コルヒチン,ビンブラスチンおよびサイトカラシンDのリンパ球幼若化反応に対する阻止効果

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The Inhibitory Effects of Colchicine, Vinblastine and Cytochalasin D on Lymphocyte Blastogenic Responses

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ABSTRACT. The inhibitory effects of colchicine, vinblastine and cytochalasin D on blastogenic responses of equine, bovine and canine peripheral blood lymphocytes (PBL) were investigated. These drugs inhibited blastogenic responses of each PBL. The concentrations for 50% recovery in PBL blastogenic response of colchicine and vinblastine were lower in equine and canine PBL than in bovine PBL. This suggested that microtubules may be more concerned in blastogenic response of equine and canine PBL than of bovine PBL. On the other hand, the concentration for 50% recovery in PBL blastogenic response of cytochalasin D was almost the same in the PBL of each animal. This meant that microfilaments made only a small contribution to the lymphocyte blastogenic response.—KEY WORDS: blastogenic response, colchicine, cytochalasin D, lymphocyte, vinblastine.

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In lymphocyte blastogenic response, a signal for the stimulation of blastogenesis transmitted through the following pathways [9]: (1) binding of mitogen to its receptor, (2) changes of metabolism in membrane, and (3) clustering of receptors and/or increase of ion influx. The responsiveness to mitogen as judged from DNA synthesis differs according to the animal species [7, 12]. Equine, bovine and canine peripheral blood lymphocytes (PBL) have its own optimum concentration of mitogens for blastogenic response, because they have the different optimum occupied binding sites of mitogen [13]. However, the cause of the different responsiveness to mitogen cannot be explained only by the occupied binding sites. Thus we suspected that the different responsiveness might depend on different mechanisms of signal transmission at the early stage of the blastogenic response as described above.

Cytomusculature such as microtubules and microfilaments maintains the structure of the cell. It also participates in the clustering of receptors and the transmission of signals for the activation from receptors to cytoplasmic organs [3, 9]. Colchicine and vinblastine have generally been used as inhibitors of polymerization in microtubules, and cytochalasin is used for that purpose in microfilaments. When lymphocytes are incubated with these drugs before the addition of mitogen, the blastogenic response is inhibited [3, 9, 15, 17].

In this study, the different inhibitory effects of colchicine, vinblastine and cytochalasin D on blastogenic responses of equine, canine and bovine PBL were investigated.

MATERIALS AND METHODS

Lymphocytes: A mixed breed horse (19 years old), a Holstein-Friesian cow (9 years old) and a mongrel dog (7 years old), recognized as clinically healthy by physical examination, were used in this study. Equine PBL were collected from venous blood by the Ficoll-Conray density centrifugation as described previously [12]. Bovine and canine PBL were collected by

the same method as that used for the collection of equine PBL except for the specific gravity of the Ficoll-Conray solution (specific gravity 1.087 for bovine blood and 1.082 for canine). The collected PBL was washed three times with Hank's balanced salt solution, pH 7.4 (HBSS). After washing, PBL was resuspended in RPMI 1640 containing 20% heat-inactivated horse serum. The concentration of PBL in suspension was adjusted to 1.5×10^6 cells per m*l*.

Assay for inhibition of lymphocyte blastogenic response: One hundred μl of PBL suspension was put in each well of a 96-well microculture plate and colchicine (Nakarai Chem. Co., Ltd., Kyoto), vinblastine (Kyorin Pharmaceutical Co., Ltd., Tokyo) or cytochalasin D (Sigma, St. Louis) was added. The drugs were diluted by HBSS and 20 μl of their dilutions were added to each well. The final concentration of the drugs was adjusted to be between 10^{-10} and 10^{-4} M. PBL was incubated for one hour in 5% CO_2 incubator at 37°C. After incubation, phytohemagglutinin-P (PHA, Difco, New York) or concanavalin A (ConA, Pharmacia, Milwaukee) was added to each well and incubated for 72 hours. The concentration of PHA for stimulation of equine, bovine and canine PBL was 5, 15 and 20 μg per m*l*, respectively, and that of ConA was 50, 5 and 25 μg per m*l*, respectively, as described previously [8, 10, 12].

Next, 0.5 μCi of ^{3}H-thymidine (^{3}H-TdR, Amersham Japan, Tokyo) was added to the culture. After the incubation for 24 hours, PBL was harvested on a glass fiber filter, and the radioactivity of ^{3}H-TdR incorporation was counted. The cultures were performed in triplicate. The rate of recovery in blastogenic response was calculatd by the following equation:

$$\text{The recovery rate in the blastogenic response (\%)} = \frac{^3\text{H-TdR incorporation of cultured PBL with inhibitor}}{^3\text{H-TdR incorporation of cultured PBL without inhibitor}} \times 100$$

The influence of inhibitors on the response to mitogen was compared among equine, bovine and canine PBL, based on the concentration for 50% recovery in the blastogenic response.

RESULTS

The recovery rates in blastogenic response brought about by PHA or ConA in equine, bovine and canine PBL are shown in Figs. 1 to 3. The concentration for 50% recovery in the blastogenic response of colchicine was highest in bovine PBL, followed by those in equine and canine PBL.

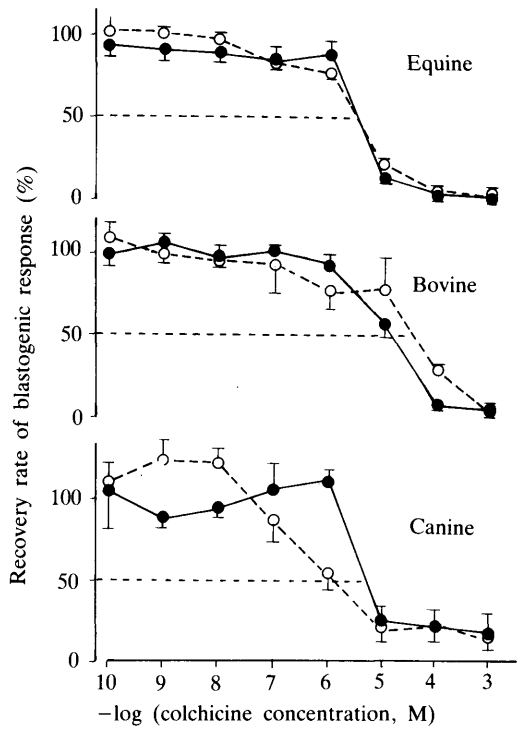

Fig. 1. The recovery rates of blastogenic response in equine, bovine and canine peripheral blood lymphocytes (PBL) by colchicine inhibition. —●—: Phytohemagglutinin-P stimulated PBL, --○--: Concanavalin A stimulated PBL. (n=3, m±SE)

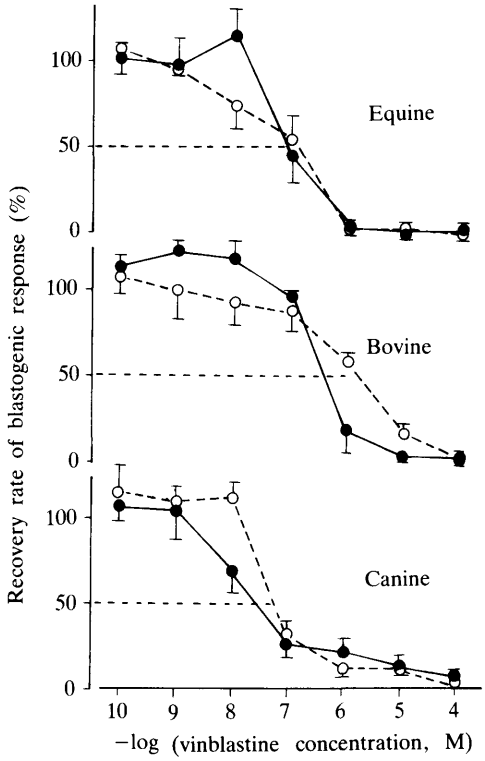

Fig. 2. The recovery rates of blastogenic response in equine, bovine and canine peripheral blood lymphocytes by vinblastine inhibiton. For key see Fig. 1. (n=3, m±SE)

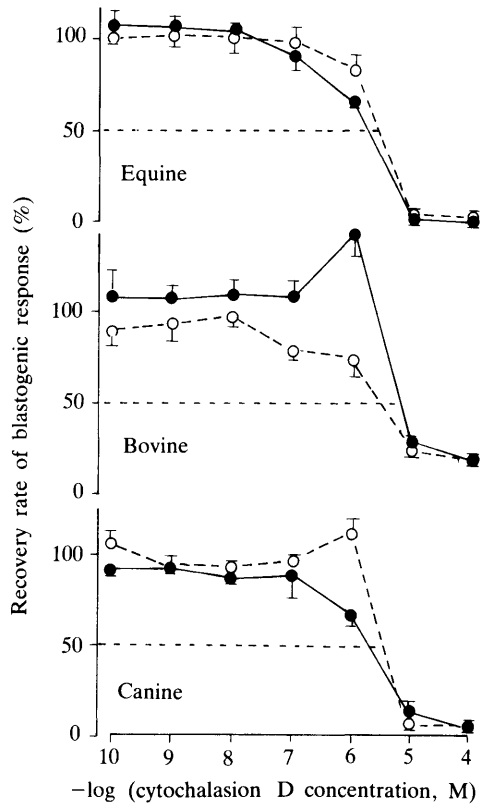

Fig. 3. The recovery rates of blastogenic response in equine, bovine and canine peripheral blood lymphocytes by cytochalasin D inhibition. For key see Fig. 1. (n=3, m±SE)

Table 1. The concentrations[a)] for 50% recovery in blastogenic response of colchicine, vinblastine and cytochalasin D

	colchicine		vinblastine		cytochalasin D	
	PHA	ConA	PHA	ConA	PHA	ConA
equine	3.2×10^{-6}	3.1×10^{-6}	8.7×10^{-8}	1.3×10^{-7}	1.8×10^{-6}	2.6×10^{-6}
bovine	1.4×10^{-5}	3.6×10^{-5}	3.8×10^{-7}	1.4×10^{-6}	6.4×10^{-6}	3.2×10^{-6}
canine	4.8×10^{-6}	1.2×10^{-6}	2.6×10^{-8}	5.4×10^{-8}	2.0×10^{-6}	3.8×10^{-6}

a) Each concentration is exhibited mole per litter (M).

That of vinblastine was highest in bovine PBL, followed by those in canine and equine PBL. That of cytochalasin D was almost the same in each animal's PBL. The inhibitor concentrations for 50% recovery in the blastogenic response for each PBL are shown in Table 1.

When colchicine or vinblastine was used as the inhibitor, the concentration for 50% recovery in blastogenic response of bovine PBL was markedly higher than that of equine and canine PBL. On the other hand,

when cytochalasin D was used , there was no marked difference in the concentration for 50% recovery in the blastogenic response of each animal's PBL.

DISCUSSION

Vinblastine, vincristine, colchicine, corcemide and podophyllotoxin have been reported to bind to microtubules and to obstruct the formation of caps by inhibition of polymerization in tubules [1, 15]. It is also clear that the inhibitory effects of the drugs cannot be ascribed to their toxicity [16]. Moreover, these effects are not attributable to the inhibition of DNA synthesis because Edelman *et al.* reported that thymidine incorporation could take place in the presence of the drugs [3].

Cytochalasin has been widely used as an inhibitor of polymerization in microfilaments. Many kinds of cytochalasin have been reported and cytochalasin B and D have generally been used as inhibitors for lymphocyte blastogenic response [18]. It was reported that cytochalasin B also inhibits the blastogenic response by inhibiting the transport of glucose [6, 11], but cytochalasin D has no detectable effect on glucose transport [5]. For investigation of the role of microfilaments in lymphocyte blastogenic response, cytochalasin D is reliable probe.

The mitogenic responses of human, mouse and rabbit lymphocytes to ConA were inhibited approximately 70 to 90% by colchicine at a concentration of 10^{-6} M [11], and the mitogenic response of human lymphocytes to PHA was inhibited approximately 75% by a 10^{-6} M concentration of colchicine or vinblastine [4]. On the other hand, it was reported that the cap formation of mouse splenic lymphocytes stimulated with ConA was inhibited approximately 60 to 80% by a 10^{-4} M concentration of colchicine, vinblastine or vincristine [3]. Since these different results might be obtained due to different sources of lymphocytes as well as to the method of the estimation, the effects of the drugs on each PBL were compared based on 50% recovery in the blastogenic response from ^{3}H-TdR incorporation in this study as in the former reports.

When colchicine was used as the inhibitor, it showed almost the same inhibitory effect on equine and canine PBL as described above, needing a higher concentration of colchicine than other animals' PBL to obtain the same inhibitory effect on bovine PBL. The concentration of vinblastine as the inhibitor was almost the same for bovine PBL as for human PBL; however, the concentration of vinblastine was lower for equine and canine PBL than for bovine PBL. When cytochalasin D was used as the inhibitor, there were no remarkable differences among the PBL of the three kinds of animals. From these results, transmissions of activating signals in equine and canine PBL were easily obstructed by inhibitors of microtubule functions, and their mitogenic responses were inhibited at lower concentrations of inhibitor. Microfilaments did not contribute to the differing responsiveness to mitogen. Consequently, it seems that microtubules control the responsiveness to mitogen more markedly in the early stage of equine and canine blastogenic response than in that of bovine one.

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要　約

コルヒチン，ビンブラスチンおよびサイトカラシンDのリンパ球幼若化反応に対する阻止効果：田島誉士・藤永　徹・大友勘十郎・小池寿男（北海道大学獣医学部家畜外科学講座）——ウマ，ウシおよびイヌの末梢血リンパ球（PBL）幼若化反応におけるコルヒチン，ビンブラスチンおよびサイトカラシンDによる幼若化阻止効果について検討した．各PBLの幼若化反応は，3種の薬剤により完全に阻止された．微小管重合阻害剤であるコルヒチンまたはビンブラスチンによるウマおよびイヌPBLの50%幼若化回復濃度は，ウシPBLのそれよりも著しく低かった．一方，微小線維重合阻害剤であるサイトカラシンDを用いた場合，ウマ，ウシおよびイヌPBLの50%幼若化回復濃度は，各種動物PBL間で大差はなかった．これらの結果から，ウマおよびイヌPBL幼若化反応においてはウシPBL幼若化反応におけるよりも，微小管が深く関与していることが示唆された．