INTRODUCTION

INTRODUCTION

A great attention has been paid to the enzyme L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) since Broome (1961) showed that it was responsible for Kidd's (1953) observed antilymphoma activity of the guinea pig serum. Several investigators also confirmed these facts (Broome, 1963; Campbell et al., 1963; Schwartz et al., 1966; Wriston et al., 1966). The low level of L-asparaginase present in guinea pig serum made it necessary to warrant a more practical source of antineoplastic enzyme. The finding by Mashburn and Wriston (1964) that L-asparaginase derived from Escherichia coli has antitumor activity similar to that of guinea pig serum opened up the possibility of large-scale production of the enzyme for clinical trial. From then the enzyme L-asparaginase has attracted much interests because of its antineoplastic activity (Oettgen et al., 1970; Crowther, 1971; Jaffe et al., 1971; 1972; Wriston and Yellin, 1973; Roberts et al., 1976; Durden and Distasio, 1980; Miniero et al., 1986; Homans et al., 1987; Ridgway et al., 1989). In addition, rat fibrosarcoma, Walker carcinosarcoma 256, Jenson sarcoma and rat Murphy - sturn lymphosarcoma are all inhibited by the enzyme (Cooney and Handschumacher, 1970). Some human cancers also respond to the enzyme (Capizzi et al., 1970; Young and Burchenal, 1971). Moreover, the enzyme has a particularly high therapeutic index when used in the treatment of acute lymphatic leukemia (Tallal et al., 1970; Ertel et al., 1979) with more than two thirds of human patients showing complete remission. Most of these remissions are, however, short-lived, and there have been a number of toxic side effects associated with the use of this enzyme (Clarkson et al., 1970; Haskel et al., 1969; Anderson et al., 1979; Miniero et al., 1986; Leonard and Kay, 1986; Ollenschlager et al., 1988; Ridgway et al., 1989). Prominent along these difficulties have been immune reactions to the foreign protein, hypersensitivity reactions and an accelerated clearance time of the enzyme from plasma have been noted with continued therapy (Capizzi and Cheng, 1981).

L-Asparaginase exerts its antitumor activity by hydrolysis of asparagine to aspartic acid and ammonia (Capizzi et al., 1970; Crowther, 1971). The depletion of asparagine selectively affects those tumor cells or leukemic cells which possess low levels of asparagine synthetase (Conney and Handschumacher, 1970; Ohnuma et al., 1971; Wriston and Yellin, 1973). In the absence of asparagine, a decrease of protein and glycoprotein synthesis occurs which ultimately leads to leukemic

cell death (Bosmann and Kessel, 1970; Kessel and Bosmann, 1970). Asparaginase has been shown to cause a number of other alterations in tumor cells which has been suggested as factors contributing to its oncolytic actions; examples are, changes in the levels of free amino acid (Broome, 1968) and increases in ribonuclease activity (Mashburn and Wriston, 1966). Other potential targets for the enzyme include the cell membrane through its action on glycoproteins or the c-terminal asparagine of proteins (Howard and Carpenter, 1972).

L-Asparaginase is widely distributed, being found in animal, microbial and plant sources (Wriston and Yellin, 1973; Yurek et al., 1983; Wriston, 1985). The need for enzyme for therapeutic work has promoted a search for L-asparaginase in micro-organisms. L-Asparaginase activity is found in varying quantities in different organisms, and appears to be more common among Gram-negative bacteria, and the members of the Enterobacteriaceae are the best producers (Peterson and Ciegler, 1969; Wade et al., 1971; Bilimoria, 1969; Arima et al., 1972; Imada et al., 1973; Wriston and Yellin, 1973).

Anti-tumor activity was shown by the enzymes from Escherichia coli (Mashburn and Wriston, 1964), Serratia marcescens (Rowley and Wriston, 1967), Erwinia carotovora (Wade et al., 1968), Er.aroideae (Peterson and Ciegler, 1969a), Mycobacterium tuberculosis (Reddy et al., 1969), Proteus vulgaris (Tosa et al., 1971), Vibrio succinogenes (Distasio et al., 1976; 1977) (renamed as Wolinella succinogenes : Ammon et al., 1985), and by a glutaminase-asparaginase enzyme from Achromobacter (Roberts et al., 1970). Except L-asparaginase from Aspergillus terreus (De-Angeli et al., 1970), none of the asparaginases from other fungal sources showed antitumor activity (Wriston and Yellin, 1973; Capizzi and Cheng, 1981).

The immunosuppressive effects of the asparaginases of E.coli (2% glutaminase) and Er.carotovara (10% glutaminase) are compared by Ashworth and MacLennan (1974). Unlike the guinea pig enzyme (Dolowy et al., 1966), clinical use of these asparaginases is associated with pronounced toxicity including liver and pancreas dysfunction, and immunosuppression (Cooney and Handschumacher, 1970; Ohno and Hersh, 1970; Ridgway et al., 1989). Many toxic effects have been noted during clinical trials with L-asparaginase, including falls in serum albumin, haemoglobin, lipoprotein and fibrinogen; abnormalities in liver function tests; increased prothrombin

times; fatty infiltration of the liver; pancreatitis; and decreased synthesis of insulin (Schein, 1969; Pratt and Johnson, 1971; Haskel et al., 1967; 1969; Jaffe et al., 1971; 1972; 1973; Leonard and Kay, 1986; Homans et al., 1987; Ollenschläger et al., 1988). Clinical and laboratory coagulation abnormalities were reported in association with the use of L-asparaginase in early studies (Barbui et al., 1983). Of particular concern are the reports of central nervous system (CNS) thrombosis and hemorrhagic infarctions occuring in patients treated with this agent (Priest et al., 1980; 1982; Steinherz et al., 1981; Packer et al., 1985).

During the evaluation of biologically active enzyme L-asparaginase in antineoplasia, for further investigations, it is proposed to seek an organism which under conditions of effective production would be low in toxigenicity or virulence (Roberts et al., 1968; Hamilton-Fairly et al., 1970; Tosa et al., 1971; Loos et al., 1972; Chong and Chang, 1977; Wriston and Yellin, 1973; Capizzi and Cheng, 1981). But in fact, by considering this view, still there is no such study by demarking explossively the toxigenicity or pathogenicity of them (Imada et al., 1972; 1973; Arima et al., 1972; Bilimoria, 1969; Wade et al., 1968; 1970; 1971; 1980; Cedar and Schwartz, 1968; Peterson and Ciegler, 1969; 1969a; Zanin et al., 1969).

The dramatic pharmocological effects of asparaginase have led quite naturally to renewed interest in other forms of enzyme therapy in human disease. Repeated administration of L-asparaginase to the blood stream caused hypersensitivity, ranging from mild allergic symptoms to anaphylactic shock in 5% to 20% of patients (Cooney and Handschumacher, 1970; Khan and Hill, 1969). To overcome this allergic responses, L-asparaginases with similar anti-tumor activity but of different antigenic structure are needed to allow continued treatment of a responding but antigenically sensitive patient (Ohnuma et al., 1972; Capizzi et al., 1970; Wade and Rutter, 1970).

Factors to consider in examining an asparaginase for potential therapeutic use are that the enzyme should be amenable to rapid isolation in high yield, active under physiological conditions, low in K_m value, devoid of glutaminase activity, resistance to sulfhydryl group inhibition, more stable, and less thermo-inactivated, cleared relatively slow from the blood stream, and not cross-react immunologically with E.coli-L-asparaginase.

All these facts promoted now to search for L-asparaginase from new source. In the present study, L-asparaginase formation of different species of Vibrio had been determined, and found that Vibrio cholerae O1 and V.cholerae non-O1 (the so called NAG Vibrio) produce the enzyme. The toxigenicity or virulence of the V.cholerae strains was evaluated, and intended to find out a relation with the enzyme production. It has been dealt with to overview the effects of some constituents or ingradients of the bacterial growth media and to specify some of the factors for stimulation or inhibition of L-asparaginase biosynthesis. A comparative study on L-asparaginase activity of sub-cellular fractions of two different toxigenic strains of V.cholerae was made. The enzyme was purified from one selected strain. The properties of the purified enzyme were studied. The antineoplastic activities of the purified enzyme were evaluated. The plasma clearance rate of the enzyme was found in mice. The immunobiological aspects for the enzyme therapy in mice had been clarified. The cross-reactivity of the purified enzyme was studied with the E.coli L-asparaginase (EC-2) antiserum.