An NADPH dependent warfarin reductase in human and rat liver and kidney soluble fraction

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Warfarin alcohols are major plasma metabolites of patients maintained on normal racemic warfarin, being present at about one third of the plasma concentration of warfarin. They are formed by reduction of the ketone group in the warfarin molecule. This reduction creates a second asymmetric centre and therefore two diastereoisomeric alcohols may be formed, warfarin alcohol$_1$ and warfarin alcohol$_2$. Each diastereoisomer exists in two enantiomeric forms, namely DL and LD for alcohol$_1$ and DD and LL for alcohol$_2$. About five times more warfarin alcohol is found in the plasma of man after a 100 mg single dose of (D)-warfarin than after an identical dose of (L)-warfarin. After (D)-warfarin the alcohol found in the plasma is warfarin alcohol$_1$ which probably has the DL configuration (Chan, Lewis & Trager, 1972; Hewick & McEwen, 1973).

We have initiated investigations regarding the origin of the warfarin alcohols. Previous workers (Ikeda, Ullrich and Staudinger, 1968) reported the formation of a non-phenolic metabolite of racemic warfarin produced in the presence of rat hepatic soluble fraction. Using hepatic and renal soluble fraction from man and the rat we have detected a number of non-phenolic warfarin metabolites. These metabolites, based on similar fluorescence properties, u.v. absorption spectra and chromatographic mobility, appear to be warfarin alcohols.

Soluble fraction, prepared from fresh rat tissue (200 g female Wistar rats) or from human samples, obtained 3.5 to 10 h after death, was incubated with (D)- or (L)-warfarin and an NADPH generating system at 37°C for 50 minutes. Warfarin alcohols formed were assayed by the method of Lewis, Ilnicki & Carlstrom (1970) as modified by Hewick & McEwen (1973).

The reductase activities of the livers of both species were similar and were about one fifth of the activities found in the kidneys. (D)-warfarin was reduced 2-8 times more readily than (L)-warfarin giving mostly alcohol$_1$, whereas alcohol$_2$ was the main product of (L)-warfarin reduction. For the four tissues the apparent Km values for the reduction of (D)-warfarin were similar, ranging from 0.63 to 1.5 mM.

Warfarin reductase activity occurring in fresh human tissue may be about twice that found in our human *post mortem* samples since we found that in 7 h rat hepatic *post mortem* samples, the reductase activity had declined by almost 60%.

These findings indicate that the relatively high concentration of warfarin alcohol (probably in the DL configuration) found in human plasma after (D)- or racemic warfarin administration may be attributable, at least in part, to stereoselective reductase enzymes in the hepatic and kidney cytosol. Alcohol dehydrogenase is probably not involved since we find ten times more warfarin alcohol is formed using NADPH compared with NADH as cofactor. Warfarin reductase however does bear some resemblance to the ketone reductases reported by Leibman (1971) in that it also occurs in liver cytosol and is NADPH linked.

References

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