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Stab Wound Age Estimation by Changes of Basophilic Granulocytes After Injection of Different Substances

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Abstract

In the present study are described the changes of basophilic granulocites' response on injection of different substances. There are evaluated possibilities to use the changes of these leukocytes as a criterion for injected stub wound age estimation. The experimental study was carried out on formalin-fixed paraffin-embedded material from rat skin. Results show that changes of basophilic granulocytes and their response depend on time since injury and injected substance and use of this particular cell response as a criterion for stub wound age estimation is recommended.

Keywords: *stub wound, age estimation, wound healing, basophilic granulocytes, labrocytes, mast cells, injection*

Introduction

Stub wound age estimation is the question of great importance in modern Forensic Medicine. Time since injury is possible to detect by the reparative processes of the wound. Unlike other mechanical injuries, the stub wound is characterized by less local inflammatory response and more quick dynamics of reparation. The changes vary according to the size of wound, the tissue, age and health of victim and so on (6). Unfortunately, biological variability introduces a wide margin of uncertainty, so that a range of probabilities can be offered and definition of time interval is almost always difficult (7). The subject is of particular interest when the question is associated with different injected substances. There are many publications and experimental works on this subject as it is a favorite topic for research (2,5), but offered schedules for stub wound age estimation are not yet sufficient and particular criteria still should be elaborated and selected.

The aim of the present study was to evaluate the changes of basophilic granulocytes and their response on mechanical injury as a possible criterion for injected stub wound age estimation after injection of different substances.

Materials and Methods

Animals: Wistar white rats of 11-12 months were obtained from the vivarium of Tbilisi State Medical University and kept under laboratory conditions.

Injection of Substances: The substances for injection have been chosen as a positive control (Turpentine), negative control (NaCl) and routine penicillin. Injection of substances had been performed on the backside of thoracic region of the animal, on midline, under the skin.

Formalin fixation: The skin specimens of 10X0,5 mm size have been taken from the back of living animals after injection, respectively, after one hour, three hours, 6 hours, 24 hours, three days, 5 days and 7 days, then put into the buffer: formalin 40% - 100ml; NaH_2PO_4 - 4g; Na_2HPO_4 - 6,5g; AD - up 1l; and incubated for 24 hours.

Paraffin embedding: Formalin fixed specimens were put for dehydration into the alcohol 70° for 24 hours, then in alcohol 90°- 24h, trice in alcohol 96° for 24 hours

each, then on room temperature in solution of alcohol-aether 1:1 for 15 min and twice in Chloroform for 3 min each, then infiltrated with I paraffin on 37°C - 30 min, II and III paraffins on 56°C for 45 min each and then blocked in paraffin on room temperature. The blocks were stored at +4°C.

Histochemistry: Paraffin blocks were sectioned on a rotation microtome. Sections were put into the thermostat on 56°C for 20 min and then dewaxed. The slides were stained with 0,5% Toluidine blue (pH 7,4) for 20 minutes (4).

Results

Results of study revealed different quantitative changes of all basophilic granulocytes, degranulated and non-degranulated cells, depending on time since injection and injected substance, as follows:

One hour after injection - In case of positive control, number of all basophilic granulocytes increases by 11% (P>0,1), degranulated cells - 52% (P<0,1) and non-degranulated - only 2,4% (P>0,1). In case of penicillin, number of all basophilic granulocytes increases by 116% (P<0,01), degranulated cells - 100% (P<0,05) and non-degranulated - 119% (P<0,01). In case of negative control, number of all basophilic granulocytes increases by 47% (P<0,01), degranulated cells rapidly increase by 408% (P<0,01), but non-degranulated decrease by 26% (P<0,05).

Three hours after injection - In case of positive control, number of all basophilic granulocytes continues to increase by 72% (P<0,02), degranulated cells decrease by 13% (P>0,1) and non-degranulated - increase by 98% (P<0,02). In case of penicillin, number of all basophilic granulocytes decreases by 21% (P<0,05), degranulated cells - 60% (P<0,05) and non-degranulated - only 14% (P>0,1). In case of negative control, number of all basophilic granulocytes does not change (P>0,1), degranulated cells rapidly decrease by 66% (P<0,01), but non-degranulated increase by 88% (P<0,02).

6 hours after injection - In case of positive control, number of all basophilic granulocytes again increases by 117% (P<0,01) and reaches the peak, degranulated cells rapidly increase by 194% (P<0,01), non-degranulated - 107% (P<0,01). In case of penicillin, number of all basophilic granulocytes again increases by 6% (P>0,1), degranulated cells rapidly increase by 230% (P<0,02), but non-degranulated continue to decrease by 13% (P<0,1). In case of negative control, number of all basophilic granulocytes again increases by 29% (P<0,02), degranulated cells continue to decrease by 74% (P<0,01) and non-degranulated continue to increase by 55% (P<0,01).

24 hours after injection - In case of positive control, number of all basophilic granulocytes begins to decrease by 36% (P<0,01), degranulated cells still hold the peak and even increase by 9% (P>0,1), but non-degranulated decrease by 44% (P<0,01). In case of penicillin, number of all basophilic granulocytes does not change (P>0,1), degranulated cells rapidly decrease by 79% (P<0,02), but non-degranulated increase by 23% (P<0,05). In case of negative control, number of all basophilic granulocytes increases only by 7% (P>0,1), degranulated cells slightly decrease (P>0,1) and non-degranulated increase by 8% (P<0,1).

3 days after injection - In case of positive control, number of all basophilic granulocytes provisionally stops to decrease (P>0,1), degranulated cells rapidly decrease by 56% (P<0,01) and non-degranulated increase by 26% (P<0,05). In case of penicillin, number of all basophilic granulocytes increases by 9% (P<0,1), degranulated cells - 164% (P<0,02) and non-degranulated do not change (P>0,1). In case of negative control, number of all basophilic granulocytes decreases by 19% (P<0,05), degranulated cells slightly decrease (P>0,1) and non-degranulated - 18% (P<0,05).

5 days after injection - In case of positive control, number of all basophilic granulocytes again decreases by 48% (P<0,01), degranulated cells - 2% (P>0,1) and non-degranulated - 54% (P<0,01). In case of penicillin, number of all basophilic granulocytes decreases by 6% (P>0,1), degranulated cells - 76% (P<0,02), but non-degranulated slightly increase by 4% (P>0,1). In case of negative control, number of all basophilic granulocytes (P>0,1), as well as degranulated (P>0,1) and non-degranulated cells (P>0,1), only slightly increases.

7 days after injection - In case of positive control, number of all basophilic granulocytes decreases by 9% (P>0,1), degranulated cells do not change (P>0,1) and non-degranulated decrease by 21% (P<0,1). In case of penicillin, number of all basophilic granulocytes again increases by 37% (P<0,02), degranulated cells do not change (P>0,1) and non-degranulated increase by 38% (P<0,02). In case of negative control, number of all basophilic granulocytes again increases by 32% (P<0,05), degranulated cells - 300% (P<0,02) and non-degranulated - 22% (P<0,05).

Discussion

Basophilic granulocytes belong to the big family of leukocytes, which perform the major part of organism defense against injurious stimuli. Basophils (tissue (labrocytes or mast cells) and blood basophilic

leukocytes) produce the tissue microcirculation and trophic regulatory bioactive substances (histamine, serotonin and etc.) which play the principal role in wound repair (1,2). They continue the inflammatory response begun by neutrophils and provide with necessary rearrangement and development of blood vessels and connective tissue (3,5,8). Release of bioactive substances occurs by degranulation of basophilic leukocytes and this process indicates to degree of bioactivity of these cells. The total number of basophilic granulocytes in wounded tissue, ratio of degranulated and non-degranulated cells and their change according to the time interval since injury and injected substance could clearly show the difference of intensity of inflammatory response in different conditions. Evaluation of these differences was a purpose of present study.

Results of study show that total number of basophilic granulocytes increases during the first six hours after injection and then slowly decreases, but couldn't reach the starting level and continues to be higher. Almost same picture is with non-granulated basophils, whereas degranulated cells increase and decrease in number much more rapidly.

Changes of leukocytes depend very much to the injected substances. In case of positive control the picture is same with total number and non-degranulated cells which reach the peak after six hours, and maximum degranulation also occurs almost for this time. The case with negative control shows less intensity of inflammatory response, total number as well as non-degranulated cells increases during 24 hours and then decreases very slowly, but degranulation occurs rapidly, within one hour after injection, and then rapidly decreases up to the levels below the initial point. The case with penicillin completely differs from others - all three "kinds" of cells' numbers increase rapidly within one hour, then slightly decrease, again increase and stay high during all experimental period, only degranulated cells decrease in number after 5 days.

These differences could be explained only by chemical and pharmacodinamic characteristics of injected substances. Turpentine - as a phlogogenic substance and positive control - causes well-developed and long-time inflammatory response, because its phagocytoses and digestion occurs slowly and completely. The response of basophilic granulocytes indicate the same process. NaCl - as a physiologic substance and negative control - doesn't change the picture of typical stub wound healing and cell response is adequate to this. That's why degranulation is maximum soon after injection and, because mechanical damage of tissues in injected wounds is less, it rapidly diminishes. Penicillin - as a salt and chemical substance - causes well-expressed local irritating changes which are reflected on changes of basophilic picture - degranulation occurs soon after injection and cell response stays high for long time, until the substance will be digested or neutralized.

Thus, changes of basophilic granulocytes described in present study have considerable value in injected stub wound age estimation. The above-mentioned changes firmly depend on injected substance. Use of this particular cell response as a criterion for stub wound age estimation is recommended.

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Установление давности колотой раны по изменениям базофильных гранулоцитов после инъекции разных веществ

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Р Е З Ю М Е

Целью исследования было определение возможности применения изменений базофильных гранулоцитов в качестве критерия установления давности колотой раны. Были изучены изменения базофильных гранулоцитов в разные интервалы времени соответственно изучаемых давностей повреждения после инъекции разных веществ. В качестве инъекционных веществ были взяты: скипидар - как позитивный контроль, физиологический раствор - как негативный контроль, и пенициллин. Исследование показало, что изменения базофильных гранулоцитов определенно зависят от давности повреждения и инъекционного вещества и могут быть применены для установления давности колотой раны.

Ключевые слова: *установление давности, колотая рана, заживление раны, базофильные гранулоциты, лаброциты, тучные клетки, инъекция*