PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 301-305 (1978)

The Role of Dextran Sulfate in Increasing the CFU_c^- Concentration in Dog Blood (40042)

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Pluripotent as well as committed stem cells are present in the peripheral blood of mammals, although in rather small number (1-4). Their presence in the circulation may well be indicative of the role they play in the maintenance of hemopoietic homeostasis amongst the various sites of hemopoiesis throughout the skeletal system. In the human and other large mammals, a quantitative determination of the pluripotent stem cell population in the blood is still a problem to be solved; we must still content ourselves with the determination of the presumably granulocytic-committed stem cell, using the agar CFUc (colony forming unit in culture) assay developed by Bradley and Metcalf (5). The number of CFUc appears to be a very good indicator for the presence of pluripotent hemopoietic stem cells in the blood of dogs, as evidenced by the fact that hemopoietic recovery in the marrow of lethally irradiated dogs is proportional to the number of blood CFUc transfused (1). In these instances, mononuclear leukocytes (MNC), including CFUc, had been collected from donor dogs via an arterio-venous shunt using the IBM Experimental Blood Cell Separator. A 5-hr leukapheresis was necessary to collect 25×10^9 leukocytes, among these being about 8×10^9 MNC, which, in turn, contained some 0.5×10^6 CFUc.

In order to increase the yield of MNC, but, in particular, of CFUc from a single leukapheresis, dextran sulfate (DS) was administered iv shortly before the onset of leukapheresis. In previous studies, Ross et al have shown that various polyanions, including DS, are able to increase the number of MNC in the blood of rats and of other mammals (6, 7). Thus, it was the objective of this study to examine in dogs the effects of administering different doses of DS on the blood levels of leukocytes, especially of MNC and of CFUc, to provide a basis for attempts to increase the yield of CFUc collected from the peripheral blood.

Materials and Methods. Two healthy beagles were used in this study, about 2 years of age and weighing 16 kg—one was male and the other female.

An anterio-venous shunt was surgically inserted between the carotid artery and the jugular vein on one side. This shunt was thoroughly cleansed (flushed with heparinised saline) twice a day to prevent blockage by fibrin buildup. It could generally be maintained free-flowing for 3-4 weeks. Antibiotics were administered only for the first few days following the operation while the wound healed.

Dextran sulfate (DS) is a synthetic polyanion of strong negative charge, composed of glucose units in a helical chain and with SO_4 groups. It was prepared for these experiments by esterifying pure dextran (m.w. 40,000, Merck) with chlorosulfonic acid, after the recipe used by Ricketts (8). On each glucose unit of dextran are three hydroxyl groups, any one or all of which may be substituted for by a sulfate group; in this case, an average of 2.3 sulfate groups were found per glucose unit, as indicated by a sulfur content of 17%. The product was neutralized, extensively dialysed against distilled water, then freeze-dried, powdered, and stored in air-tight vials at 4°. Just prior to administration, the appropriate amount of DS was dissolved (readily) in 20 ml of sterile saline solution (0.9%). It was injected over about 20 sec into the venous side of the shunt.

As can be derived from Fig. 2, each of the two dogs received on two separate occasions, saline injections (S) as a control. On four other separate occasions, 10 mg

This work was supported by Euratom-Contract No. 222-76-1 BIO-D and by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 112).

0037-9727/78/1572-0301$01.00/0

DS/kg body weight (D) were given. In two further experiments, 15 mg DS/kg body weight (D′) were given. Between one experiment (lasting 7 hr) and the next, 2–3 days were allowed to elapse in order to stabilize the blood cell levels.

The number of CFUc in the peripheral blood, as withdrawn from the arterial side of the shunt at various intervals after administration of DS, was determined by using a modified agar culture technique (4).

The levels of leukocytes (mononuclear and polymorphonuclear) and thrombocytes in the peripheral blood were also recorded, along with the hematocrit.

Results. Figure 1 illustrates the findings for the two different doses of DS and for the saline control. The data from all experiments in the two dogs were pooled for each treatment schedule.

There was no effect caused by the administration of *saline* alone, either on leukocyte numbers or on CFUc, over the 7-hr period of observation. However, after the administration of *10 mg DS/kg* body weight, within 3 hr there was a slight rise in polymorphonuclear cells (PMN), a doubling of MNC, and a sevenfold increase in CFUc (from 100 to 700 CFUc/ml blood). After the administration of *15 mg DS/kg* body weight, there was an increase in PMN of about 40% over the preinjection values. The MNC showed an increase of about 100%, double the preinjection value. The blood CFUc experienced a tenfold increase in number, from about 200 to about 2000 CFUc/ml blood. The standard error for the 3-hr mean CFUc value was fairly large, due to the relatively high values observed in the first experiment (21-fold increase) as compared to the values in the second experiment (about fivefold increase), for both dogs.

The MNC population following DS administration tends to maintain the high level beyond 3 hr, as does the PMN population. On the other hand, the CFUc population experiences a decrease after the 3-hr peak. It should also be noted that the increase in CFUc over the first 3 hr is proportionally much greater than the increase in MNC, indicating that the CFUc concentration is controlled by mechanisms other than those controlling MNC and PMN levels. Normal values for leukocytes and for CFUc are regained within 24 hr.

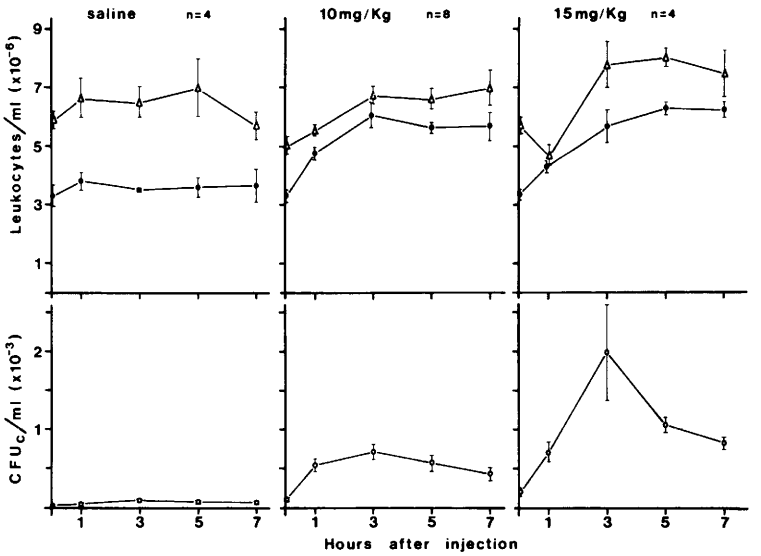

FIG. 1. Effect of dextran sulfate administration i.v. on blood leukocytes in two dogs. (a) Saline only (0.9%): $n = 4$. (b) Ten milligram DS/kg body weight (eight separate experiments, four with each dog). (c) Fifteen milligram DS/kg body weight (four separate experiments, two with each dog). Each point represents the mean; the bars indicate the standard error of the mean. — △ — PMN; — ● — MNC; — ○ — CFUc.

It is of interest, however, to note that the repeated administration (2–3 days between doses) of dextran sulfate (10 or 15 mg DS/kg body weight) results in an increase in the basic concentration of CFUc in blood (Fig. 2).

For the two dogs, the basic CFUc level (preinjection level) increased from about 65 to 230 CFUc/ml blood, a fourfold elevation from before the first dosage until 24 hr after the last. In contrast, other blood parameters remained essentially unaltered. MNC and PMN levels were unaffected. Thrombocytes displayed a slight tendency to decrease, declining to about 60% of their initial day 0 level, while the hematocrit decreased by 10%; these changes are considered to be mainly a consequence of the experimental procedures, namely the sampling.

Since it has been found by other investigators (9, 10) that the number of leukocytes and CFUc increase after injection of endotoxin, the dextran sulfate employed in these experiments was tested for endotoxin contamination by the commercially-available Limulus Assay (Associates of Cape Cod., Inc., Woods Hole, MA). Standard endotoxin from E. coli (Westphal method; code 0127:B8) was used as a positive control for a quantitative determination; it produced a positive reaction down to 0.5 ng/ml. Dextran sulfate (15 mg/ml) gave essentially a negative reaction; if endotoxin was present as a contaminant, it must have been in a quantity considerably less than 0.5 ng/ml

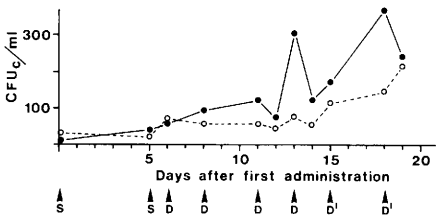

FIG. 2. CFUc concentration in peripheral blood during the 3-week period of study; effect of repeated doses of dextran sulfate on both dogs. Values shown were taken just before each administration of DS, or on another day. Note the tendency for the CFUc to increase progressively as further doses of DS are experienced. S = saline only (0.9%); D = 10 mg DS/kg body wt.; D′ = 15 mg DS/kg body wt.

which, according to the literature, would have no effect in any *in vivo* system.

Discussion. Previously, many polyanions (especially polysaccharide polysulphates, such as the heparinoids) have been observed to increase the number of MNC in the blood of mammalian species (6, 11–13). With these observations in mind, we asked ourselves the question, if stem cells (either committed or noncomitted) are part of the blood MNC population, might not polyanions be able to increase the number of stem cells in the blood too? This turned out to be indeed the case, but the CFUc increased even more than expected. While the MNC increased twofold after 10 mg DS/kg body weight, the CFUc increased some sevenfold, and after 15 mg/kg, some tenfold.

This would suggest that, although DS also affects the lymphocyte count, it is particularly effective in raising the number of CFUc in the blood within 3 hr of iv administration by 7–10 ×, depending on the dose administered.

At the present time, the mechanisms that are effective in increasing the CFUc concentration in the blood after dextran sulfate administration are far from clear. In principle, one must consider actions that lead to a "mobilization" of cells from extravascular sites by allowing increased access to the blood and thereby increasing the concentration in the blood. Along the same lines would be the argument that an increased number of CFUc in the blood could be expected if there were a marginal pool from which there was a shift of cells into the circulating pool under the influence of dextran sulfate. However, one must also entertain another possibility. If the blood transit time of blood stem cells were altered, were in effect prolonged, then one would also quickly observe an increase in the number of circulating CFUc. Bradfield and Born (14) have made a detailed investigation of the interference with lymphocyte recirculation by heparin and DS in mice and rats. This phenomenon may also apply to stem cells. In this context, it is of interest to consider ways and means by which polyanions may act on cells. Some investigations indicate that DS and other related poly-

anions may have an affinity for the membrane of target cells (15). They may in fact be attached to the cell membrane in the form of small granules (16). Reversible binding of the polyanions to the negative cell surface, perhaps by means of bridges of Ca^{2+} ions (18), would increase surface negativity. Thus, the electrophoretic mobility would be increased (17, 19). This increased negativity hinders adhesion of cells to one another as well as to the endothelium (17, 20). Such an action could result in an increased release rate from the tissues where they were lodged, and/or in an inability to "home" in these or other tissues. In this context, it has been further observed that incubation of cells with DS decreases membrane deformability, as demonstrated by the loss of the ability to adhere to glass surfaces (21). All of these factors might be involved in decreasing the anchorage of the cells outside the vascular channels and in making them more mobile for entry into the blood at the appropriate sites (as yet poorly understood) in the bone marrow.

In addition to this mechanism (or as an alternative), one must certainly consider the possibility that the CFUc increase is not due to an increase in the release rate of CFUc from extravascular sites, but rather to a decrease in their disappearance rate from the blood. If the CFUc transit time through the blood were somehow prolonged, perhaps because the CFUc were experiencing difficulties in adhering to the endothelial wall, this could give rise to the observed effect. These hypotheses can be tested by future experiments on the basis that the polyanion effect can be readily and completely neutralized by the addition of protamine (a polycation) in both the *in vivo* and the *in vitro* situations (7, 17). A final word should be said about the toxicity of dextran sulfate. It has been found that toxicity increases with increasing molecular weight but that DS of low molecular weight, such as 10,000, is no more toxic than heparin in the equivalent dosage (22). Ricketts, as well as others, have performed extensive clinical trials with DS and concluded it to be a suitable synthetic substitute for heparin (19, 23, 24).

As tentative hypothesis, then, we believe that dextran sulfate attaches itself to the CFUc cell membrane, thereby resulting in a decreased deformability and increased cell surface negativity. These changes give rise to a mobilization (apparently passive) of CFUc from extravascular sites into the circulation, and possibly also to a transient inability of these cells to leave the blood stream and to "home". This means that the observed increase in CFUc concentration in blood 3–5 hr after DS probably arises from both an elevated influx and a prolonged blood transit time of CFUc.

Summary. Intravenous administration of 10 mg or 15 mg dextran sulfate per kg body weight into dogs results in a marked increase of CFUc in the blood within 3 hr and, to a considerably lesser extent, of mononuclear leukocytes (MNC). The increase after the higher dose is more than tenfold, from less than 200 CFUc/ml blood to about 2000 CFUc/ml, within 3–5 hr; the CFUc gradually decrease thereafter to control levels several hours later. On the other hand, the MNC population experiences a two- to threefold increase which is maintained for several hours. The increase in CFUc after 10 mg/kg is less than after 15 mg/kg, indicating dose-dependence.

There is also a cumulative increase observed in the CFUc concentration in blood after repeated injections of dextran sulfate; there is no significant alteration in PMN and MNC levels and there is a slight decrease in the hematocrit and in the thrombocyte level.

The mechanism responsible for this phenomenon has not yet been clarified. Due to the probable action of polyanions on cell membranes affecting their adherence capabilities, one may consider both an increased rate of release of CFUc from a marginal or extravascular pool into the blood, as well as a prolongation of the CFUc blood transit time as being contributing factors to the observed blood CFUc increase.

We would like to express our sincere thanks to Mrs. B. Burr-Stengel and Miss E. Rüber for their excellent technical assistance, to Dr. I. Steinbach for her splendid animal care and particularly to Dr. H.-P. Schnappauf for his superb surgical skills. Dr. K. J. van den Berg of the Radiobiological Institute, TNO,

Rijswijk, Holland was kind enough to prepare the dextran sulfate.

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Received June 20, 1977. P.S.E.B.M. 1978, Vol. 157.