HPLC Determination of Antioxidant Activity of Antitubercular Drugs

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Accepted 15 September 200
Revised 21 May 200
Received 4 November 200

Antioxidant activities of some antitubercular drugs were evaluated by using a simple, accurate and reproducible method. Ascorbic acid was used as a control in the study. The antioxidant activities of antitubercular drugs were less as compared to ascorbic acid.

Mycobacterium can induce reactive oxygen species (ROS) production by activating phagocytes[1] and although it is an important part of the host defense against mycobacteria, enhanced ROS generation might also promote tissue injury and inflammation. This further contributes to immunosupression[2], particularly in those with conditions such as TB and HIV-infection[3] that impair antioxidant capacity. Moreover, malnutrition that is commonly seen in patients with TB or HIV infection may further contribute to the impaired antioxidant capacity of these patients.

The purpose of the present study was to determine the antioxidant activity possessed by some commonly used antitubercular (antiTB) drugs such as isoniazid, ethambutol, rifampicin, pyrazinamide, cycloserine and a well-known antioxidant ascorbic acid using a stable free radical α, α-diphenyl-β-picrylhydrazyl (DPPH)[4,5]. The compounds would decolourize DPPH if they possess antioxidant property.

The measurements were made using HPLC. All the compounds were scanned at 517 and 256 nm, the wavelengths at which DPPH shows strong absorption. The deep violet colour of DPPH was decolorised by the compounds and this phenomenon can be easily studied at 517 nm. Monitoring the drug and DPPH peaks at 256 nm is important, because some drugs which may not change the colour of DPPH, might still possess good antioxidant properties. Therefore to rule out the false negative results, we have studied at both wavelengths.

Drugs and DPPH solutions were prepared in methanol. For estimating of antioxidant activity, 100 µl of different concentrations of drug solutions (10, 50, 100, 500 and 1000 ng) were added to 100 µl of 0.2 mM of DPPH solution, mixed thoroughly and 20 µl of the mixture was used for HPLC analysis. Each test was performed in triplicate, which was carried out using reverse phase C18 column equipped with an LC 10A Shimadzu UV/Vis spectrophotometric detector. Isocratic elution conditions for HPLC analysis were methanol:water (85:15) monitored at 517 nm[6,7] and methanol:water (80:20) monitored at 256 nm[8]. Antioxidant activity was measured in terms of percent inhibition of DPPH peak area. The amount of drug required to produce fifty percent inhibition of DPPH peak area was taken as IC_{50}. The IC_{50} values were computed from concentration of drug and percent inhibition of DPPH peak area.

In the present study, it was observed that with increasing concentration of the test drugs DPPH peak areas were decreased as shown in fig. 1 and 2. At 517 nm the IC_{50} values were found to be as follows; for ascorbic acid, 3.14 ng, isoniazid, 7.63 ng, ethambutol, 52.9 ng, rifampicin, 199 ng, pyrazinamide, 298 ng, cycloserine, 81.30 ng and for ofloxacin, 70.20 ng. At 256 nm these were, for ascorbic acid, 3.8 ng, isoniazid, 9.54 ng, ethambutol, 53.8 ng, rifampicin, 141 ng, pyrazinamide, 206 ng, cycloserine, 74.1 ng and for ofloxacin 72.3 ng. The IC_{50} value of ascorbic acid was found to be lower than those for antiTB drugs indicating that the antioxidant activity of ascorbic acid was much higher when compared to antiTB drugs that were tested.

From this study it was evident that the order of free radical scavenging ability or antioxidant activity for various drugs tested at both wavelengths is as follows, ascorbic acidisoniazid > ethambutol > ofloxacin > cycloserin > rifampicin > pyrazinamide. From these results and the results reported earlier from this laboratory[5,9], it can be concluded that colorimetric determination may not be much

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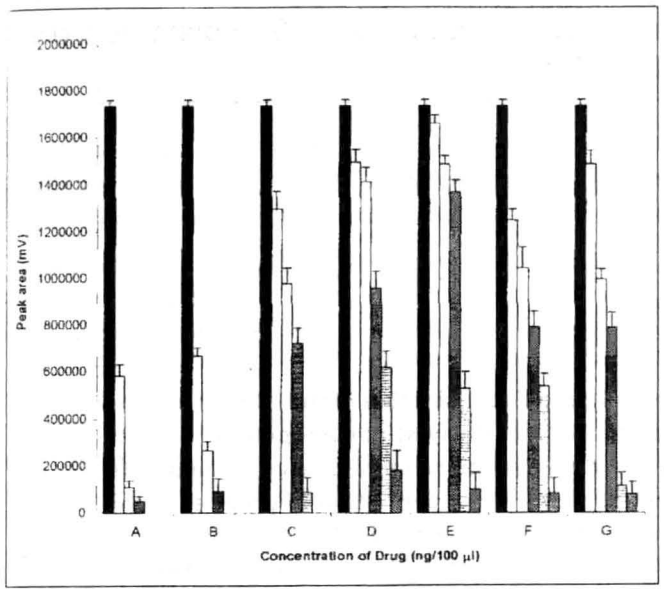

Fig. 1: DPPH peaks in presence of drugs at 517 nm

DPPH peaks in presence of drugs at 517 nm. Each bar represents Mean±S.D. A is ascorbic acid, B is isoniazid, C is ethambutol, D is rifampicin, E is pyrazinamide, F is cycloserine and G is ofloxacin. Different bars represent ■ Blank, □ 10 ng, ▨ 50 ng, ⊞ 100 ng, ▧ 500 ng and ▧ 1000 ng

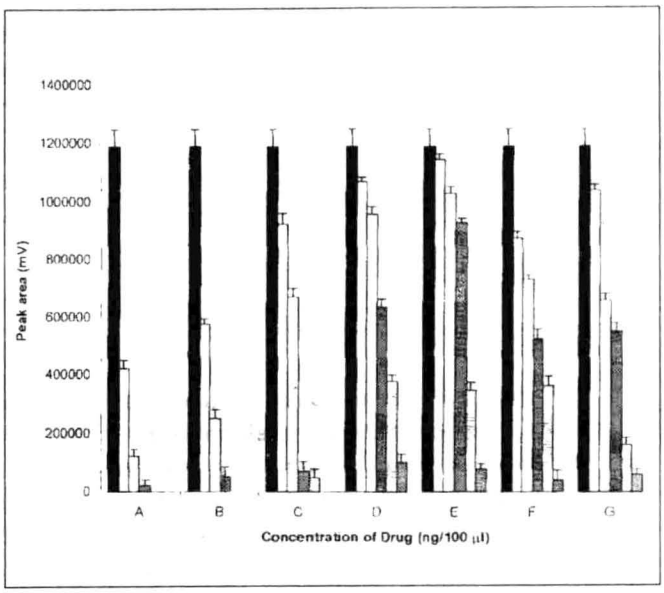

Fig. 2: DPPH peaks in presence of drugs at 256 nm

DPPH peaks in presence of drugs at 256 nm. Each bar represents Mean±S.D. A is ascorbic acid, B is isoniazid, C is ethambutol, D is rifampicin, E is pyrazinamide, F is cycloserine and G is ofloxacin. Different bars represent ■ Blank, □ 10 ng, ▨ 50 ng, ⊞ 100 ng, ▧ 500 ng and ▧ 1000 ng

useful to judge the antioxidant activity of compounds using DPPH.

Severe oxidative stress has been reported in TB patients because of malnutrition and poor immunity[10]. Low vitamin C intake might increase the susceptibility to various infections and a lower vitamin C concentration in plasma was associated with a substantially higher incidence of tuberculosis[11]. Our findings further support the importance of vitamin C as a supplement in combination with antiTB drugs.

REFERENCES

1. Attwood, E.M., Weich, D.J. and Oosthuizen, J.M., **Tuber. Lung Dis.,** 1996, 77, 341.
2. Jack, C.I., Jackson, M.J. and Hind, C.R., **Tuber. Lung Dis.**, 1994, 75, 132.
3. Muller, F., Svardal, A.M., Nordoy, I., Berge, R.K., Aukrust, P. and Froland, S.S., **Eur. J. Clin. Invest.,** 2000, 30, 905.
4. Blios, M.S., **Nature,** 1958, 181, 1199.
5. Karunakar, N., Prabhakar, M.C. and Krishna, D.R., **Drug Res.,** 2003, 53, 254 .
6. Abe, N., Nemoto, A., Tsuchiya, Y., Hojo, H. and Hirota, A., **Biosci. Biotechnol. Biochem.,** 2000, 64, 306 .
7. Koleva, I.I., Niederlaner, H.A. and VanBeen, T.A., **Anal. Chem.**, 2000, 72, 2323.
8. Muller, K. and Gurster, D., **Biochem. Pharmacol.,** 1993, 46, 1695 .
9. Kalpana, T., Karunakar, N., Mada S. R., Prabhakar, M.C. and Krishna, D.R., **Drug Res.,** 2001, 51, 633 .
10. Hemila, H., Kaprio, J., Pietmen, P., Albanes, D. and Heinonen, O.P., **Amer. J. Epidemiol.,** 1999, 150, 632.
11. Awotedu, A.A., Sofowora, E.O. and Ette, S.I., **East Afr. Med. J.,** 1984, 61, 238.