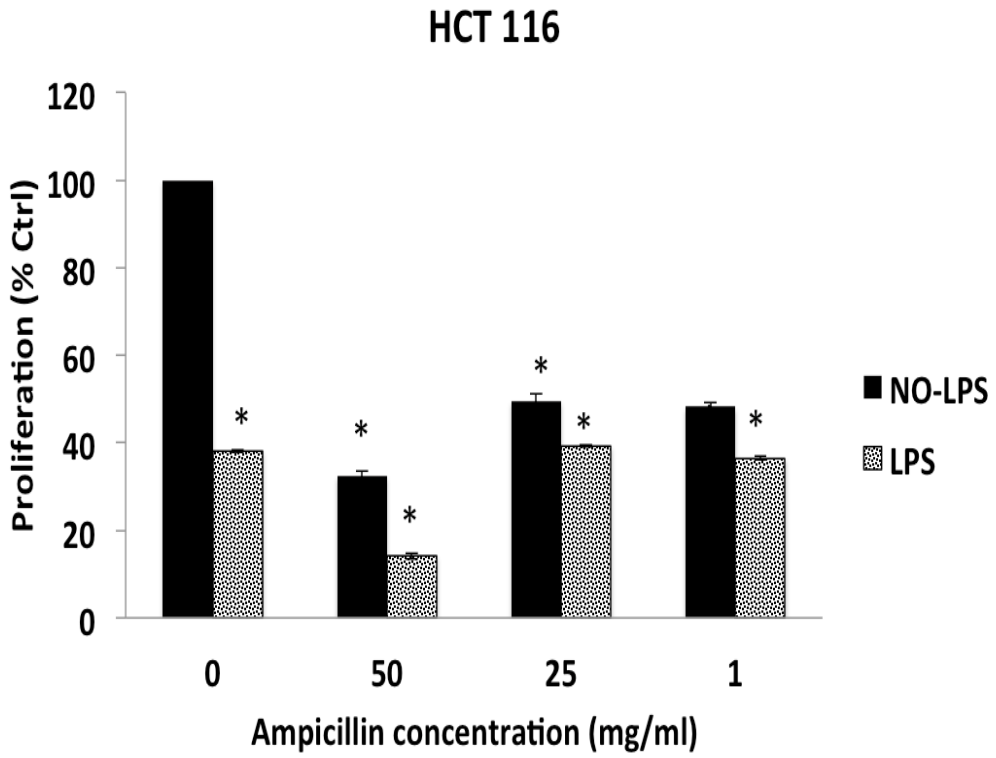
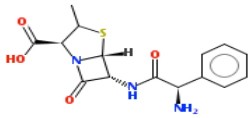


Figure 2.Growth inhibition effect of Ampicillin against Colorectal carcinoma. HCT116 cells were incubated with LPS 1µg/ml overnight in 96 well plate, then treated with 50, 25,1 mg/ml Amp for 72 h, then colorimetric MTT assay performed to measure the cellular growth at wave length (540-690)nm. The experiment performed 3 times in triplicate. Error bars represent standard deviation. P*<0.05.

Ampicillin

Isomeric SMILES CC1([C@@H](N2[C@H](S1)[C@@H](C2=O)NC(=O)[C@@H](C3=CC=C

Molecular Formula $C_{16}H_{19}N_3O_4S$



	Code	#Ligands	Reference Name	E-value	MaxTC
1	XIAP_HUMAN_1000.0	189	Inhibitor of apoptosis protein 3	4.21e-56	0.39
2	XIAP_HUMAN_100.0	75	Inhibitor of apoptosis protein 3	4.07e-55	0.38
3	XIAP_HUMAN_10000.0	316	Inhibitor of apoptosis protein 3	1.67e-42	0.39
4	BIRC2_HUMAN_10.0	35	Baculoviral IAP repeat-containing protein 2	2.03e-20	0.33
5	BIRC2_HUMAN_100.0	40	Baculoviral IAP repeat-containing protein 2	1.68e-18	0.33
6	BIRC2_HUMAN_1000.0	46	Baculoviral IAP repeat-containing protein 2	1.17e-16	0.33
7	BIRC2_HUMAN_10000.0	52	Baculoviral IAP repeat-containing protein 2	3.62e-15	0.33
8	A2RP81_CITFR_1000.0	5	PER-2 beta-lactamase	1.45e-9	0.38
9	A2RP81_CITFR_10000.0	6	PER-2 beta-lactamase	3.46e-8	0.38
10	Q5A7N4_CANAL_10000.0	10	Likely tRNA 2'-phosphotransferase	1.94e-4	0.35
11	CA2D1_MOUSE_10000.0	47	Voltage-gated calcium channel alpha2/delta subunit 1	3.72e-2	0.37
12	CATL1_RAT_10000.0	23	Cathepsin L	9.74e-1	0.31
13	NK1R_RAT_10000.0	84	Neurokinin 1 receptor	2.14e+0	0.33
14	CA2D1_MOUSE_1000.0	41	Voltage-gated calcium channel alpha2/delta subunit 1	4.77e+0	0.37

Figure 3.Possible Molecular interactions of Ampicillin. The outcome of Ampicillin subcellular ligation obtained via SEA tool as described in materials and methods

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التاثير التثبيطي لعقار الامبيسيلين على نمو خلايا سرطان القولون HCT116

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الخلاصة

الامبيسيلين هو مضاد حيوي واسع الطيف يستعمل لعلاج الاخماج المتسببة عن البكتريا الموجبة والسالبة لصبغة كرام. صممت هذه الدراسة لمعرفة تأثير الامبيسيلين على سرطان القولون ،كنموذج للسرطان الهضمي،بوجود أو غياب سموم LPS المستخلصة من بكتريا الاشريكيا القولونية. اثبتت النتائج وجود تثبيط ملحوظ لنمو الخلايا السرطانية HTC115 مع ان السم البكتيري وحده أمكنه تثبيط نمو الخلايا . ممكن ان نستنتج من هذه الدراسة ان استعمال الامبيسيلين يثبط تقدم نمو هذه الخلايا واستعماله موضعيا مع عمليات استئصال أورام الأمعاء الغليضة يساعد في كبح تقدم الورم. نوصي بإجراء المزيد من الدراسات على المستوى الجزيئي لتقصي الالية الدقيقة وراء هذا التثبيط.