The effect of methylprednisolone sodium succinate on erythrocyte and haemoglobin function

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Summary

1. As it has been suggested that the beneficial effect of methylprednisolone in shock is due to its effect on erythrocyte oxygen affinity, we studied its effect on incubated erythrocytes and on haemoglobin solution.

2. Incubation of fresh whole blood anticoagulated with acid/citrate/dextrose with methylprednisolone (7 mmol/l) produced a significant decrease in oxygen affinity, which was not seen with lower concentrations of methylprednisolone. When either acid/citrate/dextrose blood stored for 10 days or fresh heparinized blood was used, no significant increase in the partial pressure of oxygen at 50% haemoglobin saturation ($P_{50}$) was demonstrated even with methylprednisolone at 7 mmol/l. At the highest concentration achieved in plasma with standard therapeutic doses (56 pmol/l) there was no increase in $P_{50}$ under all the conditions studied.

3. Methylprednisolone reduced the oxygen affinity of haemoglobin in solution. The reduction in oxygen affinity was less than that produced by 2,3-diphosphoglycerate and more than that of either sodium succinate or sodium chloride.

4. From the results of this study we conclude that the effect observed in whole cells is probably due to a direct effect of methylprednisolone on haemoglobin. To produce a significant decrease of oxygen affinity of whole blood in vitro requires a plasma concentration of methylprednisolone above that obtained in plasma in vivo, with the currently used therapeutic doses.

Key words: erythrocyte, haemoglobin solution, methylprednisolone, oxygen affinity.

Abbreviation: 2,3-DPG, 2,3-diphosphoglycerate.

Introduction

Large doses of methylprednisolone are widely used in the shocked patient but the mechanism of its beneficial effect still remains largely unexplained (Editorial, 1977). One of the suggested mechanisms has been its effect on whole-blood oxygen affinity. It has been postulated that the decrease in oxygen affinity caused by methylprednisolone would facilitate oxygen delivery to the tissues. In two reports studying the effect of methylprednisolone on whole-blood oxygen affinity, firstly in patients with shock (McConn, 1972) and secondly after massive transfusion of stored blood (Bryan-Brown, Back, Makabali & Shoemaker, 1973), this steroid was shown to decrease oxygen affinity but the mechanism of its action was not explained. Studies by McConn & Del Guercio (1971) on whole blood incubated with methylprednisolone suggested a direct effect on the erythrocyte.

The effect of methylprednisolone on the oxygen affinity of both whole blood anticoagulated with either acid/citrate/dextrose or heparin and of haemoglobin in solution was measured. To exclude the possibility that any effect on oxygen affinity in erythrocytes is due to other intracellular factors we have measured erythrocyte 2,3-diphosphoglycerate (2,3-DPG), ATP and intracellular pH.
Methods and materials

Whole blood

Incubation studies were performed on fresh and 10-day-old blood. Fresh whole blood was anticoagulated with either acid/citrate/dextrose or heparin and 10-day-old blood was anticoagulated with acid/citrate/dextrose. Blood was incubated in an IL tonometer with 5% carbon dioxide in air at 37°C for 3 h. Oxygen affinity at 37°C and plasma pH 7.4, erythrocyte 2,3-DPG and ATP, plasma pH and intracellular pH were measured before addition of steroid and at the end of the 3 h incubation period. Three concentrations of methylprednisolone were chosen (56 μmol/l = 20 mg/l; 280 μmol/l = 100 mg/l; 7 mmol/l = 2.5 g/l) to correlate with previously published data (McConn & del Guericio, 1971) and with the plasma concentration (20 mg/l) obtained after the maximum intravenous dose used therapeutically (30 mg/kg). Sodium chloride solution (150 mmol/l/saline) was used as a control. Oxygen dissociation curve was determined by the method of Wells (1975) and the results were expressed in terms of P50. The commercial preparation of the sodium succinate derivative of methylprednisolone was used (Solu-Medrol, Upjohn Ltd, Crawley, Sussex, U.K.).

Haemoglobin solution

Haemoglobin solution was prepared from fresh blood anticoagulated with methylprednisolone was chosen (56 μmol/l = 20 mg/l; 280 μmol/l = 100 mg/l; 7 mmol/l = 2.5 g/l) to correlate with previously published data (McConn & del Guericio, 1971) and with the plasma concentration (20 mg/l) obtained after the maximum intravenous dose used therapeutically (30 mg/kg). Sodium chloride solution (150 mmol/l/saline) was used as a control. Oxygen dissociation curve was determined by the method of Wells (1975) and the results were expressed in terms of P50. The commercial preparation of the sodium succinate derivative of methylprednisolone was used (Solu-Medrol, Upjohn Ltd, Crawley, Sussex, U.K.).

Results

The effect of incubating fresh whole blood, anticoagulated with acid/citrate/dextrose, with methylprednisolone sodium succinate is shown in Table 1(a). There is no significant change in oxygen affinity except for methylprednisolone at a concentration of 7 mmol/l, where the increase in P50 (1.74 kPa; where 1 kPa = 7.52 mmHg) is particularly marked and cannot be accounted for by the changes in either 2,3-DPG or ATP (a total increase in 2,3-DPG and ATP of 1.36 μmol/l of

| TABLE 1. Effect of incubation of whole blood with methylprednisolone on 2,3-diphosphoglycerate, ATP and oxygen affinity of erythrocytes |
|---|---|---|---|---|
| Conc. of methylprednisolone (μmol/l) | Change in P50* (kPa) | Change in 2,3-DPG* (mmol/l of erythrocytes) | Change in ATP (mmol/l of erythrocytes) |
| 0 | -0.12 | 0.00 | 0.00 |
| 56 | +0.12 | 0.03 | +0.14 |
| 280 | -0.20 | +0.29 | +0.11 |
| 7000 | +1.74 | +0.72 | +0.64 |
| Fresh heparinized blood | | | |
| 0 | -0.04 | 0.08 | +0.08 |
| 56 | -0.12 | +0.08 | +0.16 |
| 7000 | +0.37 | +1.18 | +0.49 |
| 10-day-old blood anticoagulated with acid/citrate/dextrose | 0 | +0.21 | 0.00 | +0.07 |
| 56 | +0.20 | 0.00 | +0.06 |
| 7000 | +0.53 | 0.00 | +0.12 |

P50 before the addition of methylprednisolone†

(a) 3.00 ± 0.16 kPa (b) 3.83 ± 0.01 kPa (c) 2.11 ± 0.18 kPa

Plasma pH during incubation†

(a) 6.91 ± 0.04 (b) 7.38 ± 0.02 (c) 6.77 ± 0.04

ΔpH (plasma pH — intracellular pH)†

(a) 0.01 ± 0.00 (b) 0.14 ± 0.01 (c) 0.04 ± 0.02

2,3 DPG before the addition of methylprednisolone†

(a) 3.23 ± 0.33 mmol/l of erythrocytes (b) 4.26 ± 0.37 mmol/l of erythrocytes (c) 0.2 ± 0.10 mmol/l of erythrocytes

ATP before the addition of methylprednisolone†

(a) 3.30 ± 0.18 mmol/l of erythrocytes (b) 1.17 ± 0.25 mmol/l of erythrocytes (c) 0.82 ± 0.25 mmol/l of erythrocytes

*Values are quoted as mean of three incubations.
†Represented as mean ± 2 SE.
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erythrocytes would raise $P_{50}$ by approximately 0.36 kPa). There were no alterations in plasma pH and intracellular pH. Changes in $P_{50}$ with 56 and 280 μmol of methylprednisolone/l are not significant. There was only a small reduction in oxygen affinity when fresh blood, anticoagulated with heparin, was incubated with methylprednisolone (Table 1b) and this can be explained by the small increases in organic phosphates. Ten-day-old blood stored in acid/citrate/dextrose (Table 1c) showed first, a low initial $P_{50}$ (2.11 kPa) due to 2,3-DPG depletion and, secondly, only a small increase in $P_{50}$ after incubation with methylprednisolone even at the high concentration of 7 mmol/l. At the end of the incubation period there was still no detectable 2,3-DPG, although there was a small rise in ATP which did not account for the slight decrease in oxygen affinity. As with fresh blood, there was no detectable trend in intracellular pH and plasma pH during the incubation.

Results of the effect of methylprednisolone on $P_{50}$ of haemoglobin solution are shown in Fig. 1, where log $P_{50}$ has been plotted vs log concentration of the salt. With increasing concentrations of methylprednisolone sodium succinate the oxygen affinity of haemoglobin decreased, which is similar to the phenomenon observed for other salts (Rossi-Fanelli, Antonini & Caputo, 1961; Antonini, Amiconi & Brunori, 1971). Comparison of the effect of methylprednisolone sodium succinate on oxygen affinity with that of sodium chloride, sodium succinate and 2,3-DPG is also shown. The concentrations required to produce the same shift in $P_{50}$ vary for different salts, 2,3-DPG being the most effective. Methylprednisolone sodium succinate falls between 2,3-DPG and either sodium succinate or sodium chloride in its efficacy, requiring a concentration 100 times higher than 2,3-DPG for a similar reduction in oxygen affinity. Studying the effect of methylprednisolone sodium succinate on pH dependency of oxygen affinity showed that the Bohr effect is maintained in the presence of methylprednisolone sodium succinate (Fig. 2). Reductions in $P_{50}$ due to methylprednisolone sodium succinate in the presence of various concentrations of 2,3-DPG are shown in Fig. 3. By increasing the concentration of 2,3-DPG, the effect of methylprednisolone sodium succinate on oxygen affinity is progressively eliminated. In the presence of methylprednisolone sodium succinate at 10 mmol/l, 2,3-DPG does not decrease the oxygen affinity. The same characteristics were demonstrated for increasing concentrations of sodium chloride and for sodium chloride, 2,3-DPG and methylprednisolone sodium succinate in combination (Fig. 4).

Discussion

As the highest concentration of methylprednisolone achieved therapeutically is only 56 μmol/l the results of incubating whole blood in vitro (Table 1) show that, at these plasma concentrations, there is no change in whole-blood oxygen affinity. From this we conclude that the observed effects of methylprednisolone on whole-
FIG. 3. Effect of methylprednisolone in the presence of 2,3-DPG on the oxygen affinity of haemoglobin. A, No methylprednisolone sodium succinate; B, methylprednisolone sodium succinate (10 μmol/l); C, methylprednisolone sodium succinate (100 μmol/l); D, methylprednisolone sodium succinate (1 mmol/l); E, methylprednisolone sodium succinate (10 mmol/l). Other conditions were as described in Fig. 1.

FIG. 4. Effect of methylprednisolone on the oxygen affinity of haemoglobin in the presence of 2,3-DPG (20 μmol/l) and sodium chloride (0.1 mol/l) alone and in combination. Methylprednisolone sodium succinate alone (A) or B, with sodium chloride (0.1 mol/l), C, with 2,3-DPG (20 μmol/l) or D, with sodium chloride (0.1 mol/l) and 2,3-DPG (20 μmol/l). Other conditions were as described in Fig. 1.

Blood oxygen affinity in vivo (McConn, 1972; Bryan-Brown et al., 1973) are not due to a direct effect on haemoglobin function or rise in erythrocyte organic phosphates. The results on haemoglobin solution show that methylprednisolone sodium succinate affects haemoglobin function in a manner similar to that of other salts. Oxygen affinity decreases with
increasing salt concentration, but to produce the same reduction in oxygen affinity different concentrations of the various salts are required. Of the salts we examined, 2,3-DPG is the most effective salt in reducing oxygen affinity of haemoglobin. A concentration of methylprednisolone sodium succinate about 100 times that of 2,3-DPG is required to produce the same increase in $P_{50}$.

Owing to the water insolubility of methylprednisolone we used the water-soluble sodium succinate derivative, which is used clinically for parenteral therapy. Fig. 1 shows that a ten times higher concentration of pure sodium succinate compared with methylprednisolone sodium succinate is required to produce the same reduction in oxygen affinity. This suggests that the steroid itself, rather than the succinate radical, is important in determining the effect of methylprednisolone sodium succinate on oxygen affinity. The fact that another sodium salt, sodium chloride, is less effective than either sodium succinate or methylprednisolone sodium succinate further suggests that the effect of methylprednisolone sodium succinate on oxygen affinity of haemoglobin is due to the presence of the steroid ring.

Fig. 3 shows that at high concentrations of methylprednisolone sodium succinate addition of 2,3-DPG does not reduce the oxygen affinity of haemoglobin any further. Conversely, progressive addition of 2,3-DPG eliminates the influence of low concentrations of methylprednisolone sodium succinate on oxygen affinity of haemoglobin. This effect is similar to that described by Benesch, Benesch & Yu (1969) for sodium chloride and 2,3-DPG. They ascribe this effect to competition of the salts for the same binding site. The relevance of this phenomenon can be seen when extrapolated to whole blood, where at low concentrations of methylprednisolone sodium succinate 2,3-DPG will be of prime importance in determining the final $P_{50}$. By using the data of Fig. 1 and assuming an equimolar concentration of 2,3-DPG to haemoglobin in the erythrocyte an intracellular concentration of methylprednisolone sodium succinate above 2.3 mmol/l (0.82 g of methylprednisolone/l) would be needed to produce a further increase in $P_{50}$. Applying this principle to the results of fresh acid/citrate/dextrose blood (Table 1) the calculated concentration of methylprednisolone sodium succinate required to produce the shift of 1.74 kPa in $P_{50}$ is 6.3 mmol/l (2.25 g of methylprednisolone/l), which is very close to the actual concentration used. These results indicate that in fresh acid/citrate/dextrose blood the decrease in oxygen affinity could be explained by the direct effect of methylprednisolone sodium succinate on haemoglobin.

This conclusion presupposes a penetration of methylprednisolone sodium succinate through the erythrocyte membrane. There is some evidence for this as Wilson (1974) noted the presence of significant labelling in erythrocytes within 10 min of injection of [3H]methylprednisolone sodium succinate into dogs. In another study, analysing the distribution of radioactive methylprednisolone sodium succinate after administration to human volunteers (Slaunwhite & Sandberg, 1961), it was shown that a surprisingly high percentage of radioactivity was associated with erythrocytes.

However, with 10-day old acid/citrate/dextrose blood and fresh heparinized blood (Table 1b,1c) with methylprednisolone (7 mmol/l), there is only a minimal decrease in oxygen affinity above that which could be expected by the increase in phosphate esters, contrasting with results in fresh acid/citrate/dextrose blood. This surprising result may be due to the inability of methylprednisolone sodium succinate to cross the erythrocyte membrane under these conditions. It is known that certain anions inhibit ionic flux across the erythrocyte membrane (Sachs, Knauf & Dunham, 1975). It is conceivable that a polyanion such as heparin would block the membrane transport system although we can find no published evidence for this. The main differences detected between fresh and 10-day-old acid/citrate/dextrose blood were in pH and intracellular concentration of 2,3-DPG. Increased ionization of methylprednisolone sodium succinate under the conditions of low pH may account for the reduced permeability. However, it should be pointed out that although in these experiments methylprednisolone was used as a sodium succinate salt, in vivo it is hydrolysed to yield methylprednisolone. Its membrane permeability in vivo might thus be different.

Although erythrocyte 2,3-DPG is well known for its physiological role in reducing oxygen affinity of haemoglobin, its role in the shock syndrome is uncertain. The levels of 2,3-DPG were shown in serial measurements to fluctuate often independently of $P_{50}$ (McCann, 1975). Only in the late stages of sepsis were both $P_{50}$ and 2,3-DPG found to be low. We have demonstrated a slight increase in 2,3-DPG in fresh acid/citrate/dextrose blood incubated with methylprednisolone. This may contribute to the rise in $P_{50}$ in vivo, but it is unlikely to be of major benefit in the shock state.
In conclusion, the observed effect of methylprednisolone sodium succinate on erythrocytes in vitro is probably due to a direct effect of methylprednisolone sodium succinate on haemoglobin. However, the concentration required is unacceptably high for clinical use and cannot explain the beneficial effect of methylprednisolone in vivo. Further studies are required to characterize the transport mechanisms of methylprednisolone sodium succinate across the erythrocyte membrane and evaluate the potential usefulness of this steroid in reducing oxygen affinity of stored blood.

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References


