Mode of action of isonicotinic acid hydrazide

M. K. Chattopadhyay

Isonicotinic acid hydrazide (isoniazid), or INH, a front line antitubercular drug, was introduced in clinical practice in 1952. But till now its exact mode of action is not clearly known. Though majority of the *Mycobacterial* species are sensitive to 1 μg/ml or a higher concentration of INH, sensitivity of *M. tuberculosis* lies in the range of 0.02 to 0.06 μg/ml of INH[1]. This extreme susceptibility of the tubercular pathogen to INH is a riddle which is yet to be solved.

A number of papers published in the past few years have emphasized the role of the *katG* gene in INH-toxicity. This gene encodes a bifunctional enzyme with both catalase and peroxidase activities in *M. tuberculosis*. Loss of *KatG* activity due to gene deletion[2] or missense mutations[3] was found to be associated with INH-resistance of the pathogen. It was proposed that catalase-peroxidase might convert INH into a metabolically active form *in vivo* or its action on INH might generate toxic oxygen radicals which are actually responsible for the antibacterial properties of the drug. *In vitro* oxidation of INH by the enzyme catalase-peroxidase was also evidenced[4].

Investigations have been performed from time to time to elucidate the molecular nature of the intracellular target of INH or of its active form. Earlier it was known that the drug inhibited mycolic acid biosynthesis in *M. tuberculosis*[5]. Evidence obtained from further studies suggested that its primary target might be enoyl-acyl carrier protein reductase, encoded by the *inhA* gene of *Mycobacterium* and is believed to play a key role in mycolic acid biosynthesis[6,7].

However the postulation about the mode of action of INH in terms of its ability to inhibit mycolic acid biosynthesis failed to explain why *M. leprae* is far less susceptible to INH compared to *M. tuberculosis*. Involvement of some other gene was evident. INH is also inactive against *Escherichia coli* and *Salmonella typhimurium*. In both these organisms *katG* is a part of a oxidative stress regulon containing a number of genes including *aphC* which encodes the small subunit of alkyl hydroperoxide reductase. They are induced by the *oxyR* gene in response to challenge by H_2O_2. Knock out mutations of *oxyR* in *E. coli* and *S. typhimurium* is known to confer INH-susceptibility in them. In a recent investigation *oxyR* was found to be inactivated by multiple lesions in the wild type strain of *M. tuberculosis* which is INH-sensitive. The gene was present in intact form in *M. leprae*. When *oxyR* and *aphC* from *M. leprae* were inserted into *M. tuberculosis* through cosmid vector, the tolerance of the tubercular pathogen to INH was substantially increased. As a plausible explanation of the association of these two phenomena – viz. mutation in *oxyR* and susceptibility to INH – it has been proposed that the function of *aphC* and other genes induced by *oxyR* is to protect the bacterial cell from the metabolically active form of INH and from the free radicals. Due to mutation in *oxyR*, in the wild type strain the drug cannot be detoxified. A constitutive level of oxidative stress response, present in the wild type strain, scavenge the oxygen radicals produced in course of normal metabolism. But in absence of induction by *oxyR* the oxidative defense mechanism of the cell is unable to quench the extra load of free radicals generated in the presence of INH[8].

It is important to remember that though INH is believed to be converted into an active form by catalase-peroxidase, none of the metabolic products of the drug (isonicotinic acid and 4-pyridylmethanol) identified so far in *M. tuberculosis* has any antibacterial activity[9]. With the evidence available at present, toxicity of INH to the tubercular pathogen appears to be a multifactorial phenomenon. Investigation on some other aspects (e.g. mechanism of uptake of INH by *M. tuberculosis*) may provide further clues.

1. Deretic, V., Pagan-Ramos, E., Zhang, Y., Dhandayuthapani, S. and Vial, L. E., *Nature Biotechnol.*, 1996, **14**, 1557–1561.
2. Zhang, Y., Heym, B., Allen, B., Young, D. and Cole, S., *Nature*, 1992, **358**, 591–593.
3. Heym, B., Alzari, P. M., Honore, N. and Cole, S. T., *Mol. Microbiol.*, 1995, **15**, 235–245.
4. Johnsson, K. and Schutlz, P. G., *J. Am. Chem. Soc.*, 1994, **116**, 7425–7426.
5. Takayama, K., *Ann. N. Y. Acad. Sci.*, 1974, **235**, 426–438.
6. Banerjee, A., Dubnau, E., Quemard, A., *et al.*, *Science*, 1994, **263**, 227–230.
7. Dessen, A., Quemard, A., Blanchard, J. S., Jacobs Jr, W. R. and Sacchettini, J. C., *Science*, 1995, **267**, 1638–1641.
8. Deretic, V., Phillip, W., Dhandayuthapani, S., *et al.*, *Mol. Microbiol.*, 1995, **17**, 889–900.
9. Zhang, Y. and Young, D. B., *Trends Microbiol.*, 1993, **1**, 109–113.

M. K. Chattopadhyay is in the Centre for Cellular and Molecular Biology, Hyderabad 500 007, India.