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POTENTIAL TOXICITY PROBLEMS WITH HERBAL MEDICINES AND FOOD - NEW OBSERVATIONS WITH PYRROLIZIDINE ALKALOIDS

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Summary

Among the higher plants used in herbal medicine and for food which can cause chronic toxicity are those containing pyrrolizidine alkaloids. These compounds are metabolised in the liver to reactive pyrroles which then bind strongly to tissue thiol groups both in the liver and in red blood cells. Exposure to the alkaloids can be identified by recovering the S-bound pyrrolic nucleus of the alkaloid as an ethoxypyrrole after treatment of ethanolic tissue preparations with silver nitrate. Chronic and progressive injury to the liver after ingestion of the alkaloids may be associated with persistence of thiol pyrrole binding in the tissue. Persons who habitually use pyrrolizidine alkaloid containing plants should be aware of this possibility.

I. INTRODUCTION

Pyrrolizidine alkaloids are found in some 13 plant families, particularly Asteraceae, Fabaceae and Boraginaceae (Hirono 1987). The main toxic genera are Senecio, Crotalaria and Heliotropium respectively. In man the principal toxic effects following ingestion of the alkaloids is chronic liver damage, notably veno-occlusive disease. Domestic animals suffer the same effects and in some species significant lesions can occur also in the kidneys, lungs and other organs. The intoxication syndrome and associated pathology depend mainly on the type and amount of alkaloid ingested and the animal species involved but other factors such as dose, age and nutritional condition are also important (Mattocks 1986).

An unusual feature of pyrrolizidine alkaloidois in both man and animals is a tendency for the disease in the liver to be progressive even though regular intake of the alkaloid may have ceased weeks, months or even years previously (Molyneaux et al. 1988). In addition recovery from an initial exposure large enough to cause overt liver disease can occur with a relapse into liver failure months or years later (Anon 1988). The mechanism of such a disease process is not clearly understood but the possibility of such an outcome to pyrrolizidine alkaloid exposure in man needs to be kept in mind by consumers of plant material containing such compounds.

II. INTOXICATION MECHANISM

Hepatotoxic pyrrolizidine alkaloids after ingestion are carried to the liver where they mostly undergo metabolism prior to excretion mainly in the bile and urine. Pyrrolizidine alkaloids are esters and metabolic processes in the liver in which the alkaloids are involved are hydrolysis and microsomal mixed function oxygenation. Hydrolysis of the alkaloid ester is by and large a detoxication reaction. Mixed function oxygenation results in either N–oxide formation or dehydrogenation of the pyrrolizidine ring structure. N–oxides are highly water soluble and are readily excreted in the urine without injury to the liver. Dehydrogenation on the other hand results in the formation of a pyrrole which is highly electrophilic and capable of reacting with a variety of compounds including macromolecules in the liver cell in which it is formed. (Figure 1).

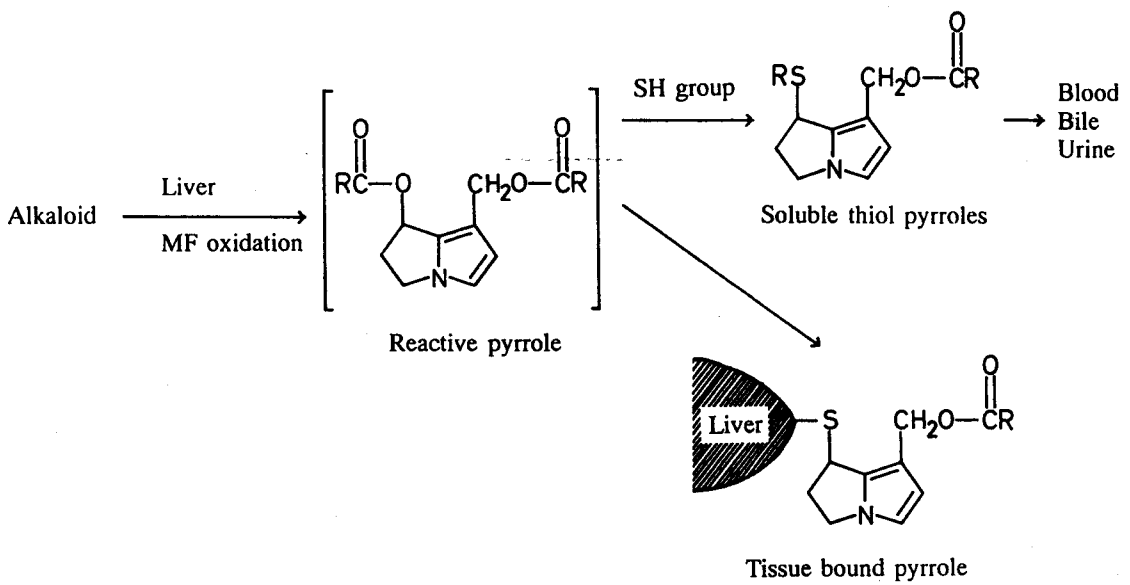

Figure 1. Hepatic metabolism of the pyrrolizidine alkaloid to give soluble and tissue bound thiol pyrroles.

While pyrroles react with amino groups to form mainly labile bonds, more importantly they react with thiol groups to form relatively stable covalent bonds. In the liver cell pyrroles can react with molecules such as glutathione to produce soluble glutathionyl pyrroles which can then be excreted in the bile and urine. In this way glutathione conjugation represents a protective mechanism against the formation of reactive pyrroles within the liver cell. Thiol binding of pyrroles can also take place on enzyme and structural proteins and this is considered to be the basis of the cytotoxic properties of these alkaloids in the liver (Mattocks 1986).

III. LIVER CHANGES

When large amounts of pyrroles are formed rapidly acute periacinar necrosis with damage to the terminal hepatic vein results. This causes extensive damage to the basic structure of this region of the liver parenchyma, simple regenerative repair cannot occur and the injured area often responds by organisation with the development of veno-occlusive disease. With injury to the liver parenchyma of slower onset, hepatocyte loss is less apparent but marked increase in perivenular fibrosis results as well as the formation of intraparenchymal dissecting fibrous tissue, with hepatocyte atrophy and cirrhosis. In affected liver cells, the pyrroles interfere with mitosis, inhibiting the cell cycle subsequent to the S-phase. This results in the formation of enlarged nuclei in giant sized liver cells normally referred to as "megalocytes". Some liver cells may escape or "throw off" the effect of the pyrroles formed in them and these may then replicate normally to give rise to regeneration nodules. This can then result in two separate populations of hepatocytes in the affected liver, megalocytes and foci of virtually normally sized hepatocytes in the regenerating areas.

IV. RESIDUAL S-BOUND PYRROLES

Recently it has been shown that residual thiol pyrrole binding can be demonstrated in the liver of animals which had at various times previously been exposed to pyrrolizidine alkaloids (Mattocks and Jukes, 1990a). Pyrroles can be demonstrated both in livers which show pathology and in livers which otherwise appear normal (Seawright et al 1991). In this test, the tissue, either fresh or fixed, is homogenised in acetone to precipitate the proteins. Following centrifugation, removal of the supernatant and resuspension and washing in absolute ethanol, the protein suspension is stirred with silver nitrate for up to one hour at room temperature. This results in the separation of the pyrrolizidine nucleus from the thiol group on the protein molecules and its reformation as an ethoxyether. Following removal of the unreacted ethanol under reduced pressure the pyrrolic ethoxyether can be removed by extraction with ether and identified by TLC, HPLC or GC-MS. So far this proceedure has been used to confirm the diagnosis of pyrrolizidine alkaloid exposure in animals such as yaks in the Himalayas, and cattle and horses in Queensland (Winter et al. 1990, Seawright et al 1991 a,b). Figure 2.

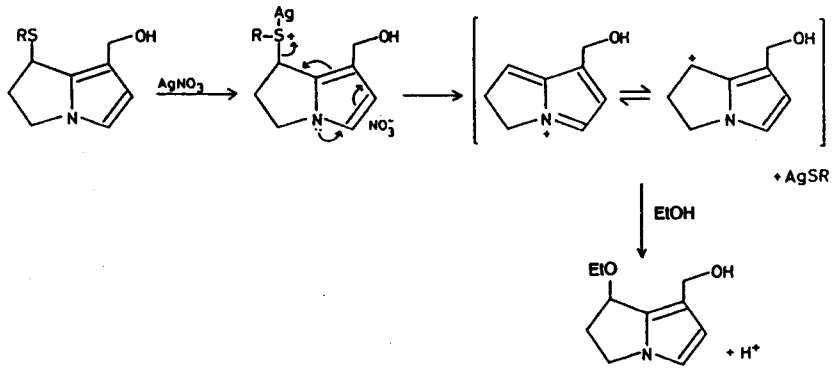

Figure 2. Suggested mechanism of cleavage of a pyrrolic thioether by silver nitrate. The cationic intermediate can react with ethanol to give the 7-ethoxypyrrole.

V. EXTRAHEPATIC EFFECTS

Activation of pyrrolizidine alkaloids for toxicity occurs only in the liver and when lesions occur in extrahepatic organs, these are considered to be due to active metabolites formed in the liver which subsequently escape into the blood flowing through the liver (Mattocks 1986). For this to occur the electrophiles formed in the liver must survive unhydrolysed for an appreciable time after their formation. This has been shown to occur for alkaloids such as monocrotaline the pyrrole of which has been demonstrated to have a half life in aqueous media of over 13 seconds (Mattocks and Jukes 1990b). Monocrotaline is well-known to be a cause of pulmonary hypertension in several species and to initiate intimal proliferation in the pulmonary arteries (Huxtable 1990). It has been shown in isolated liver perfusion studies using monocrotaline that the form in which the metabolite leaves the liver cell is 7-glutathionyldehydroretronecine (Huxtable 1990; Mattocks 1992). This compound when injected into the jugular vein causes the same lesion in the lungs as the parent alkaloid monocrotaline. 7-glutathionyldehydroretronecine is not itself an electrophile and appears to need further enzymatic metabolism in pulmonary arterial endothelial cells to cause a pathological reaction in this tissue. Suggested reactions likely to give rise to a further reactive metabolite include those with glutathione S-transferase to regenerate the original reactive pyrrolic electrophile or that with a C-S β-lyase to give a reactive mercaptan. Further studies have shown that reactive metabolites of monocrotaline derived from the liver are carried to the lungs in red blood cells (Pan et al. 1991). Some of this metabolite is available for re-release in the lungs and some is not and remains bound to the red cells. In the latter respect S-bound pyrrole on haemoglobin can be identified in the same way as it can be in the liver and this forms the basis of blood analysis as a means of diagnosing pyrrolizidine alkaloid exposure (Mattocks and Jukes 1990a).

VI. PROGRESSIVE LIVER DAMAGE

That glutathionyl pyrroles and bound pyrroles in red cells can give rise to further electrophiles in the lung circulation, presumably mainly through enzymatic metabolism, suggests that in the liver itself bound pyrroles may be re-released for binding to alternative sites within the liver cells (Mattocks 1986). Pyrroles bound initially harmlessly on various structural proteins may subsequently be released to attack more biologically significant molecules such as tubulin which is involved in the formation of mitotic spindles. This might well be the basis for mitotic inhibition in affected cells and of subsequent hepatic atrophy and other features of the pathology in this organ.

VII. THE HEALTH POSITION

Various pyrrolizidine alkaloid-containing plants are specially prized by herbalists and naturopaths for use in the treatment of sundry complaints and conditions in man and some are also favoured for inclusion in the diet. There are numerous episodes reported of serious human poisoning by these plants (Culvenor 1983). A major problem is that insidious liver damage may occur in some individuals at quite low levels of intake of the alkaloids and some of the alkaloids have been shown in experimental studies to be carcinogenic (Hirono 1987). Notwithstanding the fact that some of these plants such as comfrey have relatively low levels of the alkaloids in their leaves and roots and that by the oral route the acute toxicity of the alkaloids is moderate (Culvenor et al. 1980) compared with say monocrotaline, the main alkaloidal component of Crotalaria-derived bush teas, it would not be responsible for health authorities to pronounce them safe for internal human use. Herbal medicines and food products containing toxic pyrrolizidine alkaloids are often consumed with enthusiasm and excessive intake of the toxins could easily result.

An argument that has frequently been raised in favour of the continued use of such herbal products is that they have been used traditionally from time immemorial apparently without untoward effect. What is frequently forgotten in this context is that human life spans in westernised societies where use of such remedies has lately been popularised have considerably increased in recent decades. This increases the opportunity for the potential hepatocarcinogenic and progressive cirrhogenic actions of these alkaloids to become manifest in population groups in which extensive and regular use of these plants becomes part of the culture.

REFERENCES

ANON. (1988). 'Environmental Health Criteria 80'. Pyrrolizidine Alkaloids. p. 179. (World Health Organisation: Geneva).

CULVENOR, C.C.J., CLARKE, M., EDGAR, J.A., FRAHN, J.L., JAGO, M.V., PETERSON, J.E. and SMITH, L.W. (1980). Experientia. 36: 377.

CULVENOR, C.C.J. (1983). J. Toxicol.Environ.Health. 11: 625.

HIRONO, I. (1987). In 'Naturally Occurring Carcinogens of Plant Origin'. Chapter 2. Pyrrolizidine Alkaloids. p. 25, ed I. Hirono. (Elsevier: Amsterdam)

HUXTABLE, R.J. (1990). Pharmac. Ther. 47: 371.

MATTOCKS, A.R. (1986). 'Chemistry and Toxicology of Pyrrolizidine Alkaloids'. p. 191 (Academic Press: New York).

MATTOCKS, A.R. and JUKES, R. (1990a). Chem.-Biol. Interactions. 75: 225.

MATTOCKS, A.R. and JUKES, R. (1990b). Chem.-Biol. Interactions. 76: 19.

MOLYNEAUX, R.J., JOHNSON, A.E. and STUART, L.D. (1988). Vet. Human Toxicol. 30: 201.

PAN, L.C., LAMÉ, M.V., MORIN, D., WILSON, D.W. and SEGALL, H.J. (1991). Toxicol.Appl.Pharmcol. 110: 336.

SEAWRIGHT, A.A., HRDLICKA, J., WRIGHT, J.D. KERR, D.R., MATTOCKS, A.R. and JUKES, R. (1991a). Vet. human Toxicol. 22: 286.

SEAWRIGHT, A.A., KELLY, W.R., HRDLICKA, J., McMAHON, P., MATTOCKS, A.R. and JUKES, R. (1991b). Vet.Rec. 129: 198.

WINTER, H., SEAWRIGHT, A.A., MATTOCKS, A.R., JUKES, R., TSHWANG, U. and GURUNG, B.J. (1990). Aust. Vet. J. 67: 411.