Drug Discovery and Development

transcribed plenary speech of
Prof Iqbal Chaudry
Director, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan
iqbal.choudhary@iccs.edu

Understanding the national capacity of diseases at a molecular level and developing drugs are essential matters to be understood in the drug discovery and development process. Number of people who are contributing to the drug development process is very low since it needs quite lots of money. For an example in Belgium 1.8 billion dollars are spent and 1200 scientists are working to develop one drug molecule.

Target based screening is a one of the approaches in the drug development. In this approach people have thought that they have understood the disease at molecular level and those molecules became the target and they started to screen those in large libraries. If the molecule is fixed they could to treat the disease and that is still considered as the main state of drug development. Reduction approach is the other approach which people have considered that one disease is due to one particular molecule and then screen large libraries of compounds for that particular molecule. Prof Iqbal Caudry demonstrated that how this reduction approach is reached by using micro organisms and looking in to the drug function against them.

Drug resistance is affecting all lives and it is a major threat to survival of human beings. In the case of bacteria the drug resistance becomes complicated since bacteria tends to develop resistance much more quickly than anything else on the earth. Micro organisms develop the drug resistance in multitude ways like having mechanisms for protection of the cell wall by changing the chemistry of the cell wall and they do not let antibiotics to enter to the cell . Also they have the mechanisms to either chopping or pushing antibiotics out from the cell which is called as efflux pump. As the result this causes bedbugs which come every year. Therefore doctors having difficulty to treat infections today due to these defending mechanisms of the microorganisms.

Prof. Iqbal explained that what they have done by using three micro organisms *staphylococcus aureus*, *pseudomonas*, *E.coli* with existing antibiotic and a helper molecule. *Staphylococcus aureus* is an opportunistic parasite which attack to human body when their immune system is compromised and causes all kinds of problems.

So Prof. Iqbal demonstrated, how they are succeeded by isolating a simple compound from a well known anti infectious plant that is currently used as a topical application against infections. He explained that the isolated compound is more effective (79%) than vancomycin (21%) and further he said that they screened many of derivatives of this isolated compound. Finally they concluded that the compound containing hydroxyl group is not active or they are effective at their high doses against infection. The reason was that lipophylic part of bacterial cell wall do not allow the hydrophilic hydroxyl group to go inside the cell and it causes to block the action of the drug molecule.

Then he explained another series of compounds that was isolated from a plant which is very well known against infections. It was consisted with the monoterpine unit, isoprene unit and hydroxyl group, with the activity of 74%. When a colony is exposed to this compound all the debris was coming out from the cell which means that the cell wall chemistry has been changed.

Cell viability can be checked by the membrane potential since good membrane potential is maintained by the healthy bacterial cell. When a healthy bacterial colony is exposed to the isolated compound, the number of bacterial cell was reduced and the cell debris was started to coming out from the cell due to the effect of the compound by depolarizing cell membrane. In the experiment , the colony was exposed to the compound with a negative charged dye. Because, if the bacterial cell is healthy the negative charge of the membrane will not allow to enter it in to the cell due to the repulsion of negative charge dye. Once the bacterial cells are exposed to the compound, 92% cells were capable to have the dye and it prove that the membrane potential has been affected by the compound.

Various types of proteins like transport proteins ,receptors can be also affected by due to the depolarization of cell membrane. E-flux pumps are one of the protein types that are present on the cell

membrane and they are responsible for identifying the foreign bodies, and pump them out as a cell defending mechanism. Even though, the dye cannot enter to the bacterial cell alone, when the colony is exposed to the dye with isolated compound, the dye could enter in to the cell and at the same time antibodies could be entered to the cell which means the compound has inhibited the e-flux pump. If the e-flux pump is capable in working in the bacterial cell , antibodies could not be able to enter and be active against the bacterial cell. Therefore this isolated compound has affected to the cell in two manner. One way is the compound has depolarized the membrane and the other way is inhibiting the e- flux pump. Both mechanisms have contributed to cause many cell death and irregular cell division. When bacterial cells are observed under the atomic force microscopy, healthy cells can be seen about 275 nm in size. After exposure of the colony by the compound the cells size has reduced to 0.77 nm in size which means that the compound has affected to the cell membrane and unhealthy cell were resulted.

Prof. Iqbal said that they have discovered, the compound doing nothing but insulting the cell, which is called as the 'cell insult'. Once the cell is exposed to the compound, the cell identifies it as a species that need to be oxidized. Therefore the bacterial cell starts to produce the reactive oxygen species which are perceived by the bacterial cell and starts oxidizing the cell membrane itself. This is causing to come out all intracellular proteins and they were identified by using technique call LC/MS/MS. In the case of antibiotic, clandomycin 4000ug was needed to treat against the infection alone. But when using this isolated compound 25 ug ,the clandomycin 8 ug/ml was enough to be effective against the infection which means that this compound has helped the antibiotic to rupture the cell and let antibiotic to go inside the cell to be active against the bacterial cell. This was an important observation of their study and it was patented.

The Next example which was given by the prof. Iqbal, is the urease enzyme which is excreted by the gut micro organism call *Helicobactor pyroli*. Urease enzyme allows *Helicobactor pyroli* to surve in extreamly low acidic pH since urease create localized basic pH around them. Therefore it proves that helicobactor pyroli is unable to survive in acidic pH. Urese is a large intracellular protein and it contains two Nickle atoms and Prof. Iqbal said that they wanted to know what actually they can do to inhibit this enzyme, that is called target based drug discovery. He said that they have discovered another series of compounds that were extracted from several plants and lichens which was active against ureases. Then they founded that all the isolated compounds had caumarine structure and futher they identified that mono caumarin is not active and di caumarin is active against the urease. By synthesizing various analogues of that compound they have found that half of the molecules was unable reach the reactive site of the enzyme and only the benzene moiety could enter to the cell and reach to the Nickel atom and form aromatic metal complex by Nickel atom with benzene and block the active site of the urease enzyme. This was proved by using chemical shift of benzene in hydrogen nuclear magnetic resonance spectroscopy. So finally these were the two examples for phenotypic screening and target based screening that can be used for drug development and discovery.