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IS AZATHIOPRINE A BETTER IMMUNOSUPPRESSIVE THAN 6-MERCAPTOPURINE?

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SUMMARY

The toxicities and immunosuppressive potencies of single doses of 6-mercaptopurine and azathioprine have been compared in mice, using the 30-day mortality as a measure of toxicity, and reduction in spleen plaque-forming cells in response to sheep erythrocytes as a measure of immunosuppression. When compared on the basis of equivalent toxicity, 6-mercaptopurine was consistently the more effective agent by the intraperitoneal route. By the subcutaneous route, 6-mercaptopurine was more effective at doses above the LD-30; at lower doses, azathioprine was marginally better, but the difference was probably not significant. For the same cost in toxicity, azathioprine was six to seven times more effective as an immunosuppressive by the subcutaneous as by the intraperitoneal route.

INTRODUCTION

6-Mercaptopurine was first shown to be an immunosuppressive agent by Schwartz, Stack & Dameshek (1958) and its effects on various immune responses in experimental animals were widely investigated during the next few years. When Calne (1960) showed that it could materially prolong the survival of kidney grafts in dogs, the possibility arose that the purine antimetabolites might be useful in renal transplantation in man. The main disadvantages of 6-mercaptopurine found by Calne were its tendency to cause marrow aplasia and its inconsistency of action on graft rejection. A large number of purine analogues was therefore synthesized in the hope that a more effective and less toxic agent would be found. Nathan *et al.* (1961) examined the effects of these on haemagglutinin production in mice, and concluded that one of them, B.W. 57-322 (now known as azathioprine), was active over a wider range of doses than 6-mercaptopurine and at lower fractions of the maximally tolerated dose. Calne (1961) therefore investigated the effects of azathioprine in dogs with kidney trans-

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plants, and felt that the results with this drug were more encouraging than those with 6-mercaptopurine as it appeared to be less toxic to the bone marrow. However, like 6-mercaptopurine, its effects on graft rejection were inconsistent, and it promoted infection.

Calne (1963) emphasized the need for caution in evaluating these results in view of the small number of animals involved, the variability of management of individual animals and their unknown genetic history. Nevertheless, azathioprine was rapidly adopted in clinical practice in place of 6-mercaptopurine, and has become the standard purine analogue used for this purpose.

Apart from the preliminary and tentative findings of Nathan *et al.* (1961) and Calne (1961, 1963), there appears to be little evidence as to the comparative merits of 6-mercaptopurine and azathioprine. It was decided therefore to compare the two drugs in a relatively standardized experimental system.

MATERIALS AND METHODS

Mice. Male Balb/C mice were used, weighing 16–22 g at the start of the experiment.

Drugs. 6-Mercaptopurine and azathioprine in the form of powders (without excipient) were obtained from Burroughs Wellcome Ltd. For injection they were ground and suspended in 1% carboxymethylcellulose (Methocell, 250 C.P.S., Dow Chemical Co.) in physiological saline at concentrations that provided the required dose in 0·1 ml/10 g body weight. Suspensions were used within a few hours of preparation.

Assay of immunosuppressive activity. Mice were given 0·2 ml 10% formalized sheep red cells (Burroughs Wellcome) in saline intraperitoneally (day 0). On day +2 they were injected subcutaneously or intraperitoneally with various doses of 6-mercaptopurine or azathioprine. Spleens were removed on day +5 and the numbers of haemolytic plaque-forming cells they contained counted by Jerne's method (Jerne & Nordin, 1963; Jerne, Nordin & Henry, 1963). Results are expressed here as fractions of the geometric means of control groups given the suspending medium alone. Groups of six mice were used for each dose in each experiment.

Toxicity Assay. Mice were given various doses of 6-mercaptopurine or azathioprine subcutaneously or intraperitoneally, and the number of survivors counted thrice weekly up to 30 days after injection. Each group usually contained ten to fifteen mice, and the 30-day survivals are here given as the pooled results of one to three assays at each dose level.

RESULTS

Toxicity

The percentage of 30-day survivals after various doses of either drug are shown in Fig. 1.

The LD-50's are, after subcutaneous injection, 165 mg/kg for 6-mercaptopurine and 375 mg/kg for azathioprine and, after intraperitoneal injection, 190 mg/kg for 6-mercaptopurine and 410 mg/kg for azathioprine. As the ratio of the molecular weights of azathioprine and 6-mercaptopurine is 266:170, or 1:1·56, it appears that azathioprine is somewhat less toxic than 6-mercaptopurine on an equimolar basis. Both drugs are slightly more toxic by the subcutaneous route.

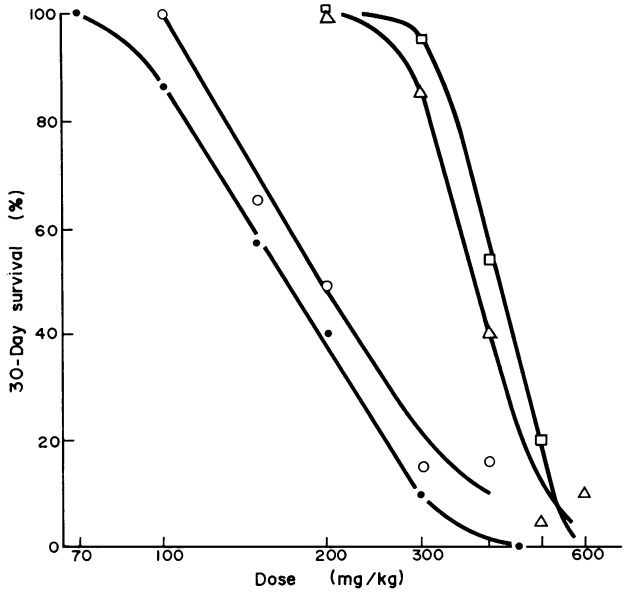

FIG. 1. 30-Day mortalities after 6-mercaptopurine or azathioprine. ○, 6-mercaptopurine i.p.; ●, 6-mercaptopurine s.c.; □, azathioprine i.p.; △, azathioprine s.c. 20–25 mice per point.

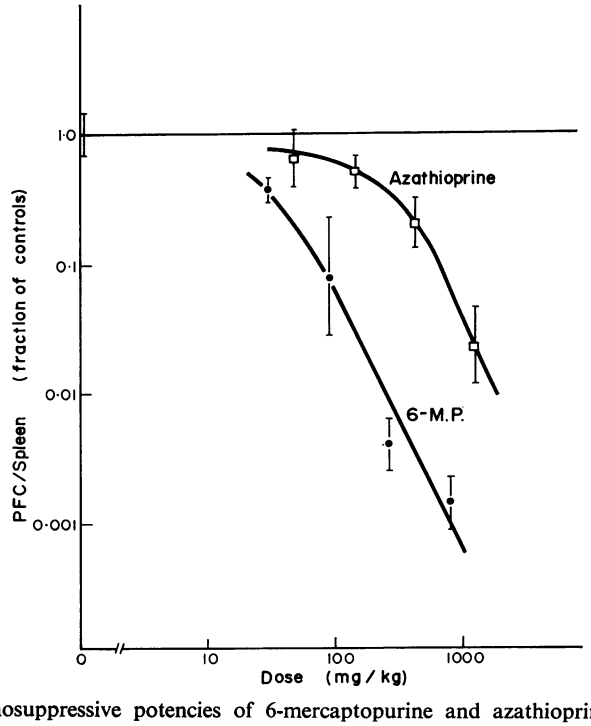

FIG. 2. Immunosuppressive potencies of 6-mercaptopurine and azathioprine given intraperitoneally. Drug given 2 days after sheep red cells and number of plaque-forming cells (PFC) per spleen counted on the 5th day. Results are expressed as fractions of the control levels. The geometric means and logarithmic standard deviations are shown.

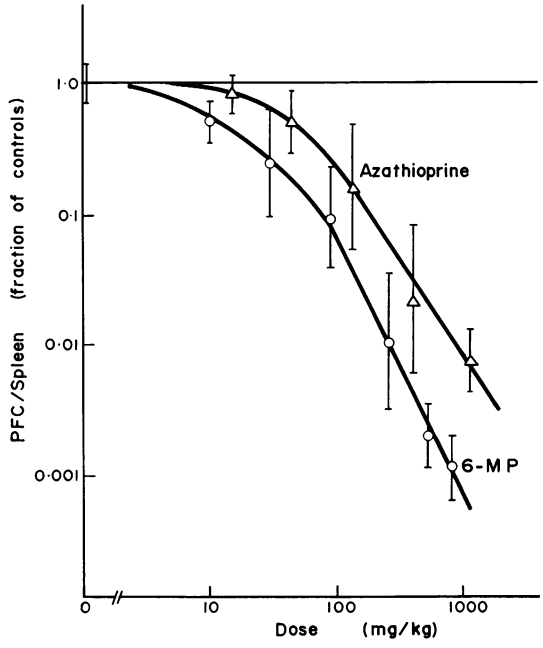

FIG. 3. Immunosuppressive potencies of 6-mercaptopurine and azathioprine given subcutaneously. Drug given 2 days after sheep red cells and number of plaque-forming cells (PFC) per spleen counted on the 5th day. Results are expressed as fractions of the control levels. The geometric means and logarithmic standard deviations are shown.

Immunosuppressive activity

The relation between dose and resulting depression in production of plaque-forming cells is shown in Figs 2 and 3. As would be expected for antimetabolites, the dose response curves are of hyperbolic type in that they tend to straighten out at high doses on a log–log plot (Berenbaum, 1969). They fit the equation

$$F = \left[\frac{D_q}{D+D_q}\right]^{\gamma}$$

where F is the number of plaque-forming cells expressed as a fraction of controls, γ the slope of the straight part of the curve, D_q the intersect of the straight part of the curve extrapolated back to the F = 1 axis and D the dose of drug. For 6-mercaptopurine the value for D_q is 38 mg/kg and for γ, 2·2 by both subcutaneous and intraperitoneal routes. For azathioprine given subcutaneously D_q is 75 mg/kg and γ is 1·8; for azathioprine given intraperitoneally D_q is 390 mg/kg and γ is 2·5. The effectiveness of 6-mercaptopurine is therefore not influenced by the route of injection but azathioprine is more effective when given subcutaneously.

Immunosuppression at doses of equivalent toxicity

In Fig. 4 the fractional survival of plaque-forming cells is plotted against fractional mortality of the animals, in other words, the degree of immunosuppression is here measured against toxicity. By the intraperitoneal route, 6-mercaptopurine is by far the more effective immunosuppressive. By the subcutaneous route it is the more effective agent at doses above

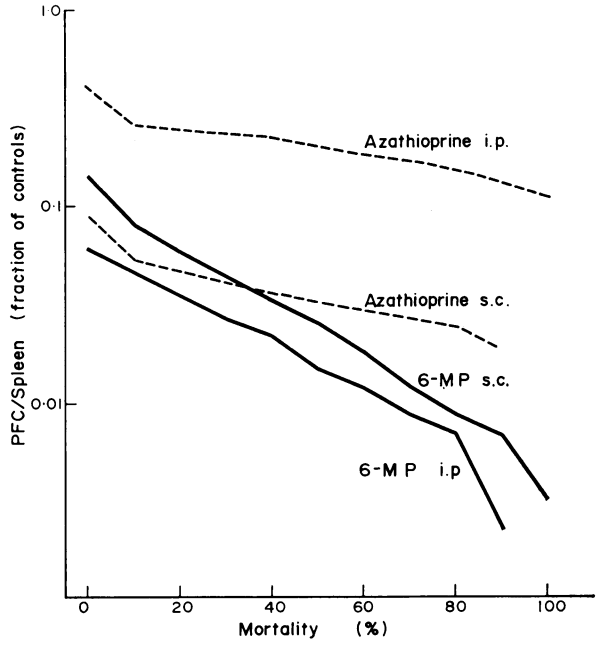

FIG. 4. Immunosuppressive potencies of 6-mercaptopurine and azathioprine at doses of equivalent toxicity. Plaque forming cells (PFC) are plotted, as a fraction of controls, against % 30-day mortalities.

the LD-30; at lower doses azathioprine is slightly more effective but, in view of the scatter of results in this dose-range (Figs 2 and 3), it is doubtful whether the difference between the two drugs is significant. Extrapolation of the curves does not suggest that the relative effectiveness of the two drugs would alter materially at doses below the LD-0.

It is interesting to note that, for the same cost in toxicity, azathioprine is some six to seven times more effective as an immunosuppressive when given subcutaneously than when it is given intraperitoneally. The reverse is true for 6-mercaptopurine, but to a lesser extent, the intraperitoneal route being 1·3-2 times as effective as the subcutaneous route at equivalent toxic doses.

DISCUSSION

The experiments reported here show that, when the immunosuppressive potencies of 6-mercaptopurine and azathioprine are compared at doses of equivalent toxicity, 6-mercaptopurine is undoubtedly the better agent over the whole lethal dose range when the intraperitoneal route is used. When the subcutaneous route is used, azathioprine is better (but not significantly so) in the low lethality dose range and 6-mercaptopurine better at high doses.

It would be rash to conclude from these results that 6-mercaptopurine is in general a better immunosuppressive than azathioprine. Indeed, as the relation between the degrees of immunosuppression and toxicity produced evidently depends on such a relatively trivial factor as the route of injection, it is clear that any comparison between the two drugs is valid only for the conditions under which it is made. It is possible therefore that the differ-

ence between the results of Nathan *et al.* (1961) and those reported here are due to differences in experimental design.

These authors measured haemagglutinins in mice after a secondary injection of sheep red cells, the drugs being given intraperitoneally for 4 days, beginning on the day of the primary antigen injection. Azathioprine was said to be more effective than 6-mercaptopurine when compared on the basis of the maximum tolerated dose. However the maximum tolerated dose was not defined and antibody levels were expressed in the form of a rather arbitrary index based on the degree of agglutination in every tube, and this method of determination does not necessarily rank antisera in the same order as the generally accepted method based on determination of the agglutination end-point. Moreover, it is questionable whether the effectiveness of drugs should be compared on the basis of a single level of toxicity. As Fig. 1 shows, the mortality curves of 6-mercaptopurine and azathioprine are most divergent in the low lethality range and approach one another as the dosage increases. Strictly speaking, therefore, their therapeutic effects should be set against toxicity for each dose level. This is not to deny that azathioprine may be more effective than 6-mercaptopurine when tested under the conditions used by Nathan *et al.* (1961) (and the results reported here certainly do not exclude this, nor the possibility that azathioprine may indeed be a better agent for use in man) but these discrepant findings emphasize the hazard in generalizing on the basis of limited experiments.

Nathan *et al.* (1961) referred to work by Calne (1961) as showing an apparent superiority of azathioprine over 6-mercaptopurine. Calne performed non-littermate transplants of kidneys to eleven dogs treated with 6-mercaptopurine and seventeen treated with azathioprine. The 6-mercaptopurine-treated animals survived 7–46 days whereas those on azathioprine survived from 2 to 122 days. In a later publication, Calne (1963) described the subsequent fates of these dogs and made up the number treated with 6-mercaptopurine to fifteen and with azathioprine to nineteen. The mean survival time of 6-mercaptopurine treated dogs was $21{\cdot}9 \pm 14{\cdot}1$ days compared with $29{\cdot}2 \pm 36{\cdot}0$ days for dogs on azathioprine. However, the latter result was largely due to weighting by a single dog that survived 164 days, the mean survival time of the remainder being $21{\cdot}7 \pm 15{\cdot}6$ days. With the benefit of hindsight we would now assume that this long survival was due to fortuitously favourable donor-recipient compatibility. However, even with this result included, the difference between the groups on the two drugs is not significant (Student's t-test, $P > 0{\cdot}1$). As regards toxicity, the number of animals with appreciable rejection, marrow failure or infection were respectively eight, six and five out of the fifteen on 6-mercaptopurine, and thirteen, four and twelve out of the nineteen on azathioprine. There was therefore no evidence from this work that azathioprine was a significantly more effective therapeutic agent than 6-mercaptopurine.

In looking back on these pioneer studies it is difficult now to see how they would, in themselves, have justified the rapid swing from 6-mercaptopurine to azathioprine that took place in clinical practice. Certainly, there has been no formal clinical trial comparing the two drugs in renal transplantation. There is no doubt that better results were obtained during the few years following the introduction of azathioprine, but the contribution that simple accumulated clinical experience made to this is often overlooked. After the adoption of azathioprine and before the introduction of antilymphocyte serum there was a period of 6–7 years during which practice in regard to immunosuppressive drugs remained virtually constant, yet results improved consistently over this period. Even when the same transplantation unit is considered, results may improve radically over the course of a few years

(Hume *et al.*, 1966). That better results were obtained with azathioprine in 1966 than with 6-mercaptopurine in, say, 1962 is therefore not surprising and is hardly evidence that one drug is a better immunosuppressive than the other. Possibly the change from 6-mercaptopurine to azathioprine was occasioned or, at least, accelerated for a reason quite unrelated to the immunosuppressive potencies of these drugs, i.e. the impression that azathioprine has a lesser tendency to cause cholestatic jaundice in man.

It is also relevant to consider here the problem of screening immunosuppressive agents. The case of 6-thioguanine, which was investigated by Nathan *et al.* (1961) and Calne (1961) is instructive. This antimetabolite was the most active of the agents tested by Nathan *et al.* (1961), having an 'immunosuppressive index' of 0·18 at maximum tolerated doses, compared with 0·32 for 6-mercaptopurine and 0·30 for azathioprine. The superiority of 6-thioguanine over 6-mercaptopurine in mice was confirmed by Frisch & Davies (1962) who measured its effect on haemagglutinin formation, and Berenbaum & Brown (1964) who compared the toxicities of the two drugs as well as their abilities to depress the response to T.A.B. vaccine. However, Calne (1961) found that two dogs treated with this drug died of bone marrow depression within a week, although graft rejection was not prevented. Thioguanine has occasionally been used successfully to treat clinical autoimmune disease but the danger of serious bone marrow damage in man has discouraged its use (Damashek & Schwartz, 1960; Goodman *et al.*, 1963). It is clear that, because of species differences in the effectiveness and toxicity of drugs, animal screens can do little more than indicate agents worth testing in man, and that the ranking of active agents in a single type of animal test may bear little relation to their order of clinical usefulness.

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